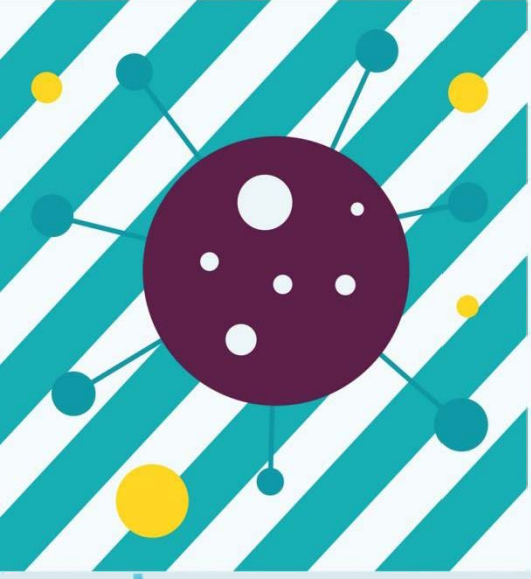
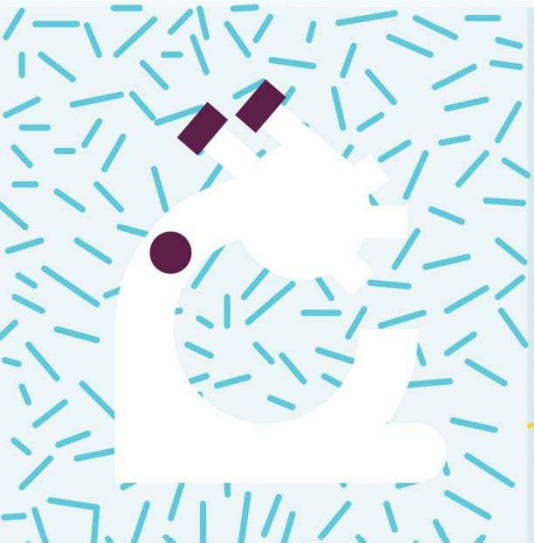


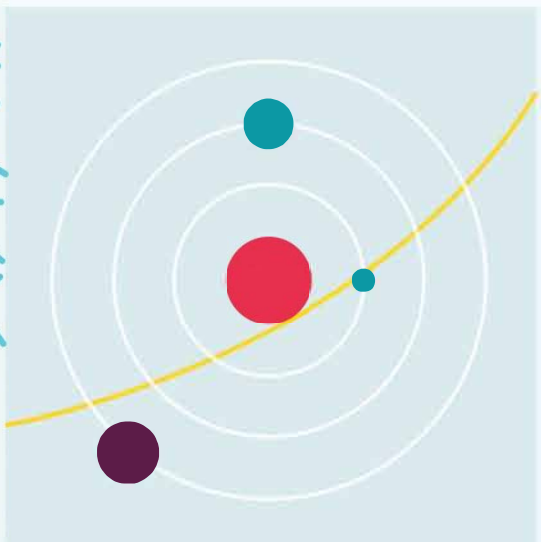
ISRES

2025

CURRENT STUDIES IN LIFE SCIENCES

EDITOR
PROF.DR. MEHMET OZASLAN





Current Studies in Life Sciences

EDITOR

Prof. Dr. Mehmet ÖZASLAN

2025

Current Studies in Life Sciences – 2025

Editor
Prof. Dr. Mehmet ÖZASLAN

Language Editors
Lecturer Ceren DOĞAN

Cover Design and Layout
Mehmet SÖNERCAN

This book was typeset in 10/12 pt. Times New Roman, Italic, Bold and Bold Italic.

Current Studies in Life Sciences – 2025

Published by ISRES Publishing, International Society for Research in Education and Science (ISRES)

Includes bibliographical references and index.

ISBN
978-625-6959-89-7

Date of Issue
October / 2025

Address: Askan Mah. Akinbey Sok. No:5/A Meram/Konya/Türkiye

E-mail
isresoffice@gmail.com

www.isres.org

Current Studies in Life Sciences-2025

PREFACE

Welcome to **"Current Studies in Life Sciences – 2025."** This volume stands as a testament to the ever-evolving landscape of scientific inquiry, presenting a comprehensive view of the latest advancements, innovative research, and critical explorations in the fields that shape our understanding of health, life, and well-being. As we navigate the complexities of modern science, this book serves as a valuable resource for researchers, practitioners, and students seeking insights into the forefront of contemporary discoveries.

In today‘s rapidly advancing world, the realm of health and life sciences continues to expand at an unprecedented pace. Each year, dedicated researchers, scholars, and professionals push the boundaries of knowledge, unveiling novel findings that deepen our understanding of the human body, the environment, and the intricate relationships between them. This compilation reflects the spirit of scientific curiosity and innovation, presenting a diverse collection of studies that capture the multifaceted dimensions of life sciences.

The chapters contributed by distinguished authors span a wide range of disciplines, highlighting **the interdisciplinary nature** of modern scientific research. From cutting-edge developments in medicine and dentistry to ecological studies exploring the delicate balance of our ecosystems, each contribution offers a unique perspective on the challenges and opportunities shaping the future of health and life sciences.

One of the central themes of this volume is **the interconnectedness** of health, dentistry, and life sciences, emphasizing how progress in one field often fuels advancements in another. By bringing together diverse insights, this book underscores the importance of collaborative research in addressing global health concerns and enhancing the quality of life.

As we embark on this intellectual journey, we celebrate the dedication, perseverance, and creativity of the researchers whose contributions enrich the collective knowledge of humanity. **"Current Studies in Life Sciences – 2025"** is more than a compilation of scholarly works—it is a reflection of the indomitable human spirit that drives us to explore the unknown and develop solutions to the complex challenges of our time.

We hope that this book inspires curiosity, sparks innovative ideas, and fosters a deeper appreciation for the remarkable strides being made in understanding, preserving, and improving life in all its forms.

Prof. Dr. Mehmet Özaslan

Department of Biology, University of Gaziantep, Turkiye

Website: mehmetozaslan.com e-mail: ozaslanmd@gmail.com

Table of Content

Avian Influenza Virus: Epidemiology, Evolution, Antigenicity, and Control Strategies in Pakistan

Abdul REHMAN
Muhammad SAFDAR
Mehmet OZASLAN

1. Introduction

Avian influenza viruses (AIVs) are members of the Orthomyxoviridae family and are classified into subtypes based on the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) (Krammer et al., 2018). These viruses are categorized as either low pathogenic avian influenza (LPAI) or highly pathogenic avian influenza (HPAI), depending on their virulence in poultry (Naeem et al., 2007). Subtypes H5, H7, and H9 are of particular concern owing to their association with poultry outbreaks and zoonotic transmissions (Ali et al., 2021). AIVs demonstrate remarkable adaptability through two primary evolutionary mechanisms: antigenic drift and antigenic shift (Peacock et al., 2018). Antigenic drift arises from gradual mutations in the HA and NA genes, which progressively diminish vaccine effectiveness (Shahzad et al., 2021). In contrast, antigenic shift involves the reassortment of genetic segments between multiple influenza strains, a process that occurs frequently (Rehman et al., 2020). The H9N2 subtype, which is endemic in poultry populations across Asia, including Pakistan, significantly contributes to viral evolution by serving as a donor of internal gene segments to more virulent strains, such as H5N1, thereby amplifying its importance in global influenza dynamics (Ali et al., 2021).

Pakistan's susceptibility to AIV outbreaks stems from a confluence of environmental, industrial, and socioeconomic factors (Umar et al., 2016). Its location along major migratory bird flyways, coupled with intensive poultry farming and insufficient biosecurity practices, fosters an environment conducive to viral persistence and evolution (Ali & Akhtar, 2022). The poultry industry plays a vital role in Pakistan's economy, yet AIV outbreaks impose substantial financial burdens through decreased productivity, elevated mortality rates, and international trade restrictions (Umar et al., 2016). For instance, H9N2 outbreaks result in millions of dollars in annual losses within the sector (Umar et al., 2016). Moreover, the zoonotic potential of AIVs poses a significant public health threat, as evidenced by mutations in LPAI H9N2 strains that enhance their infectivity and replication in humans (Ahmed et al., 2024). Surveillance efforts in live bird markets (LBMs), where close human-poultry interactions occur, highlight the elevated risk of spillover events (Chaudhry et al., 2020).

2. Epidemiology of Avian Influenza in Pakistan

Avian influenza (AI), caused by influenza A viruses, represents a major public health and economic challenge in Pakistan, where interfaces between the poultry sector, wild bird populations, and human activities facilitate viral transmission, evolution, and persistence (Rehman et al., 2020). This section examines key epidemiological aspects, drawing on recent studies. The initial AI outbreak in Pakistan was recorded in 1995, with subsequent events underscoring profound economic and public health repercussions (Naeem et al., 2007). The 2006-2007 H5N1 outbreak

was especially severe, necessitating extensive poultry culling and imposing trade embargoes that inflicted millions of US dollars in losses, particularly in key production regions such as Punjab and Sindh (Naeem et al., 2007). These incidents also compromised food security, disproportionately affecting small-scale farms and backyard operations, and highlighted the urgent need for enhanced surveillance and biosecurity protocols (Hussain et al., 2020) as shown in Figure 1.

Table 1 presents that surveillance systems in Pakistan have consistently identified H5 and H9 subtypes in both commercial and backyard poultry settings (Abid et al., 2017). The H9N2 strain, now endemic, exhibits rising prevalence in densely populated areas like Lahore, Faisalabad, and Multan (Abid et al., 2017). Data from LBMs reveal seropositivity rates exceeding 20%, positioning these markets as critical hotspots for viral amplification and dissemination (Chaudhry et al., 2020). Nonetheless, limited surveillance capabilities in rural regions and inconsistent reporting hinder precise epidemiological mapping (Hussain et al., 2020).

Table 1. Major Avian Influenza Outbreaks in Pakistan; affected regions, estimated economic losses, and key characteristics based on published surveillance and outbreak reports.

Year / Period	Subtype	Affected Regions	Estimated Losses	Key Notes
1995	H7	Punjab, Sindh	Not documented	First recorded AI outbreak in Pakistan
2003–2004	H7N3	Multiple provinces	Millions of USD	Trade restrictions imposed
2006–2007	H5N1	Punjab, Sindh	> US$ 10 million	Severe outbreak, culling of thousands of birds
2010–Present	H9N2 (endemic)	Lahore, Faisalabad, Multan	Millions annually	High seropositivity in LBMs
2016	H5N8	Migratory bird habitats	Localized impact	Linked to intercontinental migration
2020–2023	H9N2, H5N8	Various	Continuing	Detected in wild and domestic birds

Migratory birds traversing the Indus Flyway serve as vectors for transmitting AIVs to domestic poultry, with waterfowl and shorebirds playing central roles in viral introduction through wetland interactions (Tahir et al., 2020). Unmanaged poultry systems exacerbate risks of viral reassortment and evolution at these interfaces (Ali & Akhtar, 2022).

The zoonotic capabilities of AIVs, notably H5N1 and H9N2, are well-documented, with human infections typically linked to direct contact with infected birds or contaminated

environments (Ahmed et al., 2019). In Pakistan, seropositivity for H9N2 has been observed among LBM and farm workers, indicating occupational vulnerabilities (Tahir et al., 2020). Genetic markers in H9N2 isolates suggest adaptation to mammalian hosts, fueling concerns over pandemic potential (Iqbal et al., 2022). Although human-to-human transmission remains limited, sustained vigilance and proactive public health measures are essential (Sharma et al., 2019).

3. Mechanisms

AIVs primarily evolve through genetic reassortment, wherein co-infecting strains exchange genomic segments within a host, generating novel genotypes with potentially heightened virulence or expanded host ranges (Rehman et al., 2020). Such events are confined to specific ecosystems shaped by host interactions and environmental conditions (Ahmed et al., 2024).

The inherently high mutation rate of AIVs, driven by their error-prone RNA-dependent RNA polymerase, facilitates antigenic drift (Peacock et al., 2018). Mutations in HA and NA glycoproteins enable immune evasion, sustaining recurrent outbreaks (Shahzad et al., 2021). Evidence indicates that targeted point mutations have promoted H9N2 adaptation to mammalian hosts in Pakistan (Iqbal et al., 2022).

Phylogenetic analyses of clades offer insights into AIV evolutionary patterns:

1. H5N1 strains circulating in Pakistan exhibit genetic similarities to those in Southeast Asia, emphasizing regional connectivity and the global pandemic risks posed by reassortment (Ali et al., 2021).
2. H5N8, first identified in Pakistani migratory bird habitats, shares high sequence identity with European variants, illustrating the influence of intercontinental migration routes on viral dissemination (Ali et al., 2021).
3. Endemic H9N2 in Pakistan demonstrates adaptability to poultry and mammals, with frequent reassortment enhancing its fitness across diverse hosts (Abid et al., 2017).

Migration along the Central Asian flyway significantly contributes to AIV propagation:

1. Waterfowl, such as ducks and geese, act as reservoirs for LPAI viruses that may evolve into pathogenic forms; genomic evidence confirms migratory introductions of H5N8 in Pakistan (Ali & Akhtar, 2022).
2. Strains detected in Pakistan often align with those from the Middle East, Central Asia, and Europe, underscoring the necessity for international surveillance to address transboundary spread (Gilbert et al., 2008).

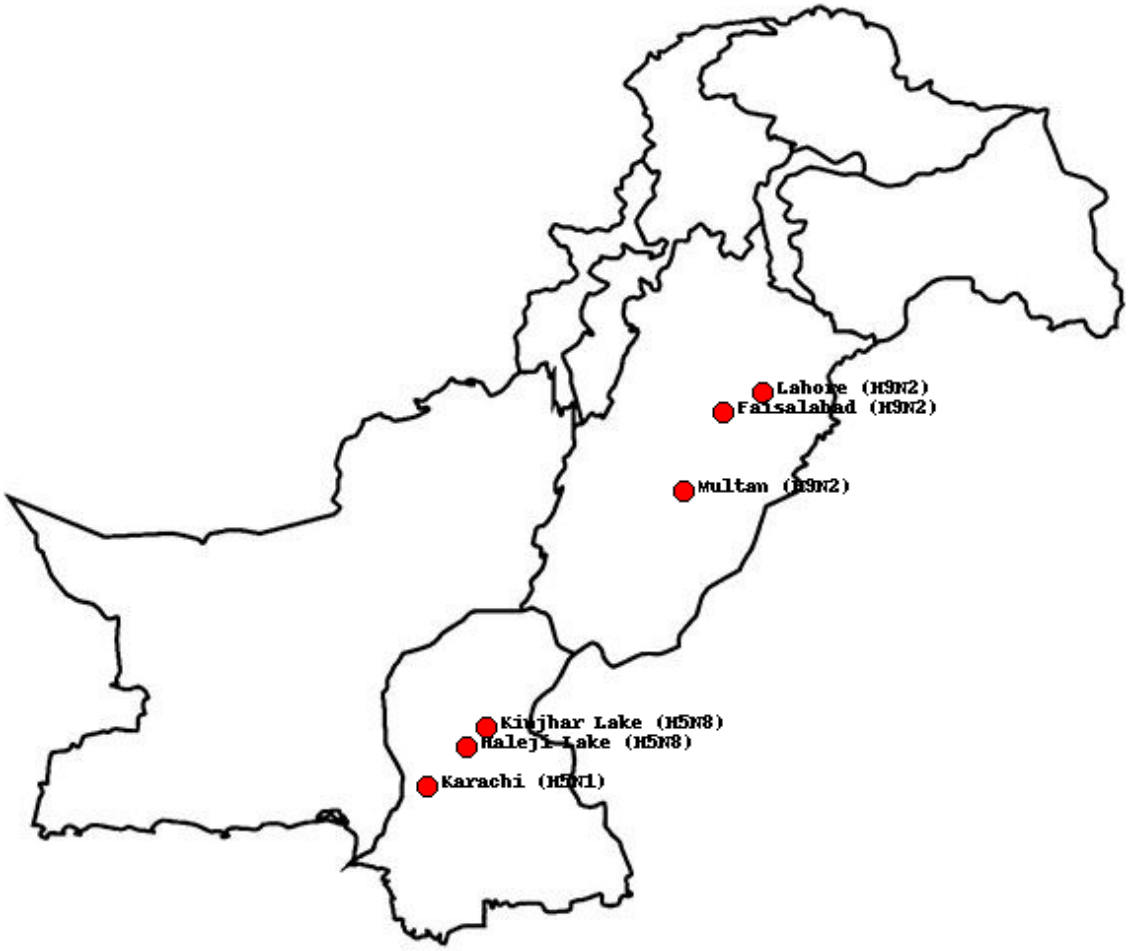


Figure 1. Map showing major avian influenza outbreak areas in Pakistan

4. Antigenic Variability and Immune Defense

AIVs display substantial antigenic variability, a hallmark of their adaptability that complicates vaccine development and control efforts, particularly in endemic areas like Pakistan where multiple strains co-circulate (Peacock et al., 2018).

HA and NA glycoproteins are central to antigenic properties and immune evasion, evolving under selective pressures from vaccination, cross-species transmission, and host immunity: a. Antigenic Drift: Involves point mutations in HA and NA genes that modify epitopes, as observed in Pakistani H9N2 strains where alterations in HA receptor-binding sites and NA activity facilitate immune escape and diminished vaccine performance (Peacock et al., 2018). b. Antigenic Shift: Occurs via gene segment reassortment in mixed bird populations, potentially yielding pandemic strains; frequent H9N2 reassortment in Lahore has led to highly pathogenic variants (Rehman et al., 2020).

Antigenic drift directly undermines vaccine efficacy:

1. Strain Mismatch: Global H9N2 vaccines offer limited protection in Pakistan due to region-specific HA genetic variations (Shahzad et al., 2021).

2. Elevated Mutation Rates: The absence of proofreading in AIV RNA polymerase accelerates divergence, necessitating ongoing monitoring and tailored vaccines (Peacock et al., 2018).

Advances in antigenic mapping, including hemagglutination inhibition assays and next-generation sequencing, have revealed:

a) Geographical variability in H9N2 antigenic profiles, reflecting local adaptations (Rafique et al., 2018).
b) Increased zoonotic potential from enhanced receptor-binding avidity in recent H9N2 isolates (Iqbal et al., 2022).
c) Novel HA and NA mutations, such as K113R and G228S, critical for evasion and transmissibility (Shahzad et al., 2021).

5. Molecular Mechanisms of Pathogenicity in Avian Influenza Virus

Interactions between AIVs and hosts are multifaceted, influenced by species, receptor specificity, and immune responses (Klenk et al., 2008). Wild waterfowl, as natural reservoirs, typically experience mild or asymptomatic infections, whereas domestic poultry suffer severe outcomes from HPAIV (Krauss et al., 2007). HA binding to sialic acid receptors determines tissue tropism and virulence; wild birds predominantly express α2,3-linked receptors, while poultry display both α2,3- and α2,6-linked, enabling wider infection (Watanabe et al., 2010). AIV adaptation in poultry increases HA affinity for respiratory epithelium, promoting systemic dissemination (Klenk et al., 2008).

Comparative analysis highlights LPAIV replication efficiency in wild birds without systemic disease, versus HPAIV transition in poultry through HA and NA modifications, allowing multi-organ replication (Hatta et al., 2001). Interspecies transmission poses zoonotic risks, with PB2 and HA mutations enhancing pathogenicity in humans for strains like H5N1 and H7N9 (Li et al., 2005).

Key genetic determinants of pathogenicity include:

1. HA multibasic cleavage site enabling cleavage by ubiquitous proteases for systemic infection (Hatta et al., 2001).
2. Polymerase complex mutations (e.g., PB2-E627K) improving mammalian replication efficiency (Li et al., 2005).
3. NS1 protein suppressing host interferon responses for immune evasion (Qi et al., 2012).
4. NA stalk deletions boosting viral fitness in poultry by supporting replication in respiratory and intestinal tracts (Qi et al., 2012).

Antiviral resistance presents treatment challenges: a. M2 channel mutations render amantadine and rimantadine ineffective (Bukhsh et al., 2023). b. Neuraminidase inhibitors like oseltamivir retain utility but are threatened by mutations (e.g., H275Y) (Bukhsh et al., 2023).

Emerging strategies focus on host pathway targeting and combination therapies to prevent resistance development (Krammer et al., 2018).

6. Risk Assessment for Transmission to Humans

The zoonotic transmission potential of AIVs is a global priority, especially in Pakistan, where extensive poultry farming and human-bird interfaces heighten risks (Ahmed et al., 2019). AIVs can adapt to cross species barriers, leading to infections and potential pandemics (Sharma et al., 2019). This section explores transformative molecular, epidemiological, and strategic dimensions of human transmission risks.

AIV human infectivity depends on mutations improving receptor-binding specificity (Watanabe et al., 2010). Avian strains bind α-2,3-linked sialic acids in bird tracts, while human-adapted viruses prefer α-2,6-linked in upper human airways (Watanabe et al., 2010). HA mutations in the binding site can enable this shift, elevating zoonotic threats (Iqbal et al., 2022).

Recent Pakistani surveillance has detected H5N1 and H9N2 strains with human-receptor-affining mutations (e.g., HA Q226L and G228S in H9N2) (Iqbal et al., 2022). Pakistan's endemic status includes documented zoonotic cases, primarily from LBMs and backyard flocks (Ahmed et al., 2019). H9N2, known as a gene donor for reassortants like H7N9, is widespread; human cases are mild but occupational risks are high for workers (Sharma et al., 2019). Seroprevalence studies in Lahore indicate 10% antibody presence in poultry workers, suggesting underreported subclinical infections (Malik et al., 2018).

Pandemic preparedness in Pakistan is hampered by weak biosecurity, limited vaccine access, and infrastructure deficits (Hussain et al., 2020). Vaccination is key for H5 and H9 control, but coverage is only 40-50% in commercial farms, with drift reducing efficacy (Hussain et al., 2020). Laboratory and personnel shortages impede response (Hussain et al., 2020). International support is vital for diagnostics and early warning (Gebreyes et al., 2014).

Recommendations include:

1. Bolstering molecular surveillance for emerging mutations (Rehman et al., 2020).
2. Improving biosecurity in LBMs and farms (Abbas et al., 2020).
3. Investing in broad-protection vaccines (Krammer et al., 2018).
4. Training healthcare workers for zoonotic detection and management (Gebreyes et al., 2014).

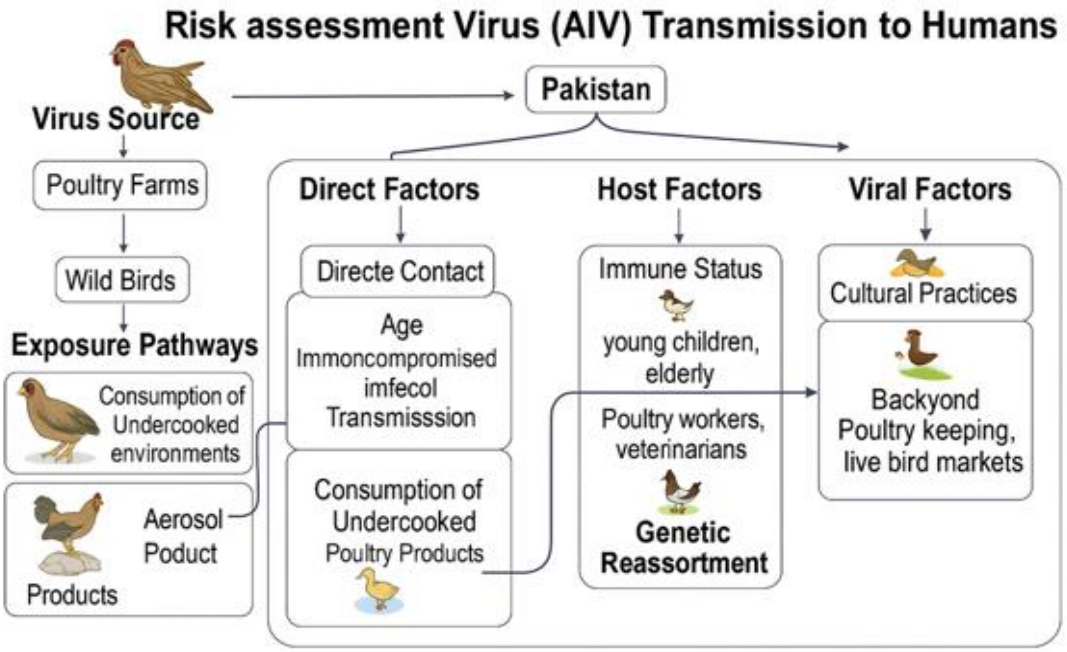


Figure 2. Risk assessment framework for Avian Influenza Virus (AIV) transmission to humans in Pakistan, illustrating virus sources, exposure pathways, and contributing direct, host, and viral factors.

7. Current Control Strategies in Pakistan

AIV outbreaks pose ongoing threats to Pakistan's poultry industry, public health, and economy (Umar et al., 2016). Control efforts encompass vaccination, biosecurity, surveillance, and collaborative initiatives, yet implementation gaps limit their success (Hussain et al., 2020).

Vaccination, using inactivated formulations against HPAI (e.g., H5N1) and LPAI (e.g., H9N2), effectively lowers mortality and viral shedding, mitigating economic impacts (Umar et al., 2016). However, challenges persist: a. Antigenic drift causes mismatches between vaccines and field strains, particularly for diverse H9N2 variants (Peacock et al., 2018). b. Cold chain disruptions compromise vaccine integrity in rural settings (Hussain et al., 2020). c. Lack of uniform policies; commercial farms vaccinate more consistently than smallholders, who face awareness, cost, and logistical barriers, fostering viral reservoirs (Hussain et al., 2020).

Biosecurity practices—access restrictions, disinfection, waste management, and wild bird separation—are essential for preventing introduction and spread (Abbas et al., 2020). Commercial operations generally maintain higher standards, but small farms and backyard systems often fall short, contributing to outbreaks via poor carcass disposal and multi-species housing (Chaudhry et al., 2020). Targeted education and incentives for smallholders could enhance compliance (Gebreyes et al., 2014).

Surveillance, supported by government and international bodies like FAO and WHO, involves diagnostic labs in high-risk zones (Rehman et al., 2020). Yet, underreporting, rural capacity limitations, and funding shortages impede early detection (Abbas et al., 2020). Advanced

tools like NGS could improve real-time monitoring but are constrained by costs and expertise (Rehman et al., 2020).

Government actions include culling, movement controls, and farmer compensation to encourage reporting (Naeem et al., 2007). Enforcement struggles with funding and resistance (Hussain et al., 2020). International partnerships with FAO, WHO, and WOAH provide technical and financial aid, including training, but a unified long-term strategy is needed, incorporating public-private collaboration and community involvement (Gebreyes et al., 2014).

8. Innovative Approaches to AIV Control

As AIV threats evolve, traditional methods like culling and strain-specific vaccines prove insufficient against antigenic variability and unpredictable outbreaks in settings like Pakistan (Krammer et al., 2018). This section discusses transformative innovations: CRISPR genome editing, universal vaccines, and AI for prediction.

CRISPR-Cas9 enables precise host genome modifications to confer AIV resistance, such as disrupting ANP32A, a gene vital for viral polymerase, reducing replication without affecting bird health (Bukhsh et al., 2023). Transgenic poultry could minimize transmission and culling needs, but regulatory, ethical, and acceptance hurdles remain (Bukhsh et al., 2023). In Pakistan's poultry-dependent economy, investments in research infrastructure and training are crucial for adoption (Hussain et al., 2020).

Universal vaccines target conserved viral elements (e.g., HA stalk, M2e) for cross-subtype protection, less susceptible to mutations (Krammer et al., 2018). mRNA and nanoparticle platforms offer rapid development and robust immunity, showing promise in trials (Krammer et al., 2018). For recurrent HPAIV outbreaks, these could revolutionize control, though scaling and accessibility for small farmers require government and private sector support (Hussain et al., 2020).

9. Environmental and Ecological Considerations

Environmental and ecological dynamics are integral to AIV epidemiology and management (Gilbert et al., 2008). In Pakistan, with its critical wetlands and migratory routes, understanding host-ecosystem interactions is essential for effective strategies (Ali & Akhtar, 2022).

Wetlands and sanctuaries act as reservoirs, sustaining AIV in wild waterbirds (Anseriformes and Charadriiformes) where the virus persists in cool, neutral-pH water (Krauss et al., 2007; Stallknecht & Brown, 2010). Sites like Haleji Lake, Kinjhar Lake, and the Indus Delta facilitate high-density bird mixing during migrations, promoting genetic reassortment and spillover risks (Ali & Akhtar, 2022).

Climate change, urbanization, and agricultural intensification alter transmission patterns by shifting migration routes and degrading habitats, bringing wild birds closer to farms (Gilbert et al., 2008). Deforestation near the Indus Basin fragments habitats, while poultry litter pollution contaminates water sources (WWF Pakistan, 2021). Seasonal practices like rice farming create temporary reservoirs aligning with migration cycles (Stallknecht & Brown, 2010).

Sustainable wildlife surveillance integrates conservation and prevention, emphasizing early detection and genetic characterization via NGS (Gebreyes et al., 2014). Regional networks targeting wetlands are recommended, with community engagement and One Health education fostering collaboration among stakeholders (Gebreyes et al., 2014).

10. Future Perspectives

Managing AIV epidemiology, control, and containment in Pakistan demands a multifaceted strategy, given the virus's complexity and the country's robust poultry sector (Bukhsh et al., 2023). Prioritizing emerging strains, One Health integration, and research-policy frameworks is imperative to avert outbreaks (Gebreyes et al., 2014).

AIV evolution—characterized by genetic diversity, antigenic drift, and reassortment—poses persistent challenges, exacerbated by migratory introductions and LBM reservoirs (Rehman et al., 2020). Inadequate surveillance and mismatched vaccines hinder responses; advanced genomic tools like NGS could enhance detection but require resource investment (Rehman et al., 2020).

The One Health paradigm, linking human, animal, and environmental health, is vital (Gebreyes et al., 2014). Wetland monitoring in high-risk zones can mitigate transmission at interfaces (Ali & Akhtar, 2022). Interdisciplinary training and community interventions, inspired by successes in Bangladesh and Vietnam, could strengthen Pakistan's capabilities (Gebreyes et al., 2014).

Comprehensive policies and research are essential:

a) Advance molecular epidemiology to track strain evolution and establish databases for global sharing (Rehman et al., 2020).
b) Develop locally adapted vaccines using reverse genetics to address antigenic mismatches (Krammer et al., 2018).
c) Promote biosecurity and awareness through education for farmers, reducing exposure risks (Abbas et al., 2020).
d) Allocate funding for infrastructure and foster collaborations among agencies and international partners (Gebreyes et al., 2014).

Emulating integrated frameworks from Southeast Asia could yield effective, sustainable AIV control (Bukhsh et al., 2023).

References

Abbas, M. A., Ahmad, M., & Rashid, H. B. (2020). Avian influenza at the animal-human interface: One-health challenge in live poultry retail stalls of Chakwal, Pakistan. *Influenza and Other Respiratory Viruses*, 14(5), 527–533.

Abid, M., Khan, M. S., & Ahmad, N. (2017). Characterization and phylogenetic analysis of avian influenza virus subtype H9N2 in Pakistan. *Hosts and Viruses*, 6(1), 1–10.

Ahmed, A., Saqlain, S., Rasool, A., & Javeed, A. (2024). Avian influenza virus (H5N1) was not detected among dairy cattle and farm workers in Pakistan. *Influenza and Other Respiratory Viruses*, 18(5), e13345.

Ahmed, Z., Malik, M. I., & Siddiqui, T. (2019). H9N2 avian influenza in humans: A growing zoonotic risk in Pakistan. *Frontiers in Public Health*, 7, 211.

Ali, A., & Akhtar, N. (2022). Role of migratory birds in the epidemiology of avian influenza in Pakistan. *Pakistan Journal of Zoology*, 54(3), 345–358.

Ali, M., Yaqub, T., Mukhtar, N., Imran, M., Ghafoor, A., & Shahid, M. (2021). Genetic characterization of highly pathogenic avian influenza A (H5N8) virus in Pakistani live bird markets. *Viruses*, 13(8), 1633.

Bukhsh, A., Khan, M., & Ahmad, S. (2023). *Animal influenza: An eco-health outlook*. Unique Scientific Publishers.

Chaudhry, M., Ahmad, M., & Rashid, H. B. (2020). Avian influenza at the animal-human interface: One-health challenge in live poultry retail stalls of Chakwal, Pakistan. *Influenza and Other Respiratory Viruses*, 14(5), 527–533.

Gebreyes, W. A., Dupouy-Camet, J., Newport, M. J., & Oliveira, C. J. (2014). The global One Health paradigm: Challenges and opportunities for tackling infectious diseases at the human, animal, and environment interface. *The Lancet Infectious Diseases*, 14(11), 1205–1217.

Gilbert, M., Slingenbergh, J., & Xiao, X. (2008). Climate change and avian influenza. *Revue Scientifique et Technique (International Office of Epizootics)*, 27(2), 459–466.

Hatta, M., Gao, P., Halfmann, P., & Kawaoka, Y. (2001). Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. *Science*, 293(5536), 1840–1842.

Hussain, M., Khan, M. I., & Khalid, A. (2020). Challenges in controlling avian influenza in Pakistan: Vaccination and surveillance gaps. *Avian Pathology*, 49(5), 479–488.

Iqbal, M., Rehman, M. Z., & Shah, M. A. (2022). Genetic evolution and zoonotic potential of avian influenza viruses in South Asia. *Journal of Virology Research*, 16(3), 134–145.

Klenk, H. D., Matrosovich, M., & Garten, W. (2008). Molecular mechanisms of pathogenesis and host range. In *Avian Influenza: Molecular Mechanisms of Pathogenesis* (pp. 45–67). Caister Academic Press.

Krammer, F., Smith, G. J. D., Fouchier, R. A. M., & Peiris, J. S. M. (2018). Influenza virus vaccines: Current status and future prospects. *Nature Reviews Microbiology*, 16(2), 60–74.

Krauss, S., Walker, D., Pryor, S. P., & Webster, R. G. (2007). Influenza A viruses of migrating wild aquatic birds in North America. *Vector-Borne and Zoonotic Diseases*, 7(1), 1–12.

Li, Z., Chen, H., Jiao, P., & Deng, G. (2005). Molecular basis of replication of duck H5N1 influenza viruses in a mammalian model. *Journal of Virology*, 79(18), 12058–12064.

Malik, M., Rehman, A., & Saeed, T. (2018). Serological evidence of H9N2 influenza virus infection among poultry workers in Lahore, Pakistan. *Influenza and Other Respiratory Viruses*, 12(3), 315–320.

Naeem, K., Siddique, N., Ayaz, M., & Jalalee, M. A. (2007). Avian influenza in Pakistan: Outbreaks of low- and high-pathogenicity avian influenza in Pakistan during 2003-2006. *Avian Diseases*, 51(s1), 189–193.

Peacock, T. P., Harvey, W. T., & Sadeyen, J. R. (2018). The molecular basis of antigenic variation among A (H9N2) avian influenza viruses. *Emerging Microbes & Infections*, 7(1), 1–12.

Qi, W., Jia, W., & Liu, D. (2012). Molecular basis of efficient replication and pathogenicity of H9N2 avian influenza viruses in mice. *PLoS ONE*, 7(7), e40118.

Rafique, S., Rashid, H., & Ahmad, S. (2018). Seroprevalence and molecular characterization of infectious bronchitis virus variants from poultry in Pakistan. *Pakistan Journal of Zoology*, 50(4), 1235–1242.

Rehman, Z. U., Mukhtar, N., & Yaqub, T. (2020). Molecular characterization of avian influenza viruses in Pakistan: A five-year perspective. *Frontiers in Veterinary Science*, 7, 12.

Shahzad, R., Irshad, S., Mehmood, M. S., & Amin, F. (2021). Adaptive mutations in nuclear export protein and non-structural 1 protein of avian influenza A H9N2 virus circulating in Punjab, Pakistan. *Advancements in Life Sciences*, 8(2), 123–130.

Sharma, S., Bano, S., & Shahid, A. (2019). Zoonotic transmission of avian influenza viruses in South Asia: A public health concern. *Asian Journal of Infectious Diseases*, 4(2), 101–110.

Stallknecht, D. E., & Brown, J. D. (2010). Tenacity of avian influenza viruses. *Avian Diseases*, 54(1), 102–109.

Tahir, M. F., Abbas, M. A., & Akram, Q. (2020). Seroprevalence and risk factors of avian influenza H9 virus among poultry professionals in Rawalpindi, Pakistan. *Journal of Infection and Public Health*, 13(6), 850–856.

Umar, S., Sarwar, M., & Mushtaq, M. H. (2016). Emerging threat of H9N2 viruses in poultry of Pakistan and vaccination strategy. *World's Poultry Science Journal*, 72(2), 333–342.

Watanabe, T., Watanabe, S., & Kawaoka, Y. (2010). Understanding the molecular basis of influenza virus pathogenicity in humans. *Nature Reviews Microbiology*, 8(7), 509–518.

WWF Pakistan. (2021). *Wetlands of Pakistan: Challenges and strategies for conservation*. WWF Annual Repor

About The Authors

Abdul Rehman received his graduate degree in Applied Microbiology from Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan. He has completed his internship at the renowned National Institute of Health Pakistan, where he worked on various microbiological studies. Abdul has written a thesis based on his work during this internship. He has also authored several research and review articles and contributed a chapter on Foot and Mouth Disease in animals and livestock, which is set to be published soon. His research interests encompass viral immune evasion, antimicrobial resistance, and diagnostic advancements.

E-mail: jami.abdrehman@gmail.com ORCID: 0009-0000-7588-0127

Dr. Muhammad SAFDAR earned his PhD in molecular biology and genetics from Gaziantep University, Turkey. He is a lecturer in the Breeding and Genetics department at Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, Pakistan. His research interests are molecular genetics and genomics, nutrigenomics, nano-genomics, bioinformatics, biotechnology, and their applications. He has published more than 70 research articles in national and international journals. He has also written many book chapters as well as an edited book. He is an associate editor for international journals.

E-mail: drmuhammadsafdar8@gmail.com, ORCID: 0000 0002 3720 2090

Prof. Dr. Mehmet OZASLAN received his PhD in 1995 Institute of Natural Sciences at Cukurova University, Turkiye. He is a Professor in Molecular Biology and Genetics. His research interests are included Cancer Genetics, Molecular Virology, Molecular Genetics, Microbiology, and Genetic mutations etc. He has published more than 200 research articles in national and international well reputed journals. He also has written many book chapters as well as edited books. He is an editor and editor in chief of many well reputed national and international journals.

E-mail: ozaslanmd@gantep.edu.tr, ORCID: 0000 0001 9380 4902

To Cite This Chapter

Rehman, A, et al. (2025). Avian Influenza Virus: Epidemiology, Evolution, Antigenicity, and Control Strategies in Pakistan. In Current Studies in Life Sciences (pp.1-12). ISRES Publishing.

Genetic Basis of Immunity and Disease Resistance in Livestock

Shahnaz
Mehmet OZASLAN
Muhammad SAFDAR

1. Introduction

Livestock, such as cattle, sheep, goats, and pigs, are vital for global food security and sustainable agriculture, providing meat, milk, and other products essential for human nutrition. However, infectious diseases like bovine tuberculosis, mastitis, and foot-and-mouth disease threaten animal health, reduce farm productivity, and cause significant economic losses. Understanding the genetic factors that enable livestock to resist these diseases is crucial for breeding healthier animals that require fewer treatments, such as antibiotics or vaccines. Recent advancements in genomics—the study of an organism‘s complete set of genes—have revealed how specific genes influence the immune system, offering new opportunities to develop disease-resistant livestock through selective breeding and cutting-edge technologies like gene editing. This review explains the genetic mechanisms behind livestock immunity, explores tools like molecular markers and breeding strategies, and discusses challenges and future directions in a clear and accessible way, suitable for researchers, farmers, and students alike (Behl et al., 2012; Bishop & Woolliams, 2014).

2. Genetic Foundations of Immune Response

The ability of livestock to fight diseases depends on a complex network of genes that control the immune system. A key player in this network is the major histocompatibility complex (MHC), called bovine leukocyte antigen (BoLA) in cattle, which helps the immune system recognize and respond to harmful pathogens like bacteria and viruses. Variations in BoLA genes, particularly BoLA-DRB3, are linked to resistance against diseases such as mastitis (an udder infection in dairy cows) and bovine leukemia virus (BLV), a viral disease affecting cattle (Oliveira et al., 2024). For example, certain BoLA-DRB3 gene variants enable cattle to better detect and eliminate pathogens, reducing disease severity.

Modern genetic studies, known as genome-wide association studies (GWAS), have identified specific DNA changes, called single nucleotide polymorphisms (SNPs), associated with disease resistance. In dairy cattle, an SNP in the Toll-like receptor 4 (TLR4) gene (rs8193069) reduces the risk of mastitis while maintaining milk production (Doeschl-Wilson et al., 2021). In African cattle breeds like N‘Dama, genes such as RSAD2 and CMPK2 enhance immune responses and help animals cope with environmental stresses, such as high temperatures and prevalent diseases like trypanosomiasis (Nguyen & Smith, 2023). Additionally, studies of proteins in tick-resistant cattle have identified markers like complement factors (C3, C4) and alpha-1-acid glycoprotein (AGP), which indicate a strong immune response (Islam et al., 2020). These discoveries highlight how genetics can be used to breed livestock that are naturally more resistant to diseases, benefiting both farmers and animals (Figure 1).

Figure 1. Genetic Basis of Immunity and Disease Resistance in Livestock

3. Major Histocompatibility Complex (MHC) in Livestock

The MHC is a group of genes critical for helping livestock identify and fight infections. In cattle, this system is called BoLA and is located on chromosome 23. It includes three types of genes: Class I, Class II, and Class III. Class I genes produce proteins that alert immune cells to infections inside cells, such as viruses, by presenting viral pieces to cytotoxic T cells. Class II genes, including BoLA-DRB3, help immune cells recognize external pathogens, like bacteria, by presenting their fragments to helper T cells, which coordinate broader immune responses. Class III genes support other immune functions, such as producing complement proteins that attack pathogens (Behl et al., 2012).

The MHC genes are highly variable, meaning they differ significantly between animals, which allows livestock to recognize a wide range of pathogens. In cattle, specific BoLA-DRB3 variants are linked to resistance against mastitis, BLV, and theileriosis (a tick-borne disease). For instance, the BoLA-DRB3*010:01 variant promotes mastitis resistance, while *015:01 increases BLV susceptibility (Oliveira et al., 2024). In goats, MHC gene variations help resist Johne‘s disease, a chronic bacterial infection (Ossa et al., 2020). Buffaloes with certain MHC-DRB3 variants show improved resistance to mastitis (Ossa et al., 2020). Sheep with variable ovine

leukocyte antigen (OLA) genes are better protected against parasites like worms and ticks (Proudfoot et al., 2019). In pigs, swine leukocyte antigen (SLA) variations reduce susceptibility to porcine reproductive and respiratory syndrome virus (PRRSV), a major swine disease (Proudfoot et al., 2019).

These variations provide an advantage by enabling animals to recognize diverse pathogens, a concept called heterozygote advantage. Over time, natural selection maintains this diversity to ensure populations can adapt to new diseases (Behl et al., 2012). By identifying these MHC variants, farmers can breed animals with stronger immunity, reducing disease outbreaks.

4. Innate and Adaptive Immunity Genes

The immune system in livestock operates in two main phases: innate and adaptive immunity. Innate immunity acts quickly to detect pathogens using receptors like Toll-like receptors (TLRs). For example, a variation in the TLR4 gene (rs8193069) in Holstein cows enhances bacterial detection, reducing mastitis risk (Doeschl-Wilson et al., 2021). Cytokine genes, which produce signaling molecules like interleukin-10 (IL-10), regulate immune responses. Variations in IL-10 or its receptor (IL10Ra) can increase susceptibility to Johne‘s disease in cattle by altering immune signaling (Islam et al., 2020).

Adaptive immunity provides long-term protection by targeting specific pathogens. MHC genes are central to this process, but other genes, like HSF1 and NFκB1, help animals cope with environmental stresses, such as heat, which can weaken immunity (Islam et al., 2020). GWAS in Holstein cattle have identified SNPs in the BoLA region that enhance immune responses to diseases like bovine tuberculosis (Nguyen & Smith, 2023). Additionally, epigenetic changes—modifications that affect gene activity without altering DNA—play a role. For example, DNA methylation patterns in cattle influence disease resistance traits, offering new avenues for genetic improvement (Bishop & Woolliams, 2014). Together, these genes and mechanisms provide multiple targets for breeding programs to enhance livestock health.

5. Genomic Tools and Molecular Markers

Molecular markers are specific DNA segments used to identify traits like disease resistance. The most common markers include single nucleotide polymorphisms (SNPs), which are single DNA base changes, and simple sequence repeats (SSRs), which are repeating DNA patterns. SNPs are widely used because they are abundant and stable, making them ideal for mapping quantitative trait loci (QTL)—regions of DNA linked to specific traits (Mulder & Rashidi, 2020). For instance, SNPs associated with bovine respiratory disease (BRD) resistance help farmers select healthier cattle early, reducing treatment costs (Nguyen & Smith, 2023).

Genomic selection (GS) is a powerful tool that uses thousands of SNPs to predict an animal‘s genetic potential, known as genomic estimated breeding values (GEBVs). This method allows breeders to select animals with strong disease resistance before symptoms appear, speeding up breeding progress. GS has improved resistance to mastitis, BRD, and leptospirosis in cattle and PRRS in pigs (Mulder & Rashidi, 2020). However, challenges include the high cost of genetic testing, which can be a barrier for small-scale farmers, and the need to maintain genetic diversity to

avoid problems like inbreeding (Liu et al., 2022). These tools make breeding more precise but require careful management to be effective.

6. Breeding Strategies for Disease Resistance

Breeding strategies aim to produce livestock with natural disease resistance, reducing reliance on medications. Traditional selective breeding chooses animals with desirable traits, like mastitis-resistant cows, but progress is slow because resistance often involves many genes (Mulder & Rashidi, 2020). Marker-assisted selection (MAS) speeds up this process by using molecular markers to identify animals with resistance genes, as seen in programs targeting bovine tuberculosis (Nguyen & Smith, 2023).

Crossbreeding combines traits from different breeds. For example, crossing N'Dama cattle, which resist trypanosomiasis, with high-yielding Friesian cattle produces offspring that are both disease-resistant and productive (Bishop & Woolliams, 2014). Genomic selection uses SNP data to predict resistance across multiple diseases, improving outcomes for complex traits like PRRS resistance in pigs (Mulder & Rashidi, 2020). These strategies help create healthier livestock, supporting sustainable farming by reducing disease-related losses.

7. Biotechnological Approaches Including Gene Editing

Biotechnology offers innovative ways to enhance disease resistance. Immunogenomics combines genomics and immunology to identify genes involved in immune responses, using technologies like RNA sequencing to study how genes behave during infections (Islam et al., 2020). For example, this approach has pinpointed genes linked to bovine tuberculosis resistance.

CRISPR-Cas9, a precise gene-editing tool, allows scientists to add or remove specific genes to improve resistance. For instance, inserting the NRAMP1 gene in cattle enhances tuberculosis resistance, while deleting the CD163 gene in pigs prevents PRRS infection (Proudfoot et al., 2019). Base editing, a newer technique, corrects small DNA errors without breaking the DNA strand, offering a safer way to fix disease-related mutations (Liu et al., 2022). Gene editing libraries, which test many genes at once, have identified resistance genes for diseases like avian influenza (Proudfoot et al., 2019). Combining these technologies with breeding strategies like MAS creates powerful tools for developing disease-resistant livestock, making farming more efficient and sustainable.

8. Challenges and Future Directions

Applying genetic discoveries to livestock breeding faces several hurdles. Disease resistance often involves many genes, making it hard to identify reliable markers. Additionally, enhancing resistance can sometimes reduce other traits, like milk production in dairy cows (Doeschl-Wilson et al., 2021). Limited genetic data for less-studied breeds, especially in developing countries, slows progress (Bishop & Woolliams, 2014). Analyzing complex genetic data requires advanced computing tools, which can be costly and inaccessible in some regions (Liu et al., 2022).

Gene editing also raises ethical and regulatory concerns. Some worry about the welfare of genetically modified animals or public acceptance of their products. Different countries have

varying rules, complicating trade (Proudfoot et al., 2019). In developing regions, limited access to research facilities and funding hinders progress, as noted at the 2023 International Veterinary Immunology Symposium (Islam et al., 2020).

Looking ahead, combining genomics with other fields like proteomics (protein studies) and transcriptomics (gene expression studies) can provide a fuller picture of immunity, helping identify new resistance markers (Islam et al., 2020). Creating databases for different breeds and fostering global research collaborations will speed up discoveries. Developing affordable tools and training programs for farmers in low-resource areas will ensure these advances benefit everyone (Liu et al., 2022). Clear regulations will also support safe and ethical use of gene editing.

9. Summary

Understanding how genetics influence livestock immunity is key to creating healthier, more resilient animals that support sustainable agriculture. Tools like MHC gene analysis, molecular markers, and CRISPR-Cas9 gene editing enable breeders to develop livestock resistant to diseases like mastitis and tuberculosis. By combining traditional breeding with modern technologies, farmers can reduce disease impacts, use fewer antibiotics, and improve food security. Overcoming challenges through global cooperation, advanced research, and accessible tools will ensure these benefits reach farms worldwide, creating a stronger and more sustainable future for livestock production.

References

Behl, J. D., Verma, N. K., Tyagi, N., Mishra, P., Behl, R., & Joshi, B. K. (2012). The major histocompatibility complex in bovines: A review. *ISRN Veterinary Science, 2012*, Article 872710. https://doi.org/10.5402/2012/872710

Bishop, S. C., & Woolliams, J. A. (2014). Genomics and disease resistance studies in livestock. *Livestock Science, 166*, 190–198. https://doi.org/10.1016/j.livsci.2014.04.034

Doeschl-Wilson, A., Knap, P. W., Opriessnig, T., & More, S. J. (2021). Review: Livestock disease resilience: From individual to herd level. *Animal, 15*(Suppl 1), 100286. https://doi.org/10.1016/j.animal.2021.100286

Islam, M. A., Rony, S. A., Rahman, M. B., Cinar, M. U., Villena, J., Uddin, M. J., & Kitazawa, H. (2020). Improvement of disease resistance in livestock: Application of immunogenomics and CRISPR/Cas9 technology. *Animals, 10*(12), 2236. https://doi.org/10.3390/ani10122236

Liu, Z., Wu, T., Xiang, G., Wang, H., Wang, B., Feng, Z., Mu, Y., & Li, K. (2022). Enhancing animal disease resistance, production efficiency, and welfare through precise genome editing. *International Journal of Molecular Sciences, 23*(13), 7331. https://doi.org/10.3390/ijms23137331

Mulder, H., & Rashidi, H. (2020). Why breed disease-resilient livestock, and how? *Genetics Selection Evolution, 52*(1), 58. https://doi.org/10.1186/s12711-020-00580-4

Nguyen, T. T., & Smith, J. (2023). Molecular breeding of livestock for disease resistance. *Virology, 587*, 109862. https://doi.org/10.1016/j.virol.2023.109862

Oliveira, A. L., Suarez, C., Vargas, B., Velez, C., Lopez-Alvarez, J., ... Patarroyo, M. (2020). Major histocompatibility complex class II (DRB3) genetic diversity in Spanish Morucha and Colombian Normande cattle compared to taurine and zebu populations. *Frontiers in Genetics, 10*, 1293. https://doi.org/10.3389/fgene.2019.01293

Oliveira, A. L., et al. (2024). Graduate student literature review: The DRB3 gene of the bovine major histocompatibility complex—Discovery, diversity, and distribution of alleles in commercial breeds of cattle and applications for development of vaccines. *Journal of Dairy Science, 107*(12), 11324–11341. https://doi.org/10.3168/jds.2023-24628

Proudfoot, C., Lillico, S., & Tait-Burkard, C. (2019). Genome editing for disease resistance in pigs and chickens. *Animal Frontiers, 9*(3), 6–12. https://doi.org/10.1093/af/vfz008

About The Authors

Shahnaz graduated from the Islamia University, Bahawalpur. With a background in Zoology, she brings a wealth of expertise to the scientific community. She has done her postgraduate study in Microbiology from Cholistan University of Veterinary and Animal Sciences Bahawalpur. She worked on nanotechnology and bacteriology field, having research interests in public health related diseases, influenza, waterborne diseases, virology and animal related diseases.

Email: shehnazch205@gmail.com, ORCID: 0009-0003-5448-7567

Prof. Dr. Mehmet ÖZASLAN received his PhD in 1995 Institute of Natural Sciences at Cukurova University, Turkiye. He is a Professor in Molecular Biology and Genetics. His research interests are included Cancer Genetics, Molecular Virology, Molecular Genetics, Microbiology, and Genetic mutations etc. He has published more than 200 research articles in national and international well reputed journals. He also has written many book chapters as well as edited books. He is an editor and editor in chief of many well reputed national and international journals.

E-mail: ozaslanmd@gantep.edu.tr, ORCID: 0000 0001 9380 4902

Dr. Muhammad SAFDAR earned his PhD in molecular biology and genetics from Gaziantep University, Turkey. He is a lecturer in the Breeding and Genetics department at Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, Pakistan. His research interests are molecular genetics and genomics, nutrigenomics, nano-genomics, bioinformatics, biotechnology, and their applications. He has published more than 70 research articles in national and international journals. He has also written many book chapters as well as an edited book. He is an associate editor for international journals.

E-mail: drmuhammadsafdar8@gmail.com, ORCID: 0000 0002 3720 2090

To Cite This Chapter:

Shahnaz et al., (2025). Genetic Basis of Immunity and Disease Resistance in Livestock. In Current Studies in Life Sciences 2025 (pp.13-19). ISRES Publishing.

The Superbugs Challenge: The Role of AI in Driving Innovations and Diagnosing and Treating Antimicrobial Resistance

Syed Sohail AHMAD
Farah ABID
Muhammad Usman QAMAR
Nimra SHAHZADI
Humera NAZIR
Muhammad SAFDAR

Introduction

A superbug is a classification of microorganisms that cannot be neutralized by the standard antibiotic, antiviral, or other treating agents. They may be bacteria, viruses, parasites or some other microorganism not so well described in this simplified definition. It is commonly used concerning bacteria that have become resistant to many antibiotics through which diseases caused by these organisms can be treated. Antibiotic resistance arises due to selective mechanisms: these are natural selection mechanisms. These forms may remain within the body after a treatment utilizing antibiotics and multiply quickly. Antibiotic abuse or misuse may lead to an emergence of resistant strains over time reducing or eradicating the efficacy of traditional therapy (Mancuso, Midiri, Gerace, & Biondo, 2021). A superbug is a menace to the community‘s health since it may bring back diseases that had earlier been preventable. It may lead to a prolonged illness, increased health costs, and an increased likelihood of passing on the disease. Superbugs are a result of misuse of antibiotics, failure to wash, and failure to discover new treatments and precautionary methods to combat antibiotic resistance. Technically, superbugs are microbes that have become so hard to eradicate due to their resistance level to the many treatments.

Now, one of the world‘s most pressing public health threats is the development and emergence of AMR. This is how antibiotic resistance in AMR starts because anti-microbial drugs that are used to cure illnesses caused by microorganisms including bacteria, viruses, fungi, and parasites cease to work as before. AMR is estimated to contribute to 1.27 million deaths annually, globally (Murray et al., 2022). To prevent such experiences, applied studies aimed at deriving new antiviral agents and vaccines are important as depicted by the COVID-19 crisis triggered by the SARS-CoV-2 virus. Due to these events, the authorities are now forced to tackle these problems and offer appropriate diagnostic method. The different strategies will allow proper stockpiling of the correct antibiotics and the implementation of the right measures that will lessen the threat presented by AMR. This is undoubtedly the case for antibiotic resistance genes (ARGs) whose detection is challenging, as anything must be developed to be sufficiently sensitive to respond to an issue that is rapidly developing within the microbiome, and at the same time, possesses very high specificity in environments that are as informative in therapeutic frameworks (Schuler & Rose, 2017). Currently, available laboratory diagnostic and characterization techniques fail to generate sufficient data to support effective surveillance operations. Besides, a different outcome is observed depending on the laboratory conditions and environment. Population-level AMR genetic differences may be examined through laboratory and high-sequencing data such as whole-genome sequencing (Schuler & Rose, 2017).

Large volumes of data may now be handled and analyzed quickly because of recent technological advancements that have greatly improved computer power and data storage. Therefore, a variety of areas are using Artificial intelligence (AI) methodologies, particularly machine learning (ML) and Deep Learning (DL) techniques (Gupta et al., 2022). Additionally, the medical industry is using AI more and more. To provide intelligent healthcare, the metaverse is being created. Devices with AI/ML capabilities that have received Food and Drug Administration (FDA) approval across a range of medical specialties were enumerated by the authors. About 85% of all authorized devices are AI-enabled, and there has been a noticeable rise in their acceptance from 2018 forward. So far, around 531 devices using AI/ML have received approval; radiology makes up the bulk of these devices. Of the other devices mentioned, four are connected to pathology and five are related to microbiology (Joshi et al., 2024).

AI in the Field of Antimicrobials

Although a lot of research is being done to make it realistically relevant, most of the research is currently limited to laboratories and has not yet been put into practice. AMR-related potential AI applications are summed up in Figure 1. Models are created to deduce hypotheses about novel AMR genes or mutation-variation pathways. It has been attempted to use the predictions made by various models for diagnostic purposes. The use of genomics to enhance monitoring is gaining popularity. By tracking known causative resistance genes, emerging AMR trends may be shown. Additionally, transmission patterns can be uncovered, which can aid in detecting and containing resistant disease outbreaks. Growing amounts of whole-genome sequencing data enable AI algorithms to achieve high surveillance accuracy (Argimón et al., 2020). AI models can learn extremely influential qualities, allowing for the early adoption of critical actions.

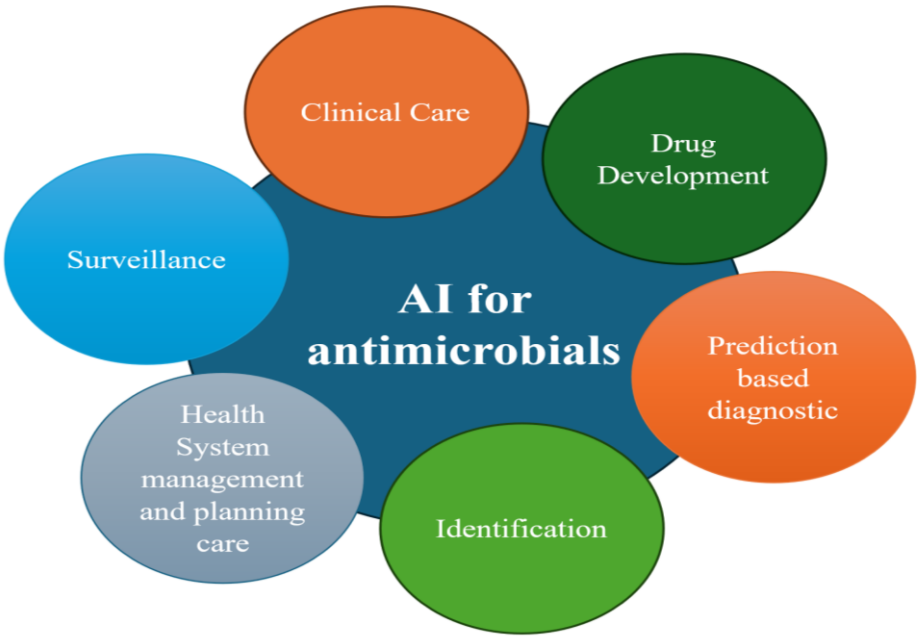


Figure 1. AI may be Used With Antibiotics to Achieve a Variety of Goals, Including Clinical Treatment, Medication Development, Monitoring, and Discovering Novel AMR, Etc.

Conventional methods for antimicrobial tests are neither quick nor easy to use. For example, the current susceptibility test takes more than twenty-four hours to complete, and the whole-genome sequence of an antibiotic susceptibility test necessitates the skill and attention of a bioinformatician with low error rates. Processing this vast amount of data takes many hours. Various investigations have been conducted to reduce the diagnosing time. For instance, ML with a flow cytometry antimicrobial susceptibility test may cut the diagnostic time down to three hours. An effective method for managing genetic data using AI is provided. The presentation of AI-based data-driven methodologies for the creation of effective antibiotic usage strategies in sepsis therapy is made (Tsoukalas, Albertson, & Tagkopoulos, 2015). AI may improve patient outcomes by positively identifying appropriate interventions and accurately predicting the duration of stay and death. With Phoenix, phenotypic test techniques that are often employed might have an accuracy of around 90%. These accuracy levels may be raised by ML/DL models with the right training methods and data. Creating a method that uses tailored treatment based on rapidly identifying a disease and its resistance profile from clinical samples would be a novel approach to antimicrobial stewardship. For instance, delaying the start of effective antibiotic treatment might have a negative effect on mortality in sepsis by up to 20% (Zasowski et al., 2020). Additionally, there is a growing interest in quick bloodstream infection tests. Patients' outcomes would improve with such actions combined with antimicrobial stewardship. Various AI methods are used in silico to forecast novel antibiotic compounds and explore the synergy resulting from medication combinations. The development and approval of around 14 novel antibiotics have occurred since 2014, and the use of AI may hasten the process of discovering and producing new antibiotics (Miethke et al., 2021).

A clean water supply and proper hygiene are also being ensured by artificial intelligence. The likelihood of antimicrobials occurring in water may be predicted by several machine-learning methods. AI has been used in another study to address the water situation. The goal of this endeavor is to stop the spread of AMR bacteria and infectious illnesses to guarantee that everyone has access to clean water and sanitation. Water resource management, pollution detection, improving effluent quality, and data monitoring are a few important real-world uses. The application of Bentham's felicific calculus paradigm to the choice to begin antibiotic therapy is shown in Figure 2. It is an AI-driven clinical decision support system (CDSS) for antibiotic optimization that takes Bentham's felicific calculus a moral framework into account. Based on the moral framework and ML result, this framework aids in the beginning of therapy. In comparison to AI-based strategies, traditional methods are inefficient, require large datasets, and take longer. Research has used supervised ML to enhance the AST technique and cut the test's typical 24-hour duration down to only 3 hours. Similar to this, Lechowicz et al. created an IR-spectrometer test based on artificial neural networks that cut down on the AST time to only 30 minutes (Ali, Ahmed, & Aslam, 2023; Stuart, 2000).

Variables	Description	AMR Treatment	Ad hoc utility value
Intensity	…….	…….	Highly positive
Duration	…….	…….	Positive
Certainty	…….	…….	Neutral
Propinquity	…….	…….	Neutral
Fecundity	…….	…….	N/A
Purity	…….	…….	N/A
Extent	…….	…….	Immense negative

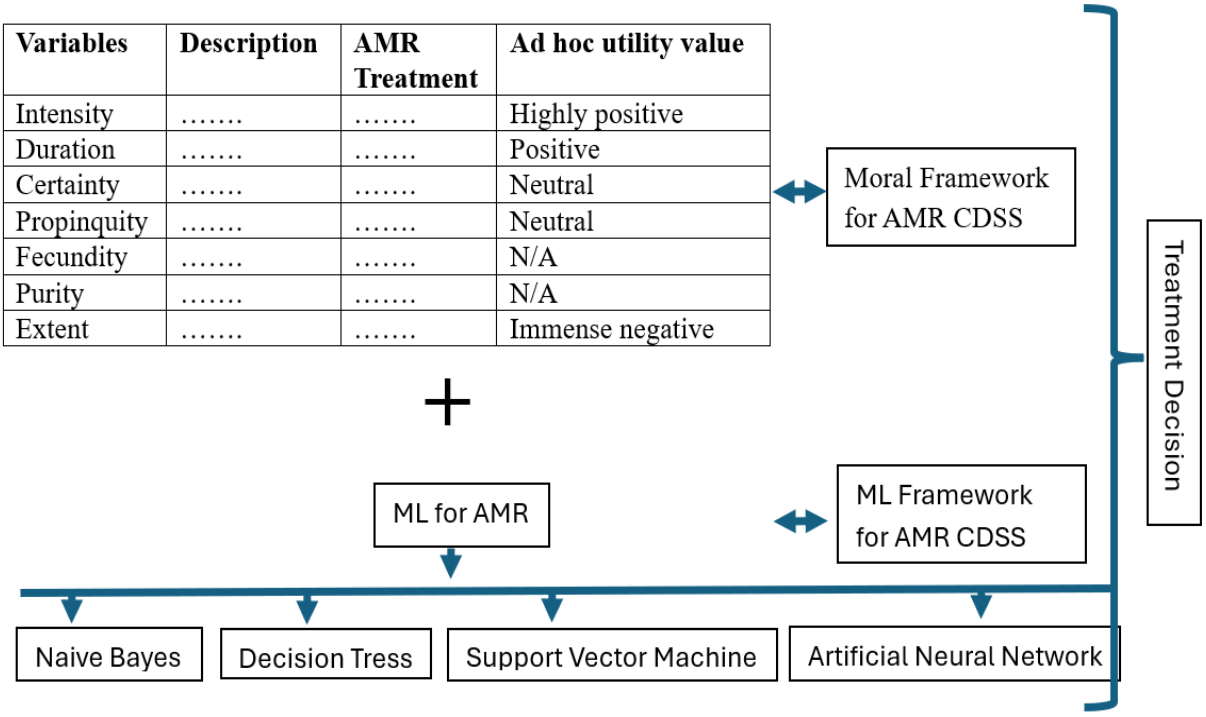


Figure 2. A combined framework of Moral and AI for CDSS.

AI and the Health Sector

By gathering patient data, using X-ray, CT, and MRI scans, early sickness detection and prevention, cutting costs, and boosting medicine prescriptions, AI plays a critical role in improving the health sector (Liu et al., 2018). AI technologies will make life easier, safer, and more productive. AI can greatly lower healthcare costs while improving patient care. AI has already digitally transformed several industries' manual health systems into automated ones. In some applications, people are relieved of more basic obligations related to medical practice, such as patient and supply management (Comito, Falcone, & Forestiero, 2020). Predicting medical needs, autonomous radiation analysis, robotic surgery, ophthalmology, lowering AMR, biofilm detection using beta-lactamase inhibitors, AMR labeling, novel medication development, and patient safety are all being worked on. Applications of AI have certain benefits and drawbacks. In Figure 3, the applications of AI in several health fields are summarized. Early automated decision-making, early diagnosis, great systematic work, data interchange, time savings, and improved performance are often helped by benefits. Challenges include areas where judgments may be made better, such as patient safety, data leaks, human error in drug prescription, and a shortage of trained workers in the health industry to use AI (Masud, Fahim, Rana, Islam, & Tanvir, 2023).

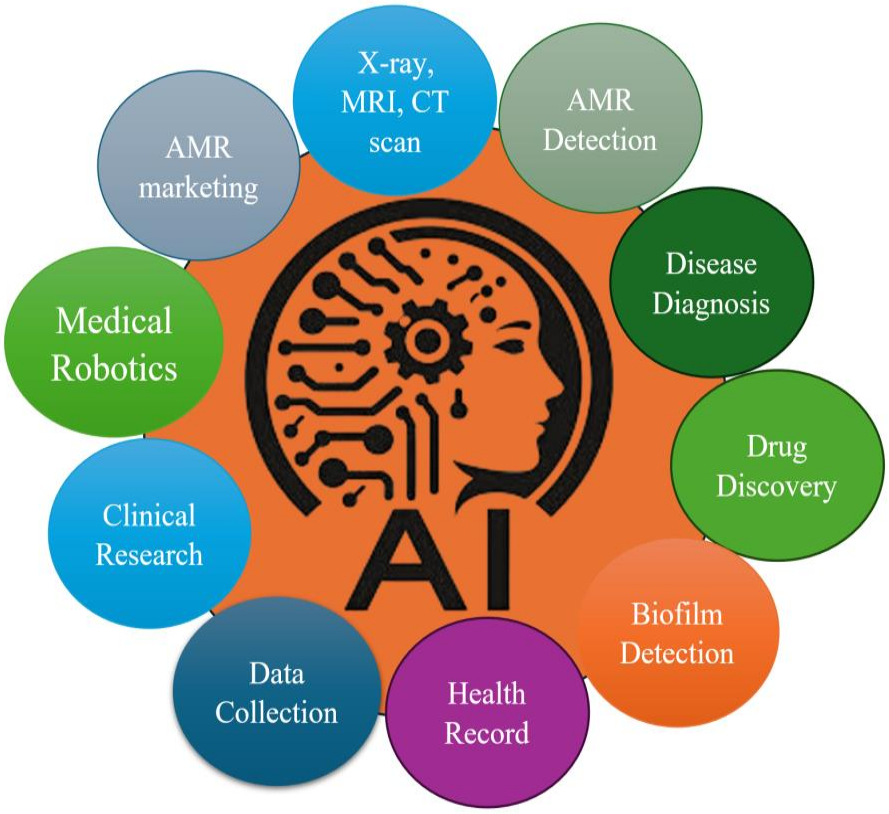


Figure 3. AI is being used in several health-related fields to expedite accurate and thorough diagnostic and treatment processes.

AI and Identification of AMR Markers

AMR indicators are characteristics or genetic elements connected to bacterial or other microorganism antibiotic resistance. These indicators are used in diagnostic procedures to determine and find AMR in microorganisms. It is crucial to identify the resistance indicators found in the pathogen when a patient has a bacterium that is resistant to a certain antibiotic. Medical personnel may choose the best antibiotics to treat the illness with the use of this information. Choosing the proper medicines may raise the odds of a good result while decreasing the possibility of further promoting AMR. There are several methods for locating the responsible genes, and scientists are constantly creating new tools. The most widely used methods of identification mostly rely on growing these microorganisms in certain conditions. Even if they are clear-cut and easy to apply, certain parts can be more advantageous. The presence of live, non-cultivable microbes or the amount of time it takes for certain organisms to grow in the environment are obstacles to some research initiatives (Braga et al., 2013).

AI-based ML and DL techniques (Figure 4) have shown significant improvements in AMR management in recent years. Examples of these techniques include sequencing-based AI applications and the collection of clinical data for the creation of clinical decision support systems. Numerous AI methods, including NB, SVM, DT, RF, and ANN, are often utilized for AMR identification. Additionally, the method has been effectively used to identify dysfunctions of the thyroid, kidney, liver, lung, gastrointestinal tract, and heart (Chen et al., 2013).

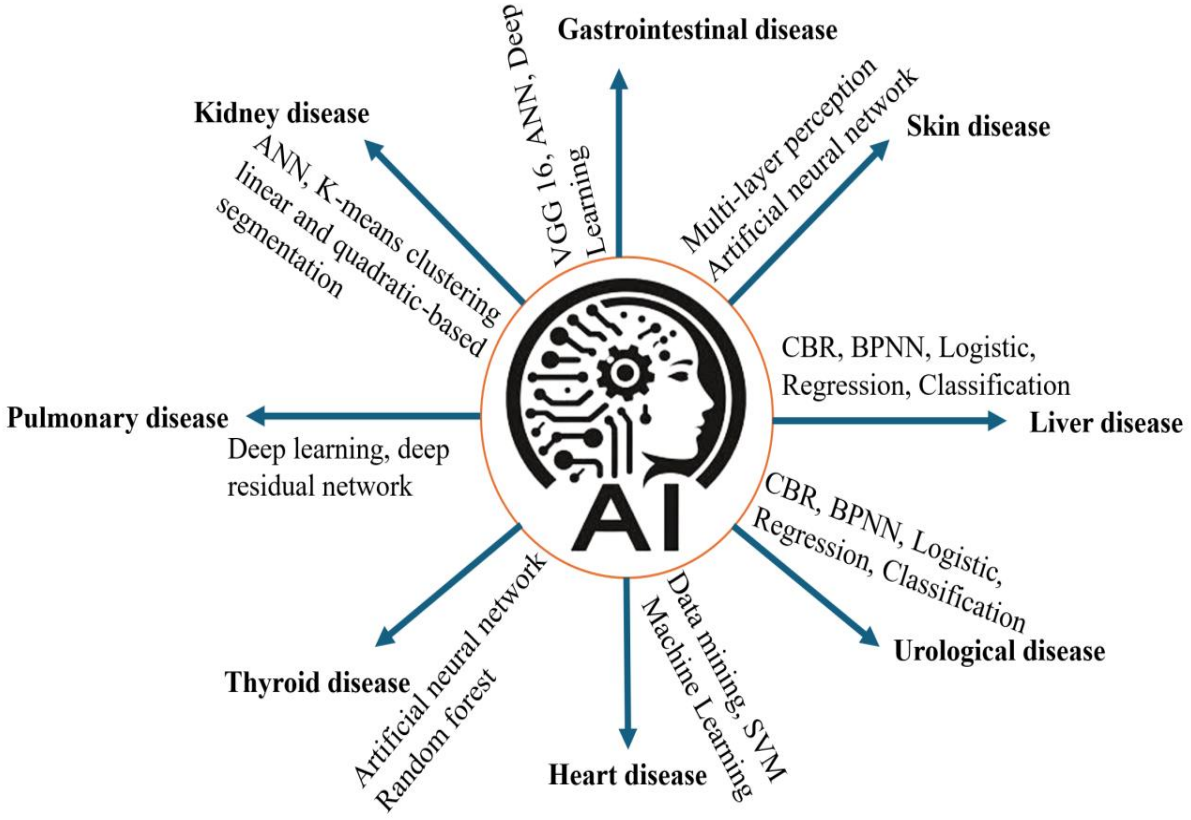


Figure 4. Different AI-based Technologies, as Previously Mentioned, are Utilized to Identify Diseases in the Body's Numerous Systems (Kumar, Koul, Singla, & Ijaz, 2023).

The resistance genes from various bacteria are effectively identified by a variety of AI-based ML and DL Techniques. 90% accuracy was achieved in identifying the precise gene and the impact of a given antibiotic on a variety of bacteria via the application of machine learning, homology modeling, and molecular docking. With an accuracy of almost 93%, models like SVM and deep convolutional neural networks (DCNN) have been used to identify particular TB genes (Oloko-Oba & Viriri, 2020). Finding AMR indicators is essential to combating AMR and giving the patient the right care. According to recent research, a class of ML techniques called DL may also increase the number of AMR genes in the catalog and improve the precision of predictions made using metagenomic data. Recent research has shown that when discovering antibiotic resistance genes based on metagenomic data, the DL technology known as Deep ARG is more accurate than more traditional methods (Arango-Argoty et al., 2018).

AI and AST

Antibiotics are a life-saving medication for treating and preventing bacterial infections in chronic infectious illnesses. Antibiotics help treat infectious infections, but when they are overused or misused by AMR, they may have potentially fatal repercussions on both human and animal health. AST may be performed in some practical methods, although it typically takes 16 to 24 hours. Phenotypic and genotypic testing are the two common forms of AST. Phenotypic methods include flow cytometry, broth dilution, Disk Diffusion Gradient Diffusion, Matrix-Assisted Laser Desorption lionization-Time of flight mass spectrometry (MALDI-TOF MS), Epsilometer testing

(EST) Agar dilution, and Beta-β-lactamase activity. In genotyping processes, loop-mediated isothermal amplification (LAMP), DNA chips, PCR, and DNA microarrays are often used. But now, new AI technology has been created that can do AST quickly. Popular AI approaches include Support Vector Machines, Genetic Methods, Artificial Neural Networks, and Quick Classifiers. These algorithms are often used by AST for machine learning.

In separate research, an ANN model was used to predict and then validate the efficacy of antibiotics in treating various bacterial species. Susceptibility was considered as the study's output, and the inputs included the name of the organism, specimen type, and antibiotic. According to the research, the suggested ANN model that makes use of the JNN tool has a 94.17% accuracy rate in determining how susceptible an organism is to antibiotics. Results in this research ranged from 60% to 100%. Here, MALDI-TOF-based medical AI (XBugHunter) was effectively employed with an accuracy of over 95% for the quick result of the AST report. As a result, using XBugHunter might help reduce the overprescription of antibiotics for individuals suffering from bacteremia caused by *S. aureus* (H.-Y. Wang et al., 2021).

AI in Antibiotic Discovery Against MRSA

The discovery of novel drugs against MRSA has been made possible in large part by the integration of AI and ML in microbiology. AI's ability to process and analyze massive data sets has accelerated the search for viable antibiotic candidates. Using features calculated by RDKit, the study technique includes training, validating, and evaluating ML models. Performance is assessed using AUPRC (Yang et al., 2019).

AI's capacity to scan and display a compound's molecular space aids in differentiating between effective and ineffective antibiotics. DL models and Monte Carlo tree searches are used to find several structural classes that hint at antibiotic activity. The results obtained from AI-based predictions are extensively tested experimentally, and emphasis is placed on the synergy between computational predictions and more traditional experimental analyses. The use of AI gives valuable recommendations concerning pharmacological characteristics, the mechanisms of resistance to the substances, and overall efficacy (Wong et al., 2021).

Due to the adoption of AI and ML, the science of microbiology has drastically changed for the better. AI‘s role is versatile and constantly evolving, it also applies to diagnostics in clinics, a speed-up in the drug development process, disease prediction, and investigations of microbial samples. Because AI and ML now play crucial roles in ongoing microbiological studies, the integration of computer procedures with biological information is creating a better framework for elaborated and precise detection and medical treatment of diseases, as well as the study of ecosystems (Tsitou, Rallis, Tsekova, & Yanev, 2024).

How AI Meets the Challenges

There has been some improvement recently on how AI deals with antibiotic resistance. For example, to help physicians develop new medications, employ the utilization of antibiotics, and prevent the spread of untreatable diseases. The development of new antimicrobial medications has

already been sped up by the AI method. A subclass of AI known as "generative models" creates predictions for the last molecule needed to make a particular new medication.

These AI models possess the ability to comprehend the properties of the underlying data and even propose previously undiscovered compounds. They search for more than just well-known compounds with the necessary properties, such as the ability to attach to and kill bacteria or viruses. Unlike searching capabilities, this design is especially transformative since there are more theoretically relevant molecules in the universe than there are atoms, making search activities impracticable (Knight et al., 2021).

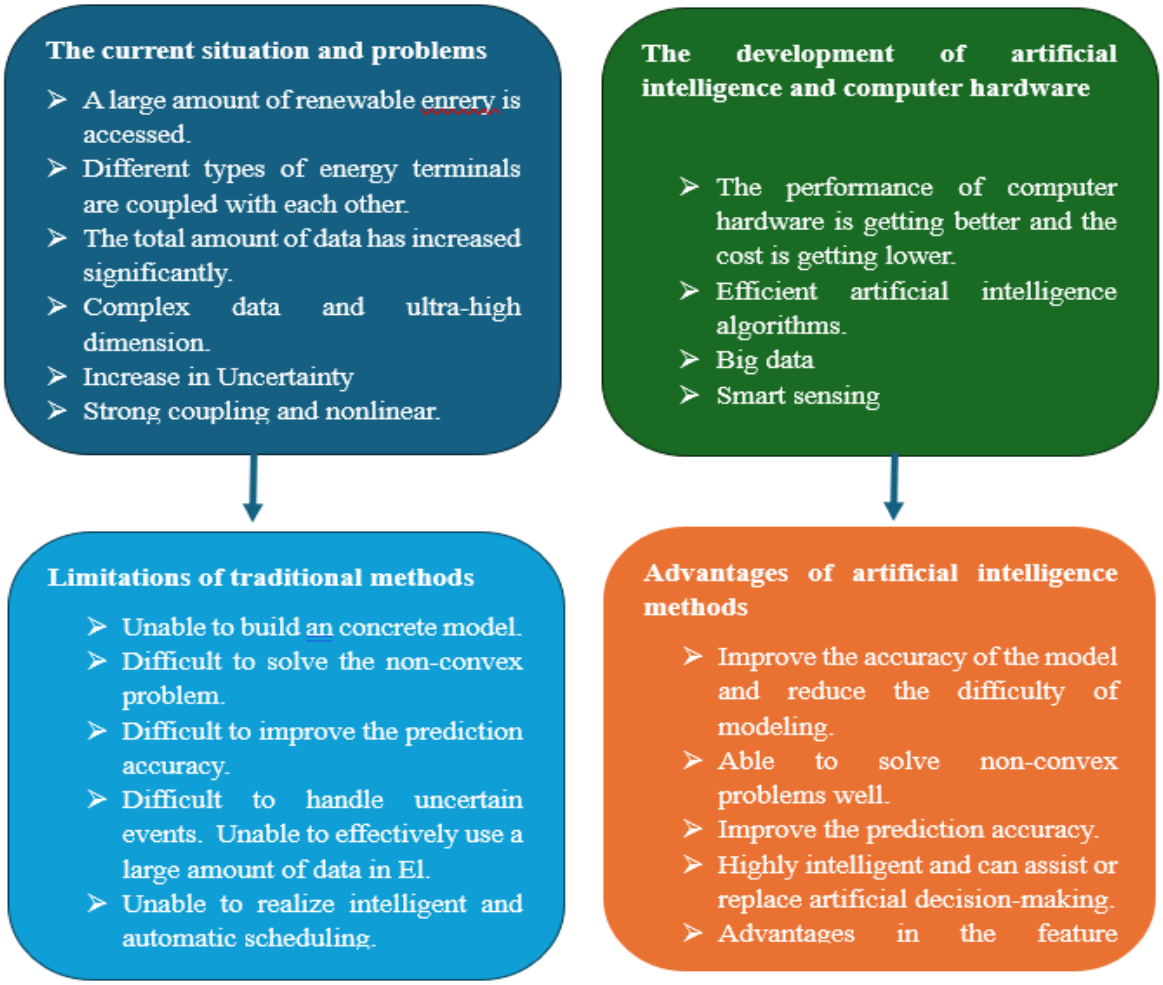


Figure 5. Traditional and AI based Methods Comparison

Application of ML in AMR

Since AMR is becoming more common, frequent AST is required to guarantee successful treatment. Phenotypic testing is the gold standard for AST. For non-fastidious bacterial illnesses, however, the process of bacterial isolation, culture, and subsequent antibiotic exposure may take two days, whereas it can take several weeks for slow-growing bacteria like *Mycobacterium tuberculosis*. It is not often done, despite the well-documented growth in resistance in certain illnesses, such as *Neisseria gonorrhoeae*. A second method of evaluating AMR that is becoming

more and more common is based on the microbial genotype rather than the phenotype and is made possible by declining sequencing costs and improvements in analytical methods (Weis et al., 2020). In addition to being speedier than phenotypic procedures, genotypic methods reduce the need for laboratory culture. However, they may also help clarify the processes behind AMR facilitate the early identification of transmission events, and provide vital ancillary data such as virulence factors and bacterial strain (Shankarnarayan, Guthrie, & Charlebois, 2022).

In the literature, several ML models are put out to analyze the AMR. For the AMR analysis, these studies often used both supervised and unsupervised ML models. This study focuses on the identification, susceptibility gene classification, antibiotic resistance gene (ARG), and infected sample classification. Figure 2 displays the entire architecture for AMR analysis utilizing machine learning.

Table 1. Different ML/DL Models with Their Merits and Demerits in AMR Applications

Technique	Algorithm	Advantages	Disadvantages
Decision Tree	Assume a target-based prediction. In the model, nodes correspond to characteristics, while leaf nodes correspond to class labels.	Table to assess characteristics The model may be tracked Interaction between features	The model may have problems when features get more complex or multivariate.
Neural Networks	1. These models learn by adjusting weights until the desired outcome is attained, simulating the functioning of the human brain. 2. The performance improves with better data. 3. Capable of working with multidimensional data.	1. Able to resolve challenging issues. 2. Interaction between features able to assess characteristics 3. Able to handle multivariate characteristics	A rise in layers and nodes corresponds to an increase in model complexity. There is no way to track the model.
Logistic Regression	The logistic curve linked to every input feature	Evaluation of features	Untraceable It is not feasible for features to interact.

ML Models for AMR Analysis

ML techniques can potentially improve antibiotic prescription procedures, identify emerging resistance patterns, and expand our knowledge of the underlying processes of AMR. Many ML techniques have been used in the study of AMR. ML techniques, including RF, DT, SVM, and LR, are some of the most popular models used recently to determine which genes in numerous bacteria are expressed differently in AMR studies (Swain, Nayak, & Swarnkar, 2023).

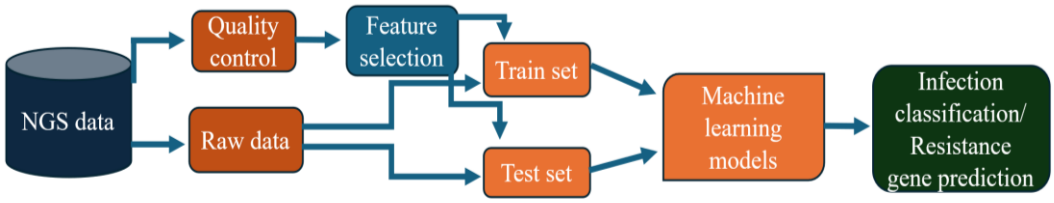


Figure 5. A General Blueprint of Various ML Models for AMR Analysis.

ML approaches have been gradually applied to different AMR datasets to tackle this significant global health concern. These techniques are critical for anticipating, understanding, and preventing AMR in a variety of data sets:

1) ***Genomic Data:*** Deep learning particularly ML has gained popularity in analyzing genomic information to find the genes and mutations that cause antibiotic resistance. It can be predicted by using classification methods that are RF, and SVMs based on the genetic information of the bacteria.

2) ***Metagenomic Data:*** Currently, when recognizing the patterns of AMR in the context of complicated microbiota, PCA or k-means are applied to the genomic data that characterize microorganisms.

3) ***Clinical Data:*** To analyze the use of antibiotics and the condition of the patients, clinical data and patients‘ case histories are employed. Clinical notes are being analyzed using Natural Language Processing (NLP) models to inform insights gathered, and logistic regression and decision tree-based approaches are used to derive a patient‘s risk of AMR.

4) ***Integration of Multi-Omics Data:*** To improve the awareness of AMR processes, DL approaches, including Multi-Omics Neural Networks, support the convergence of genome, proteome, and metabolomics data. Furthermore, feature selection, model optimization, and result interpretation are simplified by ML techniques. In the fight against AMR, these ML algorithms enable the recognition of AMR patterns, the prediction of drug susceptibility, and the development of customized therapies (Nayak, Mohapatra, Al-Dabass, & Swarnkar, 2023).

Application of DL in AMR

A recently enhanced AI has fared well in delivering high-performance results in numerous data-intensive applications, particularly in DL. Over the last few years, clinical and public health research applications have benefited from the application of DL technology. While DL algorithms effectively assess associations among numerous complex factors in clinical datasets and provide accurate predictions, it may take medical personnel months or even years to gather sufficient

experience to develop a decision-making procedure (Anahtar, Yang, & Kanjilal, 2021). Other DL algorithms, on the other hand, offer a variety of special qualities and design goals. The continuous increase in the use of DL has made several biologists adopt various DL models in AMR research in the recent past.

Based on the available histories of genomics and metagenomics, DL models might assess genomics together with other resistant genes to foresee the potential negative impact on antimicrobial therapy. They may also help in the search for new antibacterial compounds entirely. As a result, they point out that DL models are important for AMR because of the models‘ capacity to process complex biological data. They may help in the development of techniques used in identifying and predicting the pathways of AMR, which in turn help in achieving faster and more accurate susceptibility tests for antibiotics. Figure 6 shows the basic process of employing the DL models for AMR research and identifying the resistance genes (Nayak et al.).

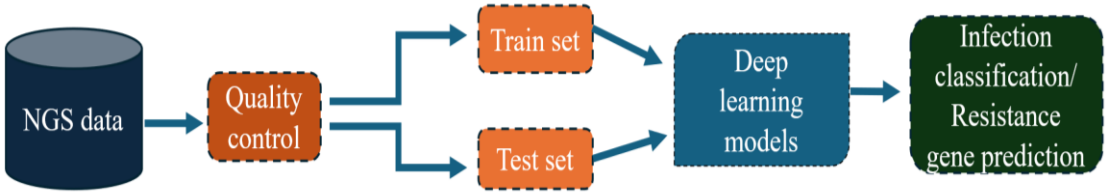


Figure 6. Pipelines of DL models in AMR Analysis

Pipeline of DL models in AMR analysis

While there are several steps to the DL models suggested for AMR analysis, the DL pipeline mentioned above primarily consists of the following stages:

1) ***Quality Control and Sequence Alignment:*** The first preparation in NGS data is filtering, which removes many low-quality reads and sequences. Subsequently, the next step is sequence alignment for aligning the reads to a reference genome or a database, which is required to complete the downstream analysis.

2) The training set and testing set should be made from the dataset with the help of a train-test split. The first dataset referred to here as the training set is employed to train the DL models, on the other hand, the second dataset commonly referred to as the test dataset is used to evaluate/ test the performances of the developed models.

3) ***DL models:*** DNNs, CNNs, and RNNs are used for training by selecting the features provided above. These models, by analyzing the data, will outline a set of conditions, and associations that point to possible antibiotic resistance.

4) ***Classification and Prediction:*** For this, the trained DL models are used to further classify and forecast the ARG that are present in the sets of NGS data. They can see what particular gene variation exists and judge the level of probable resistance by the use of learned patterns.

5) ***Model Evaluation:*** To identify antibiotic resistance genes, it is suggested that DL models be assessed on several criteria. Some of such properties are; Data splitting, Receiver Operating Characteristic (ROC) Curve, accuracy, sensitivity, specificity, Cross-validation, Concerns within the domain of application, Feature Importance, External validation, and Interpretability.

6) ***Biological Annotation:*** The outcomes bring out whether or not antibiotic resistance genes exist in the NGS data output sequence. It is important to bear in mind that biological authorization enhances the versatility of the DL model (Nayak, Das, & Swarnkar, 2022). It may be useful for medical practitioners to track these projections in an attempt to make judgment calls based on the fluctuations of AMR when prescription antibiotics (Nayak et al.).

Table 2. DL Techniques Used for AMR Analysis.

Author	Disease/Species	Dataset	Method	Analysis type
Thrift et al.	*E. coli*, and *P. aeruginosa*	Sequencing	DNN, CNN SVM	Deep neural network models can identify between treated and untreated cells in antibiotic responses in Escherichia coli and Pseudomonas aeruginosa's with more than 99% accuracy in SERS data, 10 minutes after antibiotic exposure. Quick Test for Antimicrobial Susceptibility
Chen et al.	Tuberculosis	Sequencing data	DNN	In order to identify MTB isolate resistance to ten anti-tuberculosis medications, this paper suggests a novel DL architecture using data from whole genome sequencing. The primary goal of this work was to create an incredibly precise drug resistance model using genetic data.
Brown et al.	*Staphylococcus aureus*	WGS	ANN, LR	The suggested approach demonstrates that it can complete AST far faster than the industry-standard incubation process, which takes 18–24 hours and involves visual inspection. Testing for Antimicrobial Susceptibility
Li et al.	*E. coli*, and *Pseudomonas aeruginosa*,	Raw sequence encoding	Hierarchical Multi-task DL	Annotated ARG. 99% model accuracy in the first of its kind DL model for annotating ARGs.

Pei et al.	ARG identification	CARD (v3.1.2), AMR Finder, Res Finder, Megares, deepARG, and HMD-ARG.	ARGNet	Because ARGNet accepts amino acid and nucleotide sequences of different lengths, from small (30–50 aa; 100–150 nt) sequences to full-length proteins or genes, it may be used for target and metagenomic sequencing. ARGNet outperformed other deep learning models, such DeepARG and HMD-ARG, in the majority of application situations. This was especially true when evaluating prediction consistency using quasi-negative tests and phylogenetic trees.
Poplin et al.	unspecified	WGS	Deep Variant	Small-indel and SNP calling. A deep convolutional neural network may identify genetic variation in aligned NGS read data by identifying statistical connections between photos of read pileups near suspected variations and precise genotyping calls.
Yan et al.	Candida glabrata	Sequencing	Deep-AmPEP 30	Prediction of Short Antimicrobial Peptides Based on the reduced dataset (RAAC), Deep-AmPEP30 predicts short-length AMP using a deep convolutional neural network (CNN) with a constrained AAC.

Conclusion and Recommendation

Most microbial resistances are AMR of infections that must be accurately identified to give the patients therapeutically useful medications. In this study, effort was made to present recent advancements in the use of AI in AMR detection in combination with other methods. We were pleased to learn that incorporating AI in AMR detection has been proven to be a strategic move in combating this threat to the world‘s health. AI has brought a positive change to the identification of AMR pathogens due to its capability in quick detection of the pathogens that assist medical professionals in making good decisions of the best way to handle the patient and prevent the spread of the disease. Accordingly, utilizing AI in AMR detection has consequently decreased the risk of AMR, sustained public health, and fostered rational use of antibiotics. It has also served to identify new emerging AMR strains as soon as they emerge, hence allowing superior efforts to prevent their further spread. Diagnostic assistance powered by AI similarly has decreased the use of antibiotics, hastened the choice, and enhanced the reliability of testing outcomes for microbial susceptibility.

One health alternative is to take extensive AMR monitoring that comprises the different environmental factors, animal, and human. AI may help to analyze data coming from many sources to receive a clearer picture of the existing interconnection between various variables. Research and development expenditures: As AMR is concerned with the international and national contexts the government and pharmaceutical sector should sufficiently fund the AMR and AI. This should concern the authorities. AMR is a global health problem that has consequences for human life, animal life, and ecosystems. Following these recommendations to the letter and incorporating AI more into AMR detection could perhaps enhance concerted AMR eradication attempts. Combining the potential of AI with successful public health measures and prudent antibiotic stewardship will be crucial to maintaining the efficacy of present antimicrobial agents and safeguarding the health of future generations. Together, we can overcome AMR and clear the way for a more promising and health-conscious future.

Table 3. Represents the Traditional and AI based Methods in AMR Management

Feature	**Traditional Methods**	**AI-Based Methods**	**Future Trends**
Diagnostics	Culture-based, slow (days), potential for error	Rapid (hours), genomic analysis, faster results	Increased integration of AI-powered diagnostics
Prediction	Limited, reactive	Improved accuracy, machine learning, complex pattern identification	Personalized medicine for AMR, real-time surveillance and outbreak detection
Surveillance	Manual, laborious, delayed insights	Automated, real-time monitoring, outbreak detection	AI-driven global surveillance networks
Treatment	One-size-fits-all	Personalized strategies, optimized antibiotic selection	Data-driven treatment optimization
Drug Discovery	Slow, costly	Accelerated development, identification of promising candidates	Development of novel antibiotics and alternative therapies

References

Ali, T., Ahmed, S., & Aslam, M. (2023). Artificial intelligence for antimicrobial resistance prediction: challenges and opportunities towards practical implementation. *Antibiotics, 12*(3), 523.

Anahtar, M. N., Yang, J. H., & Kanjilal, S. (2021). Applications of machine learning to the problem of antimicrobial resistance: an emerging model for translational research. *Journal of clinical microbiology, 59*(7), 10.1128/jcm. 01260-01220.

Arango-Argoty, G., Garner, E., Pruden, A., Heath, L. S., Vikesland, P., & Zhang, L. (2018). DeepARG: a deep learning approach for predicting antibiotic resistance genes from metagenomic data. *Microbiome, 6*, 1-15.

Argimón, S., Masim, M. A., Gayeta, J. M., Lagrada, M. L., Macaranas, P. K., Cohen, V., . . . Chilam, J. (2020). Integrating whole-genome sequencing within the National Antimicrobial Resistance Surveillance Program in the Philippines. *Nature Communications, 11*(1), 2719.

Aslam, B., Khurshid, M., Arshad, M. I., Muzammil, S., Rasool, M., Yasmeen, N., . . . Shahid, A. (2021). Antibiotic resistance: one health one world outlook. *Frontiers in cellular and infection microbiology, 11*, 771510.

Bolton, W. J., Badea, C., Georgiou, P., Holmes, A., & Rawson, T. M. (2022). Developing moral AI to support decision-making about antimicrobial use. *Nature Machine Intelligence, 4*(11), 912-915.

Braga, P. A. C., Tata, A., dos Santos, V. G., Barreiro, J. R., Schwab, N. V., dos Santos, M. V., . . . Ferreira, C. R. (2013). Bacterial identification: from the agar plate to the mass spectrometer. *RSC advances, 3*(4), 994-1008.

Chen, J., Liu, Y., Fang, M., Chen, H., Lao, X., Gao, X., . . . Yao, W. (2013). Combined Support-Vector-Machine-Based Virtual Screening and Docking Method for the Discovery of IMP-1 Metallo-β-Lactamase Inhibitors Supplementary Data. *American Journal of Biomedical Research, 1*(4), 120-131.

Comito, C., Falcone, D., & Forestiero, A. (2020). *Current trends and practices in smart health monitoring and clinical decision support.* Paper presented at the 2020 IEEE International Conference on Bioinformatics and Biomedicine (BIBM).

Doern, C. D., Park, J. Y., Gallegos, M., Alspaugh, D., & Burnham, C.-A. D. (2016). Investigation of linezolid resistance in staphylococci and enterococci. *Journal of clinical microbiology, 54*(5), 1289-1294.

Goralski, M. A., & Tan, T. K. (2020). Artificial intelligence and sustainable development. *The International Journal of Management Education, 18*(1), 100330.

Gupta, C., Johri, I., Srinivasan, K., Hu, Y.-C., Qaisar, S. M., & Huang, K.-Y. (2022). A systematic review on machine learning and deep learning models for electronic information security in mobile networks. *Sensors, 22*(5), 2017.

Inglis, T. J., Paton, T. F., Kopczyk, M. K., Mulroney, K. T., & Carson, C. F. (2020). Same-day antimicrobial susceptibility test using acoustic-enhanced flow cytometry visualized with supervised machine learning. *Journal of medical microbiology, 69*(5), 657-669.

Joshi, G., Jain, A., Araveeti, S. R., Adhikari, S., Garg, H., & Bhandari, M. (2024). FDA-approved artificial intelligence and machine learning (AI/ML)-enabled medical devices: an updated landscape. *Electronics, 13*(3), 498.

Kavvas, E. S., Yang, L., Monk, J. M., Heckmann, D., & Palsson, B. O. (2020). A biochemically-interpretable machine learning classifier for microbial GWAS. *Nature Communications, 11*(1), 2580.

Knight, G. M., Glover, R. E., McQuaid, C. F., Olaru, I. D., Gallandat, K., Leclerc, Q. J., . . . Van Kleef, E. (2021). Antimicrobial resistance and COVID-19: Intersections and implications. *elife, 10*, e64139.

Kumar, Y., Koul, A., Singla, R., & Ijaz, M. F. (2023). Artificial intelligence in disease diagnosis: a systematic literature review, synthesizing framework and future research agenda. *Journal of ambient intelligence and humanized computing, 14*(7), 8459-8486.

Liu, F., Zhou, Z., Samsonov, A., Blankenbaker, D., Larison, W., Kanarek, A., . . . Kijowski, R. (2018). Deep learning approach for evaluating knee MR images: achieving high diagnostic performance for cartilage lesion detection. *Radiology, 289*(1), 160-169.

Ma, L., Yi, J., Wisuthiphaet, N., Earles, M., & Nitin, N. (2023). Accelerating the detection of bacteria in food using artificial intelligence and optical imaging. *Applied and Environmental Microbiology, 89*(1), e01828-01822.

Mancuso, G., Midiri, A., Gerace, E., & Biondo, C. (2021). Bacterial antibiotic resistance: The most critical pathogens. *Pathogens, 10*(10), 1310.

Masud, R. I., Fahim, N., Rana, M. L., Islam, M. S., & Tanvir, M. (2023). Artificial intelligence, a powerful tool to combat antimicrobial resistance: an update. *J Adv Biotechnol Exp Ther, 6*, 711-727.

Miethke, M., Pieroni, M., Weber, T., Brönstrup, M., Hammann, P., Halby, L., . . . Bode, H. B. (2021). Towards the sustainable discovery and development of new antibiotics. *Nature Reviews Chemistry, 5*(10), 726-749.

Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A., . . . Wool, E. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The lancet, 399*(10325), 629-655.

Nayak, D. S. K., Das, J., & Swarnkar, T. (2022). Quality Control Pipeline for Next Generation Sequencing Data Analysis. In *Intelligent and Cloud Computing: Proceedings of ICICC 2021* (pp. 215-225): Springer.

Nayak, D. S. K., Mohapatra, S., Al-Dabass, D., & Swarnkar, T. (2023). Deep learning approaches for high dimension cancer microarray data feature prediction: A review. *Computational Intelligence in Cancer Diagnosis*, 13-41.

Nayak, D. S. K., Pati, A., Panigrahi, A., Moharana, B., Rath, A., Sahoo, S. K., & Swarnkar, T. Artificial Intelligence in Battle against Antimicrobial Resistance: Opportunities and Challenges.

Oloko-Oba, M., & Viriri, S. (2020). *Diagnosing tuberculosis using deep convolutional neural network.* Paper presented at the Image and Signal Processing: 9th International Conference, ICISP 2020, Marrakesh, Morocco, June 4–6, 2020, Proceedings 9.

Schuler, M. S., & Rose, S. (2017). Targeted maximum likelihood estimation for causal inference in observational studies. *American journal of epidemiology, 185*(1), 65-73.

Stuart, B. (2000). Infrared spectroscopy. *Kirk-Othmer encyclopedia of chemical technology*.

Tsoukalas, A., Albertson, T., & Tagkopoulos, I. (2015). From data to optimal decision making: a data-driven, probabilistic machine learning approach to decision support for patients with sepsis. *JMIR medical informatics, 3*(1), e3445.

Wang, G., Badal, A., Jia, X., Maltz, J. S., Mueller, K., Myers, K. J., . . . Yu, Z. (2022). Development of metaverse for intelligent healthcare. *Nature Machine Intelligence, 4*(11), 922-929.

Wang, H.-Y., Chung, C.-R., Tseng, Y.-J., Yu, J.-R., Chen, C.-J., Wu, M.-H., . . . Lee, T.-Y. (2021). Extensive validation and prospective observation of the impact of an AI-based rapid antibiotics susceptibility prediction platform in multiple medical centers. *medRxiv*, 2021.2006. 2023.21259391.

Weis, C. V., Jutzeler, C. R., & Borgwardt, K. (2020). Machine learning for microbial identification and antimicrobial susceptibility testing on MALDI-TOF mass spectra: a systematic review. *Clinical microbiology and infection, 26*(10), 1310-1317.

Wong, F., Stokes, J. M., Cervantes, B., Penkov, S., Friedrichs, J., Renner, L. D., & Collins, J. J. (2021). Cytoplasmic condensation induced by membrane damage is associated with antibiotic lethality. *Nature Communications, 12*(1), 2321.

Yang, K., Swanson, K., Jin, W., Coley, C., Eiden, P., Gao, H., . . . Mathea, M. (2019). Analyzing learned molecular representations for property prediction. *Journal of chemical information and modeling, 59*(8), 3370-3388.

About the Authors

Syed Sohail Ahmad completed an MPhil in Medical Microbiology from Hazara University, Mansehra. I have more than three years of teaching and research experience. Now, I am working as a Lecturer at the Rehman College of Allied Health Sciences, Rehman Medical Institute, Peshawar. My research interests are Biofilm and its eradication, Novel drugs and vaccine development for mycobacterium infections, and cancer. I worked on different anti-biofilm compounds and cancer cell lines. I have experience in in vivo and in vitro work.

Emails: syedsohailedu@gmail.com ORCID: 0009-0001-9652-7565

Dr. Farah Abid earned a Ph.D. in pharmacology from GCUF. I have more than 15 years of teaching experience. Now, I am an associate professor at the University of South Asia. My research interests are inflammation, arthritis, oxidative stress, and cancer. I have worked on different cancer cell lines and have experience in vivo.

Email: fariaayan2010@gmail.com ORCID: 0000-0001-8955-2693

Miss. Nimra Shahzadi did BS and MS in Microbiology and Molecular Genetics and Molecular Biology respectively. My research interests microbiology, molecular biology, nanotechnology, immunology, cancer and proteomics. I have experience of working on nanoparticles and proteins.

Email: shnimratalib@gmail.com ORCID: 0009-0007-4628-2292

Dr. Humera Nazir completed her PhD in medical microbiology from Quaid-i-Azam University, Islamabad. She has been teaching for more than 7 years and also has research experience. Currently, she works as an Assistant Professor at Bahuddin Zakariya University, Multan. Her research interests are investigating mechanisms of drug resistance, analysing mutations, exploring the relationship between genetic mutations and phenotypic resistance, biofilm formation in the urinary tract environment, utilising both plant extract and nanoparticles to combat resistant pathogens, and the synergistic effect of it.

Email: humera.nazir@bzu.edu.pk ORCID: 0009-0002-2667-986X

Dr. Muhammad SAFDAR earned his PhD in molecular biology and genetics from Gaziantep University, Turkey. He is a lecturer in the Breeding and Genetics department at Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, Pakistan. His research interests are molecular genetics and genomics, nutrigenomics, nano-genomics, bioinformatics, biotechnology, and their applications. He has published more than 70 research articles in national and international journals. He has also written many book chapters as well as an edited book. He is an associate editor for international journals.

E-mail: drmuhammadsafdar8@gmail.com, ORCID: 0000 0002 3720 2090

Similarity Index

The similarity index obtained from the plagiarism software for this book chapter is 7%.

To Cite This Chapter:

Ahmad, S. (2025). The Superbugs Challenge: The Role of AI in Driving Innovations and Diagnosing and Treating Antimicrobial Resistance. In S. Erten & C.A. Aksoy (Eds .), Current Studies in Life Sciences (pp.20-37). ISRES Book Series. ISRES Publishing.

Requirements For The Cultivation And Multiple Use Of Azolla

Azeddine MOUHOUS
Zahia DORBANE
Si Ammar KADI
Nacima ZEMBRI-ZIRMI
Ali BOUZOURENE
Rabia CHERFOUH
Idir MOUALEK
Hocine GUERMAH
Farid DJELLAL
Dyhia SAIDJ

A number of non-conventional feeds have the potential to be incorporated into animal feeds. One such feed is *Azolla*. This fern has been demonstrated to have a significant impact on the behaviour of the animals to which it is administered. It has been demonstrated that this species can enhance feed consumption and digestibility, thereby increasing the bioavailability of nutrients for the animals. Green *Azolla* (*Azolla pinnata*) is an incredible plant that can be used as an alternative to green fodder and as a supplementary protein feed because of its high flavor and increased yield. Its cultivation is considered the most promising due to its ease of cultivation, minimal water use, and high productivity and good nutritional value (Kathirvelan et al., 2015).

1. The Eco-physiology of *Azolla*

As outlined by Rahagarison (2005), *Azolla* necessitates a specific combination of environmental factors for the plant to thrive, develop, and grow. These factors include water, temperature, light and the pH of the water.

1.1. Water Requirements

As posited by Adzman et al. (2022), the optimal growth conditions for *Azolla* are characterized by the presence of a water layer with a depth ranging from 5 to 10 centimetres. This is advantageous for mineral nutrition, as it ensures proximity of the roots to the soil. Water is the fundamental requirement for *Azolla* growth and multiplication (Adzman et al., 2022). It is noteworthy that Azolla exhibits a high degree of susceptibility to water scarcity. It is therefore vital to maintain an optimal water level for its survival (Rajesh, 2020). The fern is unable to thrive in large lakes or turbulent waters, as the wave effect and turbulence of the water impede frond growth, leading to excessive fragmentation (Sabetraftar et al., 2013).

1.2. Humidity levels

As a purely aquatic plant, *Azolla* is unable to withstand humidity levels of less than 60%. The plant is highly susceptible to drought conditions and will perish within a few hours if the soil moisture content is reduced (Becking, 1979). The optimal range for relative humidity is 85-90%, as determined by Rajesh (2020).

1.3. Salinity

As a freshwater plant, *Azolla* can tolerate only a limited degree of salinity. Optimal growth occurs in solutions with a salinity level between 0.05% and 0.1% (Nandabalan and Kannaiyan, 1986). Growth is significantly inhibited at salinity levels above 1.3% (Sallam et al., 2024).

1.4. Temperature

The geographical distribution of the *Azolla* genus suggests that this plant can adapt to a wide range of climatic conditions (Rahagarison, 2005). Its optimal growth temperature ranges between 20 and 30°C, though certain strains exhibit temporary tolerance to extreme temperatures as low as -5°C or as high as 45°C (Rahagarison, 2005). However, some strains are notably sensitive to temperatures below 10°C, while others, like *Azolla pinnata*, can withstand temperatures above 35°C (Adzman et al., 2022). Notably, temperatures exceeding 37°C have been found to significantly hinder fern propagation (Rajesh, 2020).

1.5. Light

Azolla demonstrates optimal growth in conditions of full or partial shade, with a light intensity of between 25 and 50% of full sunlight. However, it has been observed that the growth rate declines precipitously in conditions of heavy shade (less than 1500 lux) and is significantly reduced in environments exceeding 50% of full sun, which impedes photosynthesis (Yáñez et al., 2021).

1.6. pH

Azolla demonstrates remarkable tolerance to fluctuations in environmental pH. It can survive within a broad pH range of 3.5 to 10, with minimal effects on growth. Furthermore, optimal performance and nearly identical growth characteristics are observed within the narrower pH range of 4.5 to 7 (Lumpkin and Plucknett, 1982 ; Da Silva et al., 2022).

1.7. *Azolla's* nutritional requirements

The mineral requirements of *Azolla* include the macronutrients phosphorus, potassium, calcium, and manganese, as well as the micronutrients iron (Fe), molybdenum (Mo), and cobalt (Co) (Becking, 1979; Lumpkin and Plucknett, 1982 ; Vroom et al., 2024). According to Becking (1979), deficiencies in these elements result in reduced growth rates (Table 7).

Table 7. Rate of decline in *Azolla* growth under deficiency conditions (Becking, 1979).

Nitrogen deficiency (N_2)							
Elements	Reference elements	P	K	Ca	Mg	Mn	Fe
Growth reduction rate	100% fresh weight - Minimum growth	22%	30%	5%	82%	23%	11%

2. Azolla cultivation process

According to Dhaker et al. (2021), the establishment of an *Azolla* fodder plot does not necessitate any particular expertise. Indeed, it is a relatively straight forward process that can be undertaken by farmers themselves. The following steps are recommended :

- It is recommended that the sides of the plots be raised to allow for the accumulation of stagnant water. In the event that the plot is situated in a backyard, it is necessary to level the area and cover it with bricks.
- An alternative approach involves installing the fodder plot in a pit approximately 0.2 meters deep, lined with a polyethylene sheet to retain a standing water depth of 10 cm. The bed width is maintained at 1.5 meters to facilitate access and management from both sides. The length of the plot can be adjusted according to specific fodder requirements. For instance, to meet 50% of the green fodder needs of two cows, two cultivation units—each measuring 2.5 meters in length and covering an area of approximately 8 m^2—are considered sufficient.
- Once the cultivation bed measuring 2.5 m × 1.5 m is prepared, approximately 15 kg of finely sieved soil is spread over the polyethylene lining to provide essential nutrients for *Azolla* growth. Around 5 kg of pre-decomposed cow dung (fermented for two days) is then mixed into the water, serving as a carbon source for the plant. A nutrient mixture—comprising 10 kg of rock phosphate, 1.5 kg of magnesium salt, and 500 g of muriate of potash—is subsequently added to the bed. This solution is further enriched with micronutrients in appropriate concentrations, ensuring that the nutritional requirements of the fern are met. As a result, the cultivated *Azolla* also provides enhanced nutritional value when used as livestock feed.
- The requisite volume of water is added to achieve a water level in the bed of 10 cm.
- The soil is then distributed uniformly across the base of the reservoir. The depth of the layer should be approximately 10 centimetres. The quantity of cow dung added is 1 to 1.5 kg per square metre of the tank surface area (equivalent to 2 to 3 kg of cow dung per tank).
- It is recommended that simple superphosphate (SSP) be added at a rate of 5 g per square metre of tank surface each week. (Ten grams of SSP per tank). The water tank should be filled to a level that allows for a 10 to 15 cm accumulation of water above the ground.
- Subsequent to this, the soil particles should then be permitted to settle. The fresh *Azolla* inoculum should be prepared by adding 2 g of carbofuran, which will prevent infestation by parasites. The layer of foam and scum that forms on the surface of the water should be removed, as this hinders the growth and penetration of Azolla roots. The tank should then be left to stand overnight.

On the following day, approximately 200 g of fresh *Azolla* inoculum should be spread on the surface of the water.

- It is estimated that the *Azolla* takes approximately two weeks to form a mat on the surface of the water. It is imperative to ensure that the water level in the tank remains constant, matching that of the reservoir, especially during the summer months.

The initial phase of the process is characterised by the rapid proliferation of *Azolla* across the entire surface of the bed, resulting in the formation of a dense mat within a period of seven days

(Fig. 1). The optimal result is the production of 10 kg of *Azolla* in seven days. It is imperative to maintain the water level throughout this period, which can be achieved by adding water each day.

Following a seven-day period, the *Azolla* can be harvested at a rate of 1.5 kg per day, and it is recommended that harvesting is conducted in plastic

Figure 1. *Azolla* spread over the surface of a pond (Tran, 2015).

- It is imperative that *Azolla* harvested for use in livestock husbandry be thoroughly rinsed with fresh water prior to provision. It is essential to subject the material to a washing process in order to eliminate the unpleasant odour associated with cow dung. The washings of *Azolla* can be utilised as a form of bio-manure for plants cultivated in close proximity.
- The addition of cow dung, mineral mix, soil and water is recommended at a frequency of once every seven days.
- It is recommended that the soil be removed from the bed every 60 days and 15 kg of fresh, fertile soil be added to prevent nitrogen build-up.

2.1. Precautions to be taken when carrying out the *Azolla* cultivation process

According to Dhaker et al. (2021), the following precautions should be observed during the cultivation of *Azolla*:

- It is of the utmost importance to maintain a pure crop, free from any contamination, in order to ensure a good yield.
- It is essential to harvest *Azolla* on a regular basis in order to prevent overcrowding.

The temperature is a significant factor influencing the growth process. The optimal temperature for optimal growth is approximately 35 degrees Celsius. In regions where temperatures are lower, it is advisable to cover the forage plot with a plastic sheet in order to mitigate the adverse effects of the cold.

Plots situated in areas with an abundance of direct sunlight are preferable, as shady locations tend to result in lower yields.

The pH of the environment should be maintained within the range of 5.5 to 7. The addition of appropriate nutrients, such as cow dung slurry and micronutrients, may be necessary to supplement the soil's nutritional profile.

3. The Different Systems Integrated into *Azolla* Cultivation

3.1. The Rice-Fish-*Azolla* System

One of the most successful applications of *Azolla* is as a fertiliser and/or feed in an integrated rice-fish-*Azolla* system. In such a system, the simultaneous development of rice, *Azolla* and different species of fish (planktivorous, macrophytophorous and polyphagous) is permitted. The interplay between these elements is critical to maintaining the system's equilibrium. The fish derive benefit from the *Azolla*, depending on the species. The fish waste encourages the proliferation of plankton, which is consumed by some of the fish and by others. Additionally, some fish consume the plankton, while the remainder fertilise the rice. The presence of polyphagous fish has been demonstrated to provide a protective function against a variety of harmful insects and molluscs (Hasan and Chakrabarti, 2009; Khumairoh et al., 2018).

3.2. The fish-*Azolla* system

In a fish-*Azolla* polyculture system (Fig. 2), diets containing 10-40% dried *Azolla* had no significant effect on water quality, except for nitrate and nitrogen content. All fish species exhibited significantly higher growth with diets containing up to 20% *Azolla* (Dhawan et al., 2010).

Figure 2. Fish-*Azolla* co-culture (www.theazollafoundation.org).

3.3. The canard-fish-*Azolla* system

In an integrated duck-fish-*Azolla* system (Fig. 3), Nile tilapia are stocked in ponds fertilized with a mixture of fresh pig and duck manure. This practice has been shown to lead to the complete disappearance of the fish population. In these ponds, *Azolla* is consumed at intervals of six to seven days (Gavina, 1994 ; Mansour et al., 2020).

Figure 3. The duck-fish-*Azolla* system / (www.theazollafoundation.org).

3.4. The rice-canard-*Azolla* system

In the rice-duck-*Azolla* system (Fig. 4), Becerra et al. (1995) conducted feeding trials to assess the effect of feeding *Azolla microphylla* on rice plants. The goal was to determine the impact of incorporating *Azolla microphylla* as a partial substitute for soybean protein. Additionally, the study aimed to evaluate the effectiveness of replacing protein in boiled soybeans with sugarcane juice-based diets for ducks and to assess the suitability of such diets for meat ducks. The results showed that fresh *Azolla* can replace whole soybeans at approximately 20% of the total crude protein in duck meat diets without any negative effects on growth rate or health. Moreover, this treatment resulted in the lowest feed cost per kg of weight gain and the highest net profit per bird (Nasir et al., 2022).

Figure 4. *Azolla*-rice-duck co-culture (www.theazollafoundation.org).

4. Utilization of *Azolla*

Azolla is considered one of the most nutritionally dense aquatic plants, owing to its high content of carotenoids and amino acids. Additionally, it can be incorporated into both animal feed and human food (Van Hove and Lejeune, 2002).

4.1. Use of *Azolla* in Human Nutrition

Azolla is consumed by humans, and it appears to be non-toxic, with some culinary preparations being quite palatable. However, the use of *Azolla* in human food is limited by the difficulty of removing impurities, particularly those associated with its root system (Bujak et al., 2024).

4.2. Utilization of *Azolla* in Animal Feed

Azolla can be provided to livestock either fresh or dried, and can be administered directly or incorporated into concentrates for cattle, poultry, sheep, goats, pigs, and rabbits. It is recommended that animals be gradually introduced to *Azolla* by feeding them concentrates for a few days to help them acclimatize to its taste. Thus, it is advisable to start feeding the concentrates during the initial stages. Additionally, when dung is used as fertilizer in *Azolla* ponds, it is crucial to thoroughly wash the plant with fresh water to remove any residual odor of dung (Giridhar and Rajendran, 2013).

4.2.1. Utilization of *Azolla* in Ruminant Feed

Most *Azolla* feeding trials for dairy cattle, growing buffaloes, sheep, and goats have been conducted in India since 2000 (Pillai et al., 2004). The production of *Azolla* has been shown to have the second-highest benefit/cost ratio, following the production of worms for vermicomposting (Deshmukh et al., 2013). *Azolla*, in both fresh and dried forms, can be incorporated into the feed of cattle, sheep, and goats (see Fig. 5). Despite its long-standing use in ruminant diets, there remains a lack of comprehensive data on its efficacy. Trials in India have demonstrated that fresh or dried *Azolla* can partially replace more conventional protein sources, such as groundnut meal (Tran, 2015).

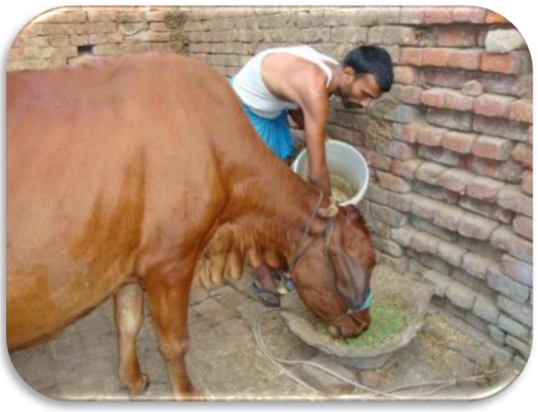


Figure 5. A cattle fed with *Azolla* (Tran, 2015).

4.2.2. The use of *Azolla* in poultry feed

4.2.2.1. The Use of Azolla in Broilers and Pullets

The inclusion of *Azolla* in the diet of broilers should be limited to a maximum of 5%, as higher inclusion rates have been shown to reduce nutrient utilization and overall performance of the birds (Parthasarathy et al., 2002; Basak et al., 2002 ; Samad et al., 2020). In pullet chicks, Azolla can be safely included at levels up to 10% (Alalade and Iyayi, 2006). Fresh Azolla (Fig. 6) has the potential to replace 20% or more of the commercial broiler feed (Namra et al., 2010 ; Samad et al., 2020).

Figure 6. Poultry consuming *Azolla* (www.theazollafoundation.org).

4.2.2.2. The utilisation of Azolla in the diet of laying hens

The inclusion of dried *Azolla* in the diet of laying hens has been demonstrated to be a viable and sustainable practice. The incorporation of dried *Azolla* in the diet of laying hens has been shown to constitute up to 15% of the diet (**Alagawany et al., 2023**). The addition of *Azolla* to the diet has been shown to have a positive effect on yolk colour (Khatun et al., 2008).

4.2.2.3. The Utilization of Azolla in the Diet of Japanese Quail

Azolla pinnata has been shown to have potential as a feedstuff for Japanese quail due to its high nutrient content. However, incorporation rates exceeding 5% have been found to negatively impact growth performance and feed conversion (Sujatha et al., 2013).

4.2.2.4. The utilization of Azolla in the diet of geese

Azolla has been utilised as a green forage for geese. A study by Zhang Zhuang et al. (1987) found that the daily weight gain of geese fed on *Azolla* was comparable to that of geese fed vegetables. It is demonstrated in this study that the dietary supplementation of Egyptian geese with 8 and 16% Azolla during the fattening stage results in enhanced growth performance and significant fluctuations in most blood biochemical parameters (Ismail et al., 2023).

4.2.3. The utilization of Azolla in rabbit nutrition

Azolla appears to be a suitable foodstuff for rabbits. A trial was conducted in which 6-week-old rabbits were fed diets containing from 0% to 36% dried *Azolla*. The results of this trial revealed that growing rabbits could be safely fed rations containing 24% dried *Azolla* hay, which has beneficial effects on the health of the rabbit. Furthermore, this hay has been shown to have beneficial effects on most production traits (Abou-Zeid et al., 2001). In a subsequent trial conducted with breeding rabbits, the substitution of 25% of the soybean meal protein with sun-dried *Azolla* protein resulted in the maintenance of feed conversion, litter size at weaning and female body weight, as well as economic performance. However, a decline in the conception rate, litter size at birth and milk production was also observed (El-Deeb et al., 2021).

4.2.4. The utilization of *Azolla* in Fish Husbandry

A series of studies conducted in aquariums have shown that cichlids (specifically those from the *Oreochromis*, *Tilapia*, and *Cichlasoma* genera) as well as a herbivorous hybrid of carp and bighead carp exhibit a preference for *Azolla*. Several researchers have demonstrated a preference for *Azolla caroliniana* over other species of Azolla (Micha et al., 1988 ; Fiogbé et al., 2004 ; Yohana et al,., 2023).

4.3. Interests Associated with the Plant

4. 3. 1. Economic Benefits

Azolla is a nitrogen fixer due to its symbiotic relationship with other organisms. It is widely used as green manure in rice fields across various Asian countries. Additionally, *Azolla* has been observed to help regulate weed growth, reduce water loss through evaporation, and improve soil structure (Rahagarison, 2005 ; Akhtar et al., 2020).

4.3.2. Environmental Benefits

Azolla reduces the intensity of underwater light, inhibiting algal photosynthesis, which in turn prevents the subsequent increase in pH and the emission of NH_3. Since up to 50% of nitrogen fertilizer applied to rice fields is lost through volatilization, *Azolla* has the potential to reduce the amount of nitrogen fertilizer needed for rice crops (Yao et al., 2018). Additionally, *Azolla* has been shown to reduce the proliferation of mosquitoes (Ravi et al., 2020 ; Wilson et al., 2023).

5. Conclusion

In summary, the findings of these studies demonstrate the significance of the endeavours undertaken to cultivate this plant (*Azolla*). The objective is to establish this plant as a viable alternative food source for animals. The findings of these studies serve as a valuable repository of knowledge, which can be utilised to devise innovative solutions that enhance the utilisation of *Azolla* in animal nutrition. Consequently, this will contribute to the enhancement of food security in countries where the production of animal products is constrained by limited resources.

References

Abou-Zeid A. E., Mohamed F. F., & RadwanM. S. M. (2001). Assessment of the nutritive value of dried azolla hay as a possible feed ingredient for growing NZW rabbits. *Egyptian J. Rabbit Sci., 11 (1): 1-21.*

Adzman, N., Goh, S., Johari, A., Alam, M., & Kamaruddin, M. (2022). Preliminary study on Azolla cultivation and characterization for sustainable biomass source. *Journal of Physics : Conference Series, 2259*. https://doi.org/10.1088/1742-6596/2259/1/012018.

Akhtar, M., Sarwar, N., Ashraf, A., Ejaz, A., Ali, S., & Rizwan, M. (2020). Beneficial role of Azolla sp. in paddy soils and their use as bioremediators in polluted aqueous environments: implications and future perspectives. *Archives of Agronomy and Soil Science*, 67, 1242 – 1255. https://doi.org/10.1080/03650340.2020.1786885.

Alagawany, M., Elnesr, S., Saleh, A., El-Shall, N., Azzam, M., Dhama, K., & Farag, M. (2023). An updated review of azolla in poultry diets. *Word's Poultry Science Journal,* 80, 155 – 170. https://doi.org/10.1080.00439339.2023.2271886.

Basak B., Pramanik A. H., Rahman M. S., Tarafdar S. U., & Roy B. C. (2002). Azolla (*Azolla pinnata*) as a feed ingredient in broiler ration. *Int. J. Poult. Sci.,* 1 (1/3): 29-34.

Bujak, J., Pereira, A., Azevedo, J., Bujak, A., Leshyk, V., Gia, M., Stadtlander, T., Vasconcelos, V., & Winstead, D. (2024). Azolla as a Safe Food: Suppression of Cyanotoxin-Related Genes and Cyanotoxin Production in Its Symbiont, Nostoc azollae. *Plants*, 13. https://doi.org/10.3390/plants13192707.

Becking, J. H. (1979). Environmental requirements of Azolla for use in tropical rice production, Nitrogen and Rice. *International rice Research Institute.* Los Banos, Laguna, Philippines, 345-374.

Becerra, M., Preston, T.R., & Ogle, B. (1995). Effet du remplacement des fèves de soja entières bouillies par de l'azolla dans l'alimentation des canards en croissance. *Recherche sur l'élevage et développement rural,* (7) : 1-10.

Da Silva, M., Mathe, L., Van Rooyen, I., Brink, H., & Nicol, W. (2022). Optimal Growth Conditions for Azolla pinnata R. Brown: Impacts of Light Intensity, Nitrogen Addition, pH Control, and Humidity. *Plants*, 11. https://doi.org/10.3390/plants11081048.

Dhaker, D.L., Devi Lal D., Rayapati K., & Shivam M. (2021). Cultivation of Azolla- A Proteinaceous Fodder for Livestock. *Agriculture and environment*, (2) :50-52.

Dhawan A., Phulia V., Ansal, M. D., 2010. Incorporation of an aquatic fern (Azolla) in fish diet - effect on water quality and fish yield. *Indian J. Ecol*., 37 (2): 122-126.

Deshmukh R. C., Balaji J., Gayatri B., 2013. Integrated farming system for strengthening rural livelihood in disadvantaged areas of Bidar district. *Int. J. Agric. Sci.,* 9(1): 57-59.

El-Deeb, M., Fahim, H., Shazly, S., Ragab, M., Alazab, A., & Beshara, M. (2021). Effect of Partially Substitution of Soybean Protein with Azolla (Azolla pinnata) on Productive Performance and Carcass Traits of Growing Rabbits. https://doi.org/10.21608/jappmu.2021.182678.

Fiogbé, E.D., Micha, J. C., & Van Hove, C. (2004). Use of a natural aquatic fern, Azolla microphylla, as a main component in food for the omnivorous-phytoplanktonophagous tilapia, Oreochromis niloticus L. *J. Appl. Ichth.,* 20 (6): 517-520.

Gavina, L.D. (1994). Pig-duck-fish-azolla integration in La Union, Philippines. NAGA, *The ICLARM Quarterly,* 17 (2) : 18-20.

Giridhar, K., & Rajendran, D. (2013).Cultivation and usage of azolla as supplemental feed for dairy cattle. In: *Value addition of feed and fodder for dairy cattle, NIANP*, June 27-July 6, 2013, 32-34 p.

Hasan, M.R., & Chakrabarti, R. (2009). Use of algae and aquatic macrophytes as feed in small scale aquaculture: *A review. FAO Fisheries and Aquaculture technical paper,* 531. FAO, Rome, Italy.

Ismail, M., Attia, A., Mohamed, L., & Alagawany, M. (2022). Effect of varying dietary Azolla levels on growth, carcass characteristics, blood biochemical parameters and digestive enzymes of growing Egyptian geese. *Animal Biotechnology,* 34, 3441 – 3448. https://doi.org/10.1080/10495398.2022.2154224.

Kathirvelan, C., Banupriya, S., & Purushothaman, M. R. (2015). Azolla-an alternate and sustainable feed for livestock. *International Journal of Science, Environment and Technology*, 4(4):1153-1157.

Khatun, M. A., Mondol, M.A.S., & Akter, Y. (2008). Effect of azolla (Azolla pinnata) based diet on the performance and egg quality of laying hens. *Int. J. Sustain. Agric. Technol*., 4 (2) : 6 12.

Khumairoh, U., Lantinga, E., Schulte, R., Suprayogo, D., & Groot, J. (2018). Complex rice systems to improve rice yield and yield stability in the face of variable weather conditions. *Scientific Reports*, 8. https://doi.org/10.1038/s41598-018-32915-z.

Lumpkin, T.A., & Plucknett, D.L. (1982). Azolla as a Green Manure: Use Management in crops production. *Westview Tropical Agriculture*. Boulder, Colorado.

Marco, A., Yáñez, Y., Gómez, P., Gajardo, J., & Espinoza, S. (2021). Growth and physiological acclimation to shade in young plants of Adesmia bijuga Phil., a critically endangered species in central Chile. *iForest-Biogeociences and Forestry.*

Mansour, E., Mounes, H., & Ahmed, K. (2020). Effect of Azolla pinnata and Nannochloropsis oculata on growth performance and immunoresponse of Nile tilapia (Oreochromis niloticus) and its resistance to bacterial infection. *European Journal of Anaesthesiology*, 10, 43-62. https://doi.org/10.21608/eja.2020.38241.1030.

Micha, J. C., Antoine, T., Wery, P., & Van Hove, C. (1988). Growth, ingestion capacity, comparative appetency and biochemical composition of Oreochromis niloticus and Tilapia rendalli fed with Azolla. In: R.S.V. Pullin, T. Bhukaswan, K. Tonguthai & J.L. Maclean, eds. The 2nd Int. Symp. On Tilapia in Aquaculture, Manila, *ICLARM Conference Proceedings*, 347-355 p.

Nasir, N., Kamaruddin, S., Zakarya, I., & Islam, A. (2022). Sustainable alternative animal feeds: Recent advances and future perspective of using azolla as animal feed in livestock, poultry and fish nutrition. *Sustainable Chemistry and Pharmacy*. https://doi.org/10.1016/j.scp.2021.100581.

Namra, M.M.M., Hataba N.A., & Wahed H.M.A. (2010). The productive performance of growing fayoumi chicks fed restricted diets supplemented with free fresh azolla. *Egypt. Poult. Sci. J.,* 30 (3): 747-762.

Parthasarathy, R., Kadirvel, R., & Kathaperumal V. (2002). Azolla as a partial replacement for fishmeal in broiler rations. *Indian Vet. J.,* 79 (2) :144-146.

Pillai, P.K., Rajamony, S., & Premalatha, S. (2004). Azolla - un substitut alimentaire durable pour le bétail avec un accent particulier sur la production à base de silpauline et la technique d'alimentation. *Technologie des biofertilisants,* 209-218 p.

Ravi, R., Rajendran, D., Oh, W., Rasat, M., Hamzah, Z., Ishak, I., & Amin, M. (2020). The potential use of Azolla pinnata as an alternative bio-insecticide. *Scientific Reports,* 10. https://doi.org/10.1038/s41598-020-75054-0.

Rahagarison, K. (2005). Etude bibliographique de l'Azolla ou la « ramilamina » plante fertilisatrice d'Azote (N2). *Mada revues*, 287-29 p.

Rajesh, S. (2020). Production of azolla as livestock feed supplement in India. Pashudhan praharee. *Indian Dairy & Poultry Industry, 43 p*.

Sabetraftar, K., Zarkami, R., Sadeghi, R., & Damme, P. (2013). A review of some ecological factors affecting the growth of Azolla spp. *Caspian Journal of Environnemental Sciences*, 11, 65-76.

Sallam, G., Helal, A., Mabrouk, H., Hermina, A., Habib, Y., Fayed, W., Dossou, S., Basuini, M., & Shehata, A. (2024). Comparative impact of replacing fish meal with azolla on growth, water quality, and physiology of red tilapia fingerlings at varying salinities. *Journal of Animal Physiology and Animal Nutrition*. https://doi.org/10.1111/jpn.13980.

Samad, F., Idris, L., Hassim, H., Goh, Y., & Loh, T. (2020). Effects of Azolla spp. as feed ingredient on the growth performance and nutrient digestibility of broiler chicken. *Journal of Animal Physiology and Animal Nutrition*. https://doi.org/10.1111/jpn.13345.

Sujatha, T., Kundu, A., Jeyakumar, S., & Kundu, M.S. (2013). Supplémentation en azolla : avantage du coût de l'alimentation dans la ration de canard aux îles Andaman. *Tamil Nadu J. Vet. Anim. Sci.,* 9 (2): 130-136.

Tran, G. (2015). Azolla. Feedipedia, INRAE, CIRAD, AFZ et FAO.https://www.feedipedia.org/node/565.

Van Hove C., Lejeune A., 2002. The Azolla: anabaena symbiosis. In : Biology and Environment : Proceedings of the Royal Irish Academy, 102 (1) :23-26).

Vroom, R., Smolders, A., Van De Riet, B., Lamers, L., Güngör, E., Krosse, S., Verheggen-Kleinheerenbrink, G., Van Der Wal, N., & Kosten, S. (2024). Azolla cultivation enables phosphate extraction from inundated former agricultural soils. *Water research*, 254, 121411. https://doi.org/10.1016/j.watres.2024.121411.

Wilson, J., Mahendran, S., Sivakumar, T., Ponmanickam, P., & Thangaraj, R. (2023). Mosquito larvicidal activity of silver nanoparticles synthesized using Azolla pinnata against Culex quinquefasciatus Say (Diptera : Culicidae). *South African Journal of Botany*. https://doi.org/10.1016/j.sajb.2023.04.019.

Yao, Y., Zhang, M., Tian, Y., Zhao, M., Zeng, K., Zhang, B., Zhao, M., & Yin, B. (2018). Azolla biofertilizer for improving low nitrogen use efficiency in an intensive rice cropping system. *Field Crops Research*, 216, 158-164. https://doi.org/10.1016/J.FCR.2017.11.020.

Yohana, M., Ray, G., Yang, Q., Tan, B., Chi, S., Asase, A., Shiyu, K., & Derrick, A. (2023). A Review on the Use of Azolla Meal as a Feed Ingredient in Aquatic Animals‘ Diets. *Aquaculture Research.* https://doi.org/10.1155/2023/2633412.

Yáñez, M.A., Gómez, P., Gajardo, J., & Espinoza, S. (2021). Growth and physiological acclimation to shade in young plants of Adesmia bijuga Phil., a critically endangered species in central Chile. *iForest* 14: 307-312. – Doi : https://doi.0org/10.3832/ifor3640-014.

Zhang Zhuang, T., Ke Yu, S ., Ling ., Duan B., & Liu X. (1987). Utilization of Azolla in agricultural production in Guangdong province, China. In : IRRI, Azolla utilization: Proc. Workshop on Azolla use, Fuzhou, Fijian, China, Manila. 31 Mars -5 Apr 1985.

About the Authors

Dr. Azeddine MOUHOUS.
Affiliation: Faculty of Biological and Agronomical Sciences. Mouloud MAMMERI University of Tizi-Ouzou, Algeria.

E-mail: azeddine.mouhous@ummto.dz ORCID: 0000-0003-0558-1919

Dr. Zahia DORBANE
Affiliation: Faculty of Biological and Agronomical Sciences. Mouloud MAMMERI University of Tizi-Ouzou, Algeria.

E-mail: zahia.dorbane@ummto.dz ORCID : 0000-0003-3093-4763

Pr. Si Ammar KADI.
Affiliation: Faculty of Biological and Agronomical Sciences. Mouloud MAMMERI University of Tizi-Ouzou, Algeria.

E-mail:siammar.kadi@ummto.dz ORCID: 0000-0002-1048-2280

Dr. Nacima ZIRMI-ZEMBRI
Affiliation: Faculty of Biological and Agronomical Sciences. Mouloud MAMMERI University of Tizi-Ouzou, Algeria.

E-mail: nacima.zembri@ummto.dz ORCID: 0009-0009-3527-1357

Mr Ali BOUZOURENE
Affiliation: Faculty of Biological and Agronomical Sciences. Mouloud MAMMERI University of Tizi-Ouzou, Algeria.

E-mail: ali.bouzourene@ummto.dz

Dr. Rabia CHERFOUH
Affiliation: Faculty of Biological and Agronomical Sciences. Mouloud MAMMERI University of Tizi-Ouzou, Algeria.

E-mail: rabia.cherfouh@ummto.dz ORCID: 0000-0001-7275-6286

Dr Idir MOUALEK
Affiliation: Faculty of Biological and Agronomical Sciences. Mouloud MAMMERI University of Tizi-Ouzou, Algeria.

E-mail: idir.moualek@ummto.dz ORCID: 0000-0002-4455-5556

Dr Hocine GUERMAH
Affiliation: Department of Agronomical Sciences, Mohamed Boudiaf University, M'sila, Algeria

E-mail: hocine.guermah@univ-msila.dz ORCID: 0000-0003-2378-1420

Dr. Farid DJELLAL
Affiliation: Departement of Agronomical Sciences, faculty of Nature and Life Sciences, Ferhat ABBAS University of Sétif1, Algeria

E-mail: fariddjellal@univ-setif.dz ORCID: 0000-0002-8066-2455

Pr. Dahia SAIDJ
Affiliation: Veterinary Sciences Institute, Saad Dahleb University, Blida, Algeria

E-mail: dyhiasdj1@yahoo.fr ORCID: 0000-0001-8380-6437

To Cite This Chapter

Mouhous, A., Dorbane, Z., Kadi, S.A., Zirmi-Zembri, N., Bouzourene, A., Cherfouh, R., Moualek, I., Guermah, H., Djellal, F., & Saidj, D. (2025). Requirements for the cultivation and multiple use of *Azolla*. Current Studies in Life Sciences 2025 (pp. 39-53). ISRES Publishing.

Impact of Environmental Factors on Human and Animal Health

Muhammad Adnan Ul Hassan
Khawar ALI
Mehmet OZASLAN

Introduction

Environmental factors significantly impact human and animal health, predominantly through air pollution and climate change, which lead to acute and chronic health issues. Air pollution is a major concern, being responsible for an increase in respiratory and cardiovascular diseases. Long-term exposure to pollutants, such as particulate matter (PM) and ozone, has been consistently linked to decreased life expectancy, studies indicating an average reduction of 1-2 years due to these environmental factors. A systematic review highlighted the acute effects of ambient PM2.5 exposure, which has been associated with elevated hospitalization rates for respiratory diseases, corroborating findings of substantial mortality linked to air pollution. Specific populations, such as those with comorbid conditions like type 2 diabetes, face exacerbated health issues due to poor air quality, resulting in higher hospitalization needs. Furthermore, air pollution exacerbates healthcare costs linked to respiratory diseases. Studies indicate that the incidence of respiratory diseases rises due to air pollution, so does the associated healthcare expenditure. This economic impact is compounded by the necessary public health responses to mitigate these environmental stressors. Additionally, the negative effects of combustion pollutants on fetal development, suggesting that air pollution poses a critical risk during pregnancy, leading to adverse outcomes such as low birth weight and stillbirth. Climate change operates as another critical environmental factor affecting health by altering disease vector populations and enhancing pathogen development. For instance, rising temperatures are linked to the expansion of vector-borne diseases, which thrive under conditions accelerated by urbanization and climate variability. Rapid urbanization increases the density of both human and animal populations, facilitating the spread of infectious diseases. Environmental changes foster conditions that predispose populations to new and resurging infectious diseases, exemplified by shifts in ecosystems driven by anthropogenic activities such as deforestation and agriculture. Moreover, the relationship between air quality and respiratory health is complex and multifaceted, involving not only direct pollution exposure but also indirect consequences of climate extremes, including heatwaves and increased allergen levels. These environmental stressors contribute to significant morbidity, particularly among vulnerable populations, reinforcing the need for targeted public health strategies and interventions tailored to mitigating risks posed by both air quality and climatic factors. Environmental factors such as air pollution and climate change significantly impact human and animal health. Addressing these issues requires a multifaceted approach that considers both immediate health effects and broader public policy implications aimed at reducing the prevalence of associated diseases and their burden on healthcare systems.

The Environment-Health Interface

Environmental conditions profoundly influence both human and animal health through shared exposures and interconnected ecosystems. This intersection of health, where environmental factors affect multiple species, underscores the necessity of a holistic approach to understanding and addressing health issues. The built environment plays a critical role in the dynamics of health behaviors and disease transmission among both humans and animals. Evidence demonstrates that elements such as urban planning, land use, and infrastructure impact individual health outcomes by shaping environmental quality and influence disease dynamics across social systems involving both humans and non-human species. Such environments can foster or hinder the transmission of infectious diseases, illustrating how structural factors interlink health dynamics within and across species. This relationship serves as a reminder of the importance of designing environments that mitigate health risks common to both humans and animals. Furthermore, the concept of "One Health" emphasizes the interconnectedness of human, animal, and ecosystem health. Rapid environmental changes, such as climate variations and habitat destruction, increase shared risks, making it essential to adopt an integrative health perspective that encompasses all living organisms. This approach has become crucial for monitoring and managing emerging public health threats, as zoonotic diseases (those transmitted from animals to humans) exemplify the intertwined nature of ecosystems and health. By understanding these links, public health initiatives can be tailored to address risks that operate across species, potentially leading to better health outcomes for all parties involved. Moreover, environmental exposure to pollutants and climate stressors affects the health of animals, which can, in turn, influence human health. Studies indicate that environmental toxins impact animal health and behavior, which may affect human interactions with them or consumption of animal products (Natterson-Horowitz et al., 2022). For instance, the health of livestock and pets can affect zoonotic disease transmission, necessitating a comprehensive approach to food safety and public health that considers animal welfare and environmental integrity. When animal health deteriorates due to environmental conditions, the resultant disease manifestations can have significant repercussions for human populations, particularly in settings where humans and animals cohabitate closely. The interaction of ecological factors drives health outcomes and shapes nutritional quality across species. The health of animal populations can significantly impact human nutrition and food security, particularly in regions where communities heavily rely on agricultural and livestock products. An enhanced understanding of animal nutrition as it relates to environmental health becomes critical, reflecting back on human health outcomes through dietary connections. The exchange of health risks among species illustrates the need for interventional strategies that mitigate the adverse effects of environmental hazards. The intricate web of interactions between environmental conditions, human health, and animal health underscores the importance of a unified perspective in public health. Recognizing that both human and animal health are influenced by shared spaces and exposures can lead to improved strategies for preventing and managing health threats that arise from environmental challenges. This integrative approach can foster healthier ecosystems and bolster health outcomes for all species involved.

Climate Change and Emerging Zoonotic Diseases

Global warming significantly alters disease ecology by influencing the dynamics of zoonotic diseases, which emerge from pathogen transmission between animals and humans. The interplay between climate change and zoonoses such as COVID-19, Nipah virus, and Rift Valley fever epitomizes this relationship; warming temperatures, changes in precipitation patterns, and habitat alterations directly affect disease transmission dynamics. One of the primary mechanisms through which climate change exacerbates zoonotic diseases is by shifting the geographical ranges of animal hosts and vectors. As temperatures rise, many species are forced to migrate to cooler regions, often leading to increased proximity between wildlife and human populations. For instance, the expansion of habitats for zoonotic vectors (such as rodents and bats) can facilitate the spillover of viruses to humans (Trebski et al., 2024). Studies show that alterations in habitat due to climate change affect the ecology of species involved in zoonotic transmission, which can lead to increased interaction rates between hosts and humans, thereby raising the risk of disease emergence. COVID-19 is a pertinent case study highlighting these dynamics. Emerging evidence suggests that human encroachment into wildlife habitats increases the likelihood of zoonotic spillover events, with some studies linking habitat degradation to the rise of zoonotic diseases like COVID-19. The dynamic nature of habitats, influenced by climate variables, underscores the urgency for predictive models to anticipate spatial shifts in disease emergence and outbreaks. For the Nipah virus, environmental changes affecting bat populations, such as extreme weather events and alterations in fruit availability, may lead to increased human-bat interactions. This concern is particularly evident in regions like Southeast Asia, where agricultural practices that favor specific crops can bring bats into closer contact with humans, raising the risk of transmission. Comprehensive analysis indicates that the changing climate impacts the epidemiology of the Nipah virus, necessitating adaptive public health measures to mitigate risks associated with these zoonoses (Hobson, 2025). Rift Valley fever exemplifies the climate-zoonosis nexus, as the disease is closely linked to rainfall patterns that facilitate the breeding of mosquito vectors responsible for transmission. Periods of heavy rainfall increase the likelihood of outbreaks, particularly as climate change contributes to the frequency and intensity of extreme weather events. Changes in land use, such as agriculture and urban development, also play a critical role in shaping the ecological dynamics of Rift Valley fever, further complicating its management. With climate change projected to influence vector habitats and populations, the potential for Rift Valley fever outbreaks is likely to increase, particularly in regions previously unaffected by the disease. Global warming fundamentally impacts disease ecology, particularly regarding zoonoses such as COVID-19, Nipah virus, and Rift Valley fever. The intersection of climate change with ecological dynamics influences pathogen circulation, host interactions, and geographical distributions of vectors, thus requiring robust, interdisciplinary health strategies to safeguard public health as these environmental changes continue to unfold.

Air Pollution and Respiratory Health in Humans and Animals

Air pollution, particularly through the prevalence of urban smog and fine particulate matter (PM2.5), has profound effects on respiratory health in both humans and animals, including companion animals and livestock. The relationship between air quality and respiratory diseases such as asthma and bronchitis has been documented, demonstrating significant public health

implications. Urban smog, primarily a mix of various pollutants including PM2.5, nitrogen dioxide (NO2), and sulfur dioxide (SO2), poses substantial risks to respiratory health. PM2.5, which refers to particles with a diameter of less than 2.5 micrometers, is particularly dangerous because it can penetrate deep into lung tissue and even enter the bloodstream. Research indicates that exposure to elevated levels of PM2.5 is associated with increased incidence of respiratory conditions, including asthma and chronic obstructive pulmonary disease (COPD). A study indicated that short-term increases in air pollution trigger many adverse health events, particularly respiratory-related issues leading to increased emergency department visits. Furthermore, epidemiological studies have linked smog to rises in hospital admissions for respiratory diseases, highlighting a public health crisis that peaks in areas with severe air pollution. In addition to the direct effects on human health, urban smog and poor air quality similarly affect companion animals and livestock. Just as humans experience respiratory issues due to exposure to pollutants in the air, animals too can suffer from conditions like bronchitis and asthma. Companion animals, such as dogs and cats, share environments with humans and can develop similar health issues due to their proximity to air pollution. A study suggests that smog could lead to increases in respiratory issues among animals housed in urban areas, where air quality is significantly compromised. This reflects broader patterns of health impacts where both human and animal respiratory health are adversely affected by the same pollutants. Livestock, such as cattle are also vulnerable to the impacts of air pollution. The stress from air contaminants can impair their respiratory function, leading to decreased productivity and increased susceptibility to infections. The economic implications are significant, as poor air quality can lead to reduced weight gain in cattle and increased veterinary costs due to respiratory diseases. In urban areas, where livestock may be housed in proximity to pollution sources, the health impacts are compounded, creating a cycle of poor health outcomes and economic losses for farmers. The impact of PM2.5 on respiratory health extends beyond immediate symptoms; it also has long-term repercussions. Chronic exposure to poor air quality has been linked to a decline in overall respiratory function in both humans and animals. This decline can manifest as chronic bronchitis, characterized by persistent cough and mucus production, which has been tied to long-term exposure to pollutants. Ongoing studies indicate that elderly populations living in areas with high pollution levels demonstrate an increased prevalence of chronic bronchitis and other respiratory symptoms, supporting the link between poor air quality and respiratory disease. The acute health effects from exposure to smog are often compounded by other socio-economic factors and health disparities. Vulnerable populations, whether human or animal, face heightened risks from poor air quality due to simultaneous health stressors and limited access to healthcare solutions. Comprehensive monitoring and intervention strategies are essential for addressing these interconnected health challenges, as improvements in air quality could lead to significant reductions in both human and animal respiratory disease burdens.

Urban smog and PM2.5 exert considerable adverse effects on the respiratory health of both humans and animals. The shared exposure to environmental pollutants necessitates comprehensive approaches to air quality management that consider the health impacts on all living beings within urban ecosystems. Addressing air pollution not only promotes human health but also contributes to the welfare of companion animals and livestock.

Water Quality and Its Role in Disease Transmission

Water quality is a critical factor in the transmission of various diseases, particularly in environments where contaminants such as pathogens, heavy metals, and antibiotic residues proliferate. The interplay between these elements has significant implications for public health, especially in urban and peri-urban areas where water resources are often compromised by anthropogenic activities. The presence of waterborne pathogens, including bacteria such as E. coli and Giardia, exposes populations to severe health risks. Studies indicate that E. coli contamination in water storage tanks increases risks for communities that rely on these water sources, particularly in regions such as Sulaimaniyah City and Bahir Dar, where fecal contamination occurs at alarming rates (Najmuldeen & Razaq, 2023). Moreover, Giardia, alongside other pathogens, is frequently linked with inadequate water treatment processes. The Protozoan Giardia lamblia is associated with gastrointestinal infections, particularly in areas with compromised water quality. Environmental studies highlight the impact of wastewater discharges on microbial dynamics within water bodies, demonstrating an elevated presence of pathogens in affected ecosystems due to insufficient filtration and disinfection measures. In parallel, heavy metals pose a significant risk to both human health and aquatic ecosystems. Pollutants such as lead, mercury, and cadmium infiltrate water supplies through various channels, including industrial discharges, urban runoff, and agricultural runoff. Research underscores the relationship between heavy metal exposure and adverse health outcomes, including neurological impairments and chronic diseases. Prolonged exposure to cadmium and lead has been linked to detrimental effects, such as kidney damage and developmental delays in children. Water contaminated with heavy metals can disrupt aquatic life, leading to bioaccumulation in food chains and health risks for human consumers, as heavy metals mobilize through biological systems. Antibiotic residues in water systems amplify the problem by fostering antibiotic resistance among microbial populations. Disinfection methods, such as chlorination, while essential for eliminating pathogens, may inadvertently enhance the survival and proliferation of antibiotic-resistant bacteria (ARB). Studies have elucidated mechanisms by which chlorine exposure leads to the uptake of antibiotic resistance genes, subsequently increasing the prevalence of ARB in treated water bodies. This phenomenon complicates disease management and presents a critical public health challenge, as common antibiotics may become less effective against resistant strains of pathogenic bacteria (Najmuldeen & Razaq, 2023). The contamination of water bodies with heavy metals and pathogens creates compounded challenges for public health. For instance, urban runoff following heavy rainfall can mobilize both heavy metals and pathogens, resulting in increased exposure risks during peak water contamination events. Efforts to improve water quality must incorporate the simultaneous treatment of physical, chemical, and biological hazards. Innovative approaches, such as biosorption using agricultural waste materials, have shown promise in mitigating heavy metal contamination. Additionally, the development of paper-based biosensors represents a cutting-edge solution for monitoring water quality, enabling rapid detection of these contaminants. Efficiencies in water treatment can be achieved through the integration of advanced filtration and disinfection technologies, significantly reducing the presence of both E. coli and Giardia in treated waters. Current recommendations include utilizing simple onsite filtration systems to enhance pathogen removal prior to water use in irrigation, thereby limiting exposure to harmful microorganisms. Additionally, targeting disinfection protocols that minimize the selection pressure for antibiotic resistance can bolster antibiotic effectiveness in clinical settings

and reduce the incidence of ARB in the environment. To address the comprehensive challenges of water quality and disease transmission, collaborative efforts involving governmental and non-governmental organizations, as well as local communities, are necessary. Stakeholder engagement in water management initiatives can lead to improved sanitation practices, increased public awareness regarding hygiene, and the implementation of policies that regulate industrial discharges into water bodies. Overall, the multifaceted nature of water quality issues necessitates an interdisciplinary approach that encompasses environmental science, public health, microbiology, and policy-making. Employing robust research to develop practical solutions is paramount in curtailing the transmission of waterborne diseases, protecting vulnerable populations, and ensuring the sustainability of water resources in the face of increasing contamination challenges.

Soil Contamination and Food Chain Bioaccumulation

Soil contamination is a persistent issue with far-reaching implications for food safety and human health. Contaminants such as lead, arsenic, and pesticides can accumulate within the soil and subsequently translocate through the soil-crop-animal-human pathways, leading to bioaccumulation and biomagnification within the food chain. This process not only jeopardizes crop yields and quality but poses significant health risks to humans and animals alike. Lead (Pb) is a widely recognized heavy metal that adversely affects human health, particularly through its entry into the food chain via contaminated soil. Crops may absorb lead from contaminated agricultural soils, particularly where industrial activities have increased soil Pb levels due to atmospheric deposition or contaminated water runoff. Research has shown that lead can accumulate in various plant tissues, raising concerns about food safety of crops grown in Pb-polluted areas. For instance, when crops such as vegetables are cultivated on contaminated soils, the absorbed lead can subsequently enter the human food web, posing risks of chronic toxicity and neurological impairment among consumers (Cioica et al., 2019). Arsenic (As), another critical contaminant, presents similar risks through its potential to bioaccumulate in crops. Studies indicate that plants can uptake arsenic from contaminated soils, especially in regions where groundwater used for irrigation contains arsenic. Varietal differences in crops may influence the extent of accumulation, indicating that some strains of rice, for example, pass higher concentrations of arsenic into the human food chain than others, exacerbating health risks associated with arsenic exposure. Long-term consumption of arsenic-contaminated produce has been linked to various health issues including carcinogenesis, highlighting the need for continued monitoring in agricultural regions with known soil arsenic pollution (Tong et al., 2014). Pesticides, while employed to protect agricultural yields, also contribute to soil contamination and bioaccumulation in crops. Residues from pesticide applications can persist in soil, being absorbed by plants, which can then be ingested by herbivorous animals, thus entering the food chain. Research shows that both organic and inorganic pesticides can accumulate in soil and subsequently transfer to crops. The bioaccumulation of pesticide residues in food crops raises significant concerns regarding human exposure, particularly through the consumption of fruits and vegetables that have not undergone thorough washing or processing. The transport mechanisms of these contaminants illustrate how soil represents a critical reservoir from which pollutants can be mobilized. Factors such as soil pH and organic matter content highly influence the behavior of heavy metals and pesticide residues, affecting their bioavailability and mobility within the soil matrix. Furthermore, anthropogenic practices, including continuous cropping and the application of inorganic fertilizers, can exacerbate

soil pollution and enhance the uptake of contaminants by plants. When animals feed on crops cultivated in contaminated soils, the bioaccumulation process continues. For instance, livestock consuming contaminated forage can exhibit elevated levels of toxic metals within their tissues, which can finally reach humans through the consumption of animal products. This cascading effect underscores the urgency required to address soil contamination issues to mitigate health risks across the food chain. Addressing soil contamination requires a multifaceted approach that includes employing clean agricultural practices, regulating pesticide usage, and promoting phytoremediation techniques to mitigate heavy metal soil pollution (Cioica et al., 2019). By enhancing soil health and monitoring contaminant levels in agricultural products, it is possible to create safer food supply chains and protect both human and ecosystem health from the dangers posed by accumulated contaminants.

Industrial Emissions and Cancer Epidemiology

Industrial emissions are a significant contributor to environmental carcinogenesis, affecting both human and animal health, particularly for populations residing near industrial zones. The International Agency for Research on Cancer (IARC) has identified over 1,000 potential carcinogens, illustrating the extensive challenge posed by industrial emissions. Specific environmental carcinogens such as polycyclic aromatic hydrocarbons (PAHs), heavy metals, and silica dust are prominent in industrial contexts. PAHs, especially benzo(a)pyrene, have been linked to various cancers, including lung and breast carcinoma (Barguilla et al., 2023). Heavy metals like cadmium and arsenic are also notorious for their carcinogenic potential. Arsenic has been confirmed as a human carcinogen associated with skin, bladder, and lung cancers, often found near industrial sites. These substances pose significant threats not only from direct exposure among workers but also environmentally through contamination of air, soil, and water sources. Epidemiological studies have underscored the correlation between cancer incidence and environmental exposure to carcinogens. For example, a study in Ontario, Canada highlighted that solar ultraviolet radiation, radon, and fine particulate matter contributed significantly to cancer burden in exposed populations. Furthermore, exposure to silica dust, a common byproduct of various industries, has been well documented to cause lung neoplasms. These findings underscore the urgent need for regulatory interventions to mitigate exposure risks. Studies on animal models has provided insights into the mechanisms of carcinogenesis induced by industrial pollutants. Studies have shown that chronic exposure to such carcinogens can result in significant mutations and changes at the cellular level, predisposing both animal models and potentially humans to cancer. Experiments have demonstrated that co-exposure to substances, such as arsenic and UV light, can synergistically enhance carcinogenic effects, complicating risk assessments and preventive strategies. In addition to direct chemical exposure, the role of biomagnification and the cumulative toxic effects of environmental carcinogens presents a compelling challenge. This is especially true in ecosystems surrounding industrial zones, where animals can accumulate these toxins, ultimately leading to the bioaccumulation of carcinogens in the food chain. Such dynamics not only threaten the health of wildlife but also present significant risks to human health through the consumption of contaminated food sources. Human health risk assessments near industrial sites often reveal unacceptable levels of exposure to heavy metals and other carcinogenic agents. For example, a study near automobile workshops indicated that exposure levels exceeded the U.S. EPA's acceptable thresholds, highlighting an urgent need for environmental monitoring and

remediation efforts. The integration of novel methodologies such as hybrid machine learning models can markedly improve the prediction of chemical carcinogenicity, providing a framework for prioritizing regulatory actions against the most harmful pollutants. Moreover, recent findings have emphasized the relevance of epigenetic alterations as a mechanism by which environmental carcinogens exert their effects. Chemicals that induce changes in DNA methylation and histone modifications can lead to long-term genomic instability, complicating the landscape of cancer epidemiology. The interplay of genetic predisposition and environmental factors is crucial in understanding the susceptibility of individuals to industrial carcinogens, highlighting the need for personalized approaches in cancer prevention. Children and fetuses represent particularly vulnerable populations concerning environmental carcinogens due to their developing systems. Evidence suggests that prenatal exposure to pollutants can significantly increase cancer risks later in life. Furthermore, chronic exposure to specific carcinogens, such as nickel and aromatic amines, has been shown to lead to lasting health impacts, including the development of cancer stem-like cells. This necessitates immediate action to reduce industrial emissions and protect at-risk populations. The challenge of managing industrial emissions is exacerbated by the complexities of regulatory frameworks and the need for public awareness. Organizations such as the EPA continue to list priority pollutants, but the ongoing presence of industrial carcinogens in the environment reveals gaps in policy implementation and the necessity for more stringent regulations (Dunnick, 2025). Strategies such as community engagement, stricter enforcement of existing laws, and the promotion of safer industrial practices are essential to mitigate risks associated with environmental carcinogens. As the landscape of industrial carcinogens evolves with the expansion of emerging contaminants, continuous research and adaptation of risk assessment methodologies become paramount. With advancements in toxicology and epidemiology, we can better understand the intricate relationships between environmental exposures and cancer incidence, thus informing future public health strategies. Industrial emissions represent a significant public health concern, particularly regarding cancer epidemiology in populations near industrial zones. The identification and understanding of environmental carcinogens coupled with community engagement and regulatory action are crucial steps in minimizing their health impact. Ongoing research, public health initiatives, and a cohesive policy approach are essential to address the multifaceted challenges presented by industrial emissions and their carcinogenic potential.

Microplastics and Nano-pollutants in the Ecosystem

Microplastics and nano-pollutants have emerged as critical environmental contaminants affecting both aquatic and terrestrial ecosystems. Their prevalence and continuous accumulation pose significant risks to organisms due to ingestion, systemic toxicity, and hormonal disruption across various species. Given the alarming rise in plastic waste, particularly in aquatic environments, the implications of these pollutants for environmental health are profound. Plastic particles, particularly microplastics (defined as less than 5 mm in diameter), are ubiquitous in marine and freshwater environments. Recent research indicates that an estimated 14 trillion microplastics enter the marine ecosystem from various sources each month, posing a considerable threat to aquatic organisms. The ingestion of microplastics by aquatic species such as fish and shellfish has been widely documented. Studies show that fish ingest plastic particles primarily by mistaking them for prey, which can lead to physical blockages and internal injuries. They can also bioaccumulate in the food chain, posing risks not only to aquatic life but also to predators,

including humans, who consume contaminated seafood. The systemic toxicity associated with microplastics and nano-pollutants is of particular concern. Evidence suggests that microplastic ingestion can lead to inflammatory responses in marine organisms, such as fish and crustaceans, potentially compromising their immune systems and overall health. Moreover, nano-pollutants like heavy metals and engineered nanoparticles can penetrate cellular membranes, directly affect cellular functioning and lead to various toxicological effects, including oxidative stress and cytotoxicity (Hermosillo-Abundis et al., 2024). For instance, studies indicate that silver nanoparticles can induce acute toxicity in aquatic organisms, disrupting normal physiological processes. Hormonal disruption is another serious consequence of exposure to microplastics and nano-pollutants. Compounds like bisphenol A (BPA), commonly found in plastics, are known endocrine disruptors that can interfere with hormonal signaling in aquatic organisms. Such disruptions can result in altered reproductive behaviors and hormone levels, leading to developmental and reproductive impairments in species such as fish. The effects can ripple through the food web, impacting ecological balance and biodiversity. In terrestrial ecosystems, the effects of microplastics are equally concerning. Microplastics have been found in soils, where they can interact with soil microorganisms and plants, potentially disrupting soil health and pollinator populations. Additionally, nano-pollutants, like engineered nanomaterials from agricultural runoff, can enter soil and water systems, bioaccumulating through plant roots and affecting herbivores and carnivores that consume them. As these pollutants interact within the soil matrix, they may affect nutrient cycling and plant health, ultimately impacting food security. As depicted in *Figure 1*, microplastics and nano-pollutants disperse through atmospheric deposition, water currents, and soil runoff, ultimately impacting biodiversity and ecosystem functioning (Boctor et al., 2025).

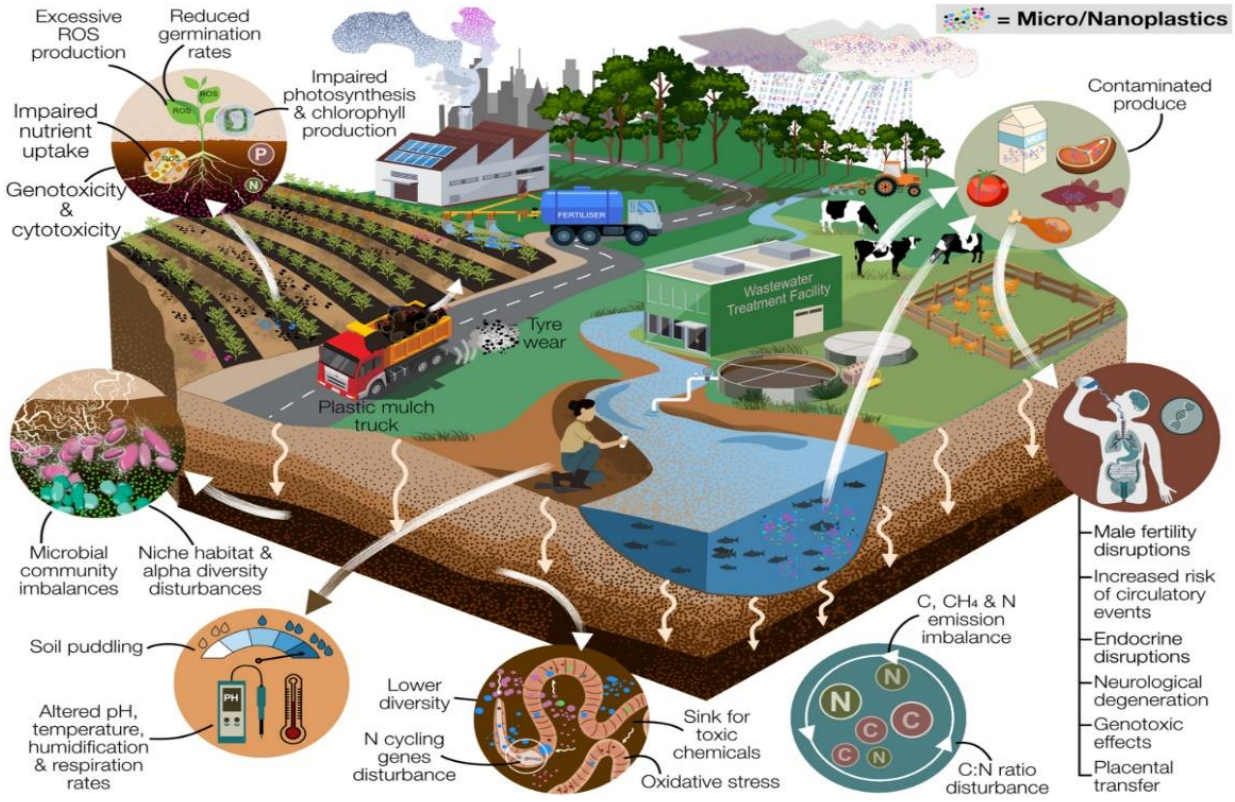


Figure 1. Microplastics and Nano-pollutants: Environmental Distribution and Ecological Implications (Boctor et al., 2025)

The persistence of microplastics in the environment poses another layer of complexity. Unlike organic pollutants that can degrade, microplastics may remain for decades to centuries, leading to chronic exposure for various organisms. Research indicates that microplastics can also serve as vectors for other toxic pollutants, such as persistent organic pollutants (POPs), enhancing their bioavailability to organisms. Hence, the risk of co-contaminants leaching from microplastics raises alarms regarding their ecological toxicity. The response of organisms to microplastics and nano-pollutants varies significantly among species. For instance, aquatic filter feeders may be more susceptible to microplastic ingestion compared to predatory species. Terrestrial species, including earthworms, have also been shown to absorb microplastics, which can affect their reproduction and growth rates. This highlights the need for species-specific research to better understand the pathways and mechanisms through which these contaminants exert their influence on various organisms across the food web. The implications of microplastics and nano-pollutants extend to natural resource management and policy-making. Global initiatives are urgently needed to regulate plastic production and usage, enhance waste management practices, and promote research focused on understanding and mitigating the effects of these compounds in the environment (Hermosillo-Abundis et al., 2024). Policies should aim not only to address the current disposal problem but also to emphasize reducing plastic consumption and supporting the development of biodegradable alternatives. Novel remediation technologies designed to manage microplastic pollution in aquatic habitats are being explored, including bioremediation and photodegradation. Such technologies hold promises for mitigating the impact of these pollutants. However, extensive research is necessary to determine their efficacy and potential drawbacks, as unintended consequences might arise during remediation efforts. The socio-economic aspects of addressing microplastic and nano-pollutants contamination pose another challenge. Communities dependent on fishing and tourism are vulnerable to the impacts of plastic pollution on marine biodiversity and ecosystem services. Greater engagement with local stakeholders is crucial for developing effective strategies to tackle pollution issues while supporting community livelihoods. Microplastics and nano-pollutants present significant challenges to environmental health, with broad implications for aquatic and terrestrial ecosystems. Their ingestion by various organisms leads to systemic toxicity and hormonal disruptions, which can jeopardize ecosystem stability and human health. Addressing this issue requires a multifaceted approach involving policy reform, community engagement, and further scientific research to not only comprehend the full extent of the impact but also to develop effective mitigation strategies.

Pesticide Exposure and Endocrine Disruption

Pesticide exposure is increasingly recognized as a significant risk factor for endocrine disruption, affecting hormone signaling, reproductive health, and fetal development across various species. These effects have been observed in both wildlife and humans, underscoring the pervasive impact of pesticides on endocrinology. Endocrine disruptors (EDCs), such as certain pesticides, can interfere with the endocrine system by mimicking or blocking hormones, thereby disrupting normal hormonal signaling pathways. A prominent example is atrazine, widely known for its ability to feminize male amphibians. Research has demonstrated that exposure to atrazine during critical developmental windows leads to alterations in hormone levels, resulting in de-masculinization and feminization of male gonads across different vertebrate classes, including

amphibians and fish, suggesting strong cross-species effects. Specifically, exposure to atrazine results in changes in testosterone and estrogen levels, which can have profound consequences on reproductive health. In vertebrates, the systemic effects of endocrine-disrupting pesticides can lead to severe reproductive dysfunction. For instance, studies on zebrafish have shown that exposure to pesticides like β-endosulfan adversely affects growth and reproduction, with significant perturbations in vitellogenin production, a key indicator of reproductive health in fish. Similarly, research on amphibian larvae exposed to atrazine highlighted changes in survivorship patterns due to hormonal disruptions that influenced reproductive capabilities. Such findings indicate that the timing and duration of pesticide exposure are critical in determining its detrimental effects, as these substances can disrupt not only reproductive signaling but also developmental pathways at various life stages. Furthermore, the implications of pesticide exposure extend beyond immediate reproductive effects to influence fetal development. Pregnant women exposed to EDCs such as chlorpyrifos have been associated with increased risks of congenital abnormalities and developmental disorders, including cerebral palsy. Animal models have demonstrated that in utero exposure to pesticides can lead to long-term reproductive dysfunction in offspring, echoing findings from environmental health studies that connect maternal pesticide exposure to adverse developmental outcomes. Such exposures disrupt key developmental processes and can alter gene expression related to hormonal regulation, leading to chronic health issues that can persist through generations (Nicolella & Assis, 2022). Gene expression studies have further elucidated the molecular mechanisms by which pesticides exert their effects as EDCs. For instance, observations of the soil invertebrate *Folsomia candida* exposed to pentachlorophenol revealed significant changes in gene expression profiles associated with reproduction and endocrine function, illustrating the genetic repercussions of pesticide exposure. This raises concerns regarding the potential for similar disruptions in other species, including humans, particularly given the conserved nature of many endocrine pathways across vertebrates. Another concerning aspect is the potential for epigenetic modifications induced by pesticide exposure. Evidence suggests that endocrine disruptors can lead to transgenerational effects, where the consequences of exposure are not limited to the individuals directly exposed but can also affect subsequent generations through epigenetic inheritance mechanisms. Such findings imply that even low-level exposures that might not cause immediate harm can have lasting repercussions on reproductive health and developmental success across generations. The intersection of pesticide exposure and endocrine disruption presents a multifaceted challenge, with significant implications for hormone signaling, reproduction, and fetal development across species. The ability of these chemicals to interfere with endocrine signaling pathways raises urgent concerns for wildlife health and human reproductive outcomes. As the consequences of such exposures continue to become clearer, there is an escalating need for comprehensive risk assessments, stringent regulatory measures, and public awareness initiatives to mitigate the widespread effects of endocrine-disrupting pesticides.

Vector Ecology and Environmental Modulators

Vector ecology, particularly concerning mosquitoes and ticks, is intricately linked to environmental changes that determine population dynamics and disease risk. Factors including climate variations, anthropogenic land use changes, and habitat fragmentation significantly influence vector distributions, which in turn affect the transmission dynamics of many vector-borne

diseases. This relationship highlights the importance of understanding how environmental modifications shape the lifecycle and behavior of these vectors, ultimately influencing human and animal health. Environmental driving forces like temperature and precipitation directly affect mosquito and tick populations. Temperature conditions are critical as they impact the development rate of mosquito larvae and adult survival. Research indicates that rising temperatures accelerate the life cycle of multiple mosquito species, including Aedes aegypti, which can enhance the potential for disease transmission by shortening the time to maturity and increasing reproductive rates. Additionally, abundant rainfall creates favorable breeding sites for mosquitoes and affects the distribution of ticks by providing conducive microhabitats for their survival and feeding. For instance, prolonged rain is associated with increased mosquito populations linked to the emergence of diseases such as Rift Valley Fever. Urbanization and land-use changes contribute to shifts in vector ecology, affecting both mosquito and tick habitats. In urban settings, socio-economic disparities can exacerbate mosquito populations in poorer neighborhoods, which often lack adequate waste management systems and water drainage, creating ideal breeding conditions. This urban phenomenon occurs alongside habitat fragmentation caused by land use changes, which can lead to increased interactions between vectors and humans. Such dynamics heighten the risk of vector-borne diseases as mosquitoes become more efficient at finding hosts in densely populated areas. Climate impacts are not only direct but also operate through indirect pathways such as alterations in vegetation and biodiversity. Evidence suggests that higher biodiversity may lower disease risk due to the "dilution effect," where increased host diversity can reduce pathogen transmission (Medeiros-Sousa et al., 2025). Conversely, habitat loss and species extinction can disrupt these ecological balances, potentially leading to a rise in vector populations and related diseases. The relationship between environmental changes and vector-borne diseases is further complicated by the effects of invasive species and pathogen evolution. The introduction of alien vector species into new ecosystems can drive up disease prevalence, as these species may carry pathogens to which native wildlife has no immunity. For example, the spread of Aedes albopictus has been linked to changes in climate and urban environments, leading to increased risks of diseases such as Zika and chikungunya. These changes in vector dynamics are critical for public health, emphasizing the need for comprehensive surveillance and adaptive management strategies. The relationships governing vector ecology are dynamic and are influenced by microclimates resulting from urbanization and land use transformations. Urban microenvironments can impact mosquito behavior, particularly blood-feeding and reproductive patterns. Additionally, factors such as socioeconomic status can modulate these ecological drivers by influencing local insecticide use and the availability of resources for vector control measures. To effectively mitigate the risks posed by vector-borne diseases, integrated management approaches that consider the socio-ecological contexts of vector populations are necessary. Understanding the interplay between environmental changes, vector behavior, and disease dynamics is essential for developing targeted public health strategies that can adapt to ongoing and future changes (Medeiros-Sousa et al., 2025) . Continuous monitoring of environmental variables and vector populations will equip policymakers and health officials with critical data to anticipate outbreaks and manage vector control efficiently. The links between environment-driven changes in mosquito and tick populations and disease risk are multifaceted. By recognizing and managing the ecological and environmental factors that influence vector behavior, control strategies can be optimized to effectively reduce disease transmission, safeguarding both human and animal health against emergent and re-emerging infectious diseases.

Environmental Allergens and Immune Sensitization

The interplay between environmental allergens and immune sensitization remains a critical area of research, particularly concerning the development and exacerbation of asthma and allergic conditions in both humans and domestic animals. Asthma is a complex, chronic respiratory disease characterized by airway inflammation, hyperresponsiveness, and obstruction. It is influenced not only by genetic predisposition but also by the environment, as factors such as pollen, mold, and air pollution significantly contribute to the prevalence and severity of this condition. Addressing these environmental triggers is essential for managing asthma effectively, particularly in at-risk populations such as children and specific geographic areas where pollution levels are high. Pollen from various plant species constitutes a significant allergenic trigger, particularly during specific seasons. In temperate climates, the peak pollen season is often marked by increased asthma exacerbations and respiratory symptoms among sensitized individuals (Sangchan et al., 2024). Environmental conditions influencing pollen dispersal, including temperature and humidity, can exacerbate allergic responses. Furthermore, specific studies have noted that children diagnosed with respiratory allergies show increasing trends of sensitization to aeroallergens such as pollen, indicating a rising burden of respiratory issues (Sangchan et al., 2024). In addition, the seasonal variability of pollen production often correlates with the timing of asthma exacerbations, underscoring the need for awareness and preparedness amongst affected communities (Sangchan et al., 2024). Mold is another potent environmental allergen that can exacerbate asthma and other allergic disorders. Indoor mold growth is often associated with dampness and poor ventilation in homes, leading to significant respiratory issues among inhabitants. Exposure to mold spores can lead to immune sensitization, whereby the immune system overreacts to these otherwise innocuous particles, further aggravating asthma symptoms. Evidence from various studies confirms that children exposed to indoor molds show increased rates of asthma and allergic rhinitis, emphasizing the importance of maintaining dry and well-ventilated living spaces (Sangchan et al., 2024). As such, proactive mold control measures in homes can be critical in mitigating asthma-related health issues, particularly in high-risk populations. Air pollution, specifically from traffic-related sources, industrial emissions, and urbanization, plays a role in increasing asthma prevalence and exacerbation episodes. Pollution from particulate matter (PM2.5), nitrogen dioxide (NO2), and other harmful pollutants has been consistently linked to increased asthma incidence. Pollutants lead to oxidative stress in the airways, which can provoke inflammatory responses that contribute to the pathogenesis of asthma. In densely populated urban environments, children and other vulnerable groups are at increased risk due to higher exposure levels. Emerging research indicates that environmental pollution not only exacerbates asthma symptoms in sensitized individuals but also potentially plays a role in the initial sensitization to common allergens, solidifying the nexus between environmental factors and immune response dynamics. The combined impact of these environmental allergens pollen, mold and air pollution demonstrates how interconnected these factors are in triggering and exacerbating asthma and allergic responses. Children are particularly susceptible, as their immune systems are still developing, and factors such as early-life exposure to these allergens can predispose them to long-term respiratory issues (Sangchan et al., 2024). The International Study of Asthma and Allergies in Childhood (ISAAC) projects that the rising prevalence of asthma may correlate with increased environmental exposures. Understanding the genetic and epigenetic mechanisms that contribute to asthma susceptibility is also vital. Studies

have indicated that certain genetic polymorphisms can influence the immune response to environmental allergens, thereby modulating the likelihood of developing asthma. Moreover, maternal health and exposures during pregnancy can extend the risk factors for childhood asthma, pointing to the importance of a comprehensive approach in tackling asthma, as prevention and intervention strategies must consider environmental, genetic, and maternal health factors. Effective management of asthma necessitates a thorough understanding of these environmental triggers and their relationship with immune sensitization. Interventions aimed at reducing exposure to known allergens, such as implementing air quality control measures in urban planning and promoting better indoor air quality through cleaning and maintenance, can significantly improve health outcomes for those affected by asthma. Furthermore, public health education emphasizing avoidance strategies for at-risk populations during high pollen seasons or poor air quality days is crucial. There is need for gathering more robust data on the interaction between various environmental factors and immune responses cannot be overstated. Continued research into the mechanistic pathways linking allergens to asthma exacerbations will inform the development of targeted therapeutic strategies. In conclusion, the triad of pollen, mold, and pollution presents a substantial threat to respiratory health, driving the need for collaborative efforts across healthcare fields, public policy, and community action to mitigate asthma's impact.

Environmental Toxins and Neurodevelopmental Disorders

The impact of environmental toxins, particularly lead and mercury, on neurodevelopmental disorders in infants and young animals has gained increasing attention due to the critical implications for public health. Both lead and mercury are recognized neurotoxicants whose exposure, particularly during prenatal and early childhood periods, has been associated with a variety of neurodevelopmental deficits including learning disabilities and attention-related disorders. Understanding how these heavy metals interact biologically and their potential to disrupt normal neurodevelopment is fundamental for preventative strategies aimed at protecting vulnerable populations. Lead exposure has been extensively studied in relation to cognitive and behavioral deficits. Epidemiological studies indicate that even low levels of lead in the blood are associated with developmental delays, lowered IQ, and an increased risk of attention-deficit/hyperactivity disorder (ADHD). The neurotoxic effects of lead primarily arise from its ability to interfere with neurotransmitter function, promote excitotoxicity, and ultimately lead to cell death within critical regions of the developing brain. Furthermore, chronic lead exposure has been linked to alterations in dopaminergic signaling pathways, which are crucial for behavior and learning. With decreasing environmental lead levels due to regulatory measures, the focus has shifted to understanding the consequences of historical exposure in children and how to mitigate these risks going forward. Mercury presents similarly dire risks, particularly methylmercury, which is a potent neurotoxin found in certain fish and seafood. Methyl-mercury affects neurodevelopment through mechanisms similar to lead, disrupting neuronal differentiation, increasing oxidative stress, and affecting synaptic plasticity and cognition. Research has demonstrated that prenatal exposure to mercury can lead to deficits in cognitive function and behavioral issues in children, with significant implications for school performance and social interactions. Additionally, studies illustrate that mercury can cause cytoskeletal rearrangement in neurons, further impairing cell function. Interactions between lead and mercury, as well as with other heavy metals, have been shown to exacerbate their

neurotoxic effects. For example, combined exposure to lead and manganese has been documented to potentiate neurotoxicity, contributing to a greater risk of learning disabilities than might be seen with either metal alone (Heng et al., 2022; Abdel-Salam et al., 2018). These findings suggest that a comprehensive understanding of the mixture effects of these metals is crucial, particularly in populations exposed to multiple environmental toxins. As shown in Figure 2, environmental toxins such as heavy metals, pesticides, and air pollutants are associated with multiple neurodevelopmental disorders through mechanisms involving oxidative stress, neuroinflammation, and disrupted neurotransmission (Nabi & Tabassum, 2022).

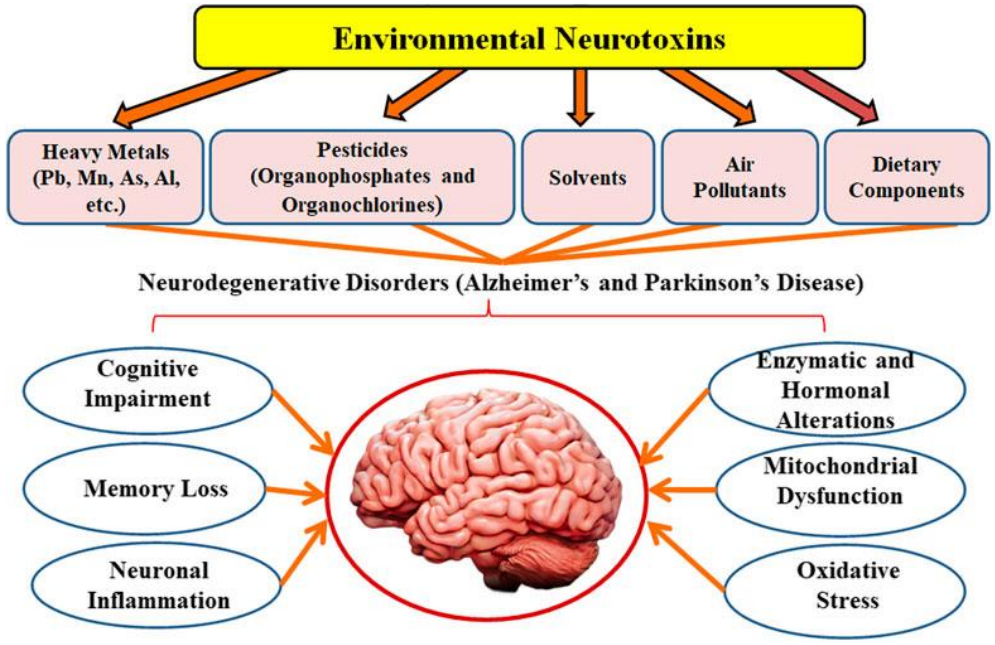


Figure 2. Neurodevelopmental Disorders Associated with Environmental Toxin Exposure (Nabi & Tabassum, 2022)

The role of gene-environment interactions in mediating the risks associated with lead and mercury exposure is also an important area of investigation. Children with specific genetic predispositions may be more vulnerable to the neurotoxic effects of these metals, which emphasizes the need for personalized approaches to prevention and intervention. Moreover, recent studies have highlighted the factors such as socio-economic status and nutritional deficiencies can modulate the severity of the neurotoxic effects related to heavy metal exposure, indicating the importance of holistic public health strategies. Lead and mercury are significant environmental neurotoxicants that contribute to developmental neurotoxicity and learning disabilities in infants and young animals. Their effects are exacerbated by interactions with other environmental toxins and are influenced by genetic and social factors. Continued research into their mechanisms of action and strategies for exposure reduction is essential to mitigate the public health impact associated with these metal toxicants.

Noise Pollution and Neurological Impacts

Chronic exposure to noise pollution is recognized as a significant environmental threat with serious implications for neurological health, particularly regarding stress, memory, and behavior patterns in both humans and animals. This overview synthesizes current research findings to

highlight the neurological impacts associated with persistent environmental noise and its association with stress-related responses and cognitive dysfunction. Exposure to noise pollution has been shown to induce stress responses in both animals and humans. Research indicates that noise can lead to increased levels of cortisol, a biomarker associated with stress, which can adversely affect various physiological processes (Shah et al., 2024). Chronic noise exposure results in a sustained cortisol response that has been linked to anxiety disorders and depressive symptoms. In animal models, stress responses induced by noise pollution are observed as alterations in behavior, including reduced exploratory behaviors and increased anxiety-related behaviors. These findings emphasize the necessity of recognizing noise as a critical factor in the environmental determinants of health, especially in urban settings. Moreover, memory deficits related to chronic noise exposure have been documented across various species. Long-term exposure to loud noise has been found to impair cognitive functions, including memory and learning abilities in rodents. The neurophysiological mechanisms involved may include structural changes in the hippocampus, a brain region essential for memory formation. Specifically, research using animal models has shown that chronic noise exposure can lead to synaptic dysfunctions and neuroinflammatory responses that contribute to cognitive impairments resembling those seen in neurodegenerative diseases. Such alterations raise concerns about the potential for chronic noise exposure to adversely impact long-term neurological health. In terms of behavioral patterns, noise pollution has been linked to significant modifications in animal behavior, particularly concerning predation and avoidance strategies. Studies have observed that chronic noise exposure affects the vigilance behavior of prey species, leading to impaired responses to predators. This change in behavioral patterns can have cascading effects on ecological interactions and population dynamics, as decreased vigilance may increase predation risk and influence reproductive success. Moreover, these changes can affect how animals interact with their environment, potentially leading to further adaptations or maladaptation in urbanized habitats. Evidence also highlights a correlation between urban noise and increased incidents of neurological disorders in human populations. Epidemiological studies have identified associations between chronic noise exposure and increased risks of conditions such as hypertension and stroke, both of which suggest broader neurological implications (Shah et al., 2024). These findings are particularly alarming in densely populated urban areas where traffic and construction noise dominate the auditory landscape. The psychological burden of living in high-noise environments can exacerbate existing health conditions and reduce the quality of life for affected individuals. Chronic exposure to noise pollution has demonstrable effects on stress levels, cognitive functions, and behavioral patterns across species. The potential for noise to induce stress responses and cognitive impairment underscores the urgent need for public health measures to manage noise pollution, particularly in urban environments. Future research should focus on elucidating the underlying mechanisms of noise-induced neurological effects and exploring effective mitigation strategies to protect vulnerable populations from its detrimental impacts.

Light Pollution and Circadian Disruption

Light pollution has become a pervasive issue in modern urban environments, significantly impacting circadian rhythms, melatonin suppression, sleep patterns, and reproductive cycles in both humans and nocturnal wildlife. The increasing use of artificial lighting at night disrupts natural light-dark cycles that are crucial for maintaining optimal health and biological functions.

The suppression of melatonin, a hormone that regulates sleep-wake cycles, is one of the most well-documented effects of light pollution. Studies demonstrate that exposure to artificial light at night (ALAN) can inhibit melatonin secretion, leading to increased difficulty in falling and staying asleep. Continuous exposure to bright light, especially blue wavelengths common in LED lighting, can disrupt the body‘s internal clock, impairing sleep quality and leading to a range of health issues, including metabolic disorders, obesity, and mood disorders. Research shows that short-wavelength light can particularly affect sleep cycle regulation, causing an increase in wakefulness and a decrease in deep sleep stages. In wildlife, particularly nocturnal species, light pollution can disrupt breeding cycles and alter behaviors. Studies have shown that artificial light exposure can desynchronize seasonal reproductive patterns among animals, as they rely on natural light cues to time their breeding activities. For example, in species such as songbirds, artificial light can lead to advanced dawn singing, which may have implications for mate attraction and territory establishment. Such disruptions not only impact individual species but can also have cascading effects on ecosystems, as altered breeding cycles can affect population dynamics and interspecies interactions. The behavioral impacts of light pollution extend beyond reproductive patterns. Animals exposed to artificial light often exhibit changes in foraging behavior, predation risk, and energy expenditure. Nocturnal animals may become more active during periods of light, which can subsequently increase their vulnerability to predators. For instance, a study on blue tits indicated that exposure to light pollution led to changes in activity patterns that could interfere with their natural behaviors, highlighting a potential mechanism through which light pollution affects wildlife fitness. Human health is also heavily impacted by these disruptions. Epidemiological evidence links long-term exposure to light pollution with increased incidences of sleep disorders, anxiety, and even certain cancers, potentially due to the lower levels of melatonin associated with disrupted sleep patterns (Menculini et al., 2024). The consequences of sleep deprivation exacerbated by light pollution can lead to cognitive impairments and reduced overall health and well-being. The cumulative effects of sleep disturbances can also influence societal health, manifesting in reduced productivity and increased healthcare costs. Light pollution poses significant threats to both human health and wildlife. The suppression of melatonin due to artificial lighting disrupts sleep patterns and negatively influences reproductive behaviors across species. Addressing light pollution through better urban planning and lighting design is crucial to mitigate its adverse effects on circadian rhythms and overall biological health.

Urbanization and Habitat Fragmentation

Urbanization leads to significant ecological changes that profoundly impact wildlife and contribute to the emergence and spread of diseases. As urban areas expand, they frequently encroach upon natural habitats, leading to habitat loss and fragmentation, which in turn disrupts ecological dynamics and facilitates increased human-animal contact. This complexity introduces multiple health concerns, particularly as wildlife displacement and novel pathogen emergence become intertwined outcomes of urban sprawl. Studies indicate that urbanization is a key driver of habitat fragmentation worldwide, significantly affecting biodiversity and species interactions. Liu et al. demonstrate that urbanization catalyzes both habitat loss and fragmentation, which can have devastating consequences for wildlife populations and their genetic diversity. Genetic responses to habitat fragmentation manifest in various vertebrate species, suggesting that isolated animal populations face increased vulnerability to health problems. In locations such as the Greater Accra

Metropolitan Area, urban expansion has been linked to severe ecological consequences, emphasizing the urgent need for conservation efforts to mitigate habitat fragmentation. The consequences of habitat fragmentation extend to the health of both wildlife and humans. For instance, fragmented habitats can augment the spread of tick-borne diseases, as shown by Vanacker et al., who explain how shifts in wildlife host assemblages in urban greenspaces can drive higher risks of pathogen spillover (VanAcker et al., 2024). Moreover, low biodiversity due to urban habitat fragmentation can exacerbate health risks, as exemplified in the findings of Hassell et al., where urbanized environments promote the sharing of pathogens between wildlife and humans. This dynamic amplifies concerns as urban megafauna often thrive in these fragmented settings, potentially harboring zoonotic pathogens of emergent significance. The case of urban heat islands and altered ecological conditions further illustrates how urbanization affects disease ecology. Urban areas with increased temperatures and modified landscapes provide suitable conditions for certain pathogens to thrive, as indicated by related research on disease dynamics in urban environments. Additionally, recent studies on urban ecological corridors highlight how maintaining connectivity in fragmented landscapes can support biodiversity and management of wildlife health, thus mitigating risks of disease spread. Simultaneously, urbanization poses direct threats to species well-being. Studies reveal that urban conditions can lead to increased stress and health issues among wildlife, as evidenced by endocrine disruptions observed in birds and other species that fail to adapt to urban environments. The presence of invasive species, often favored in fragmented habitats, can also lead to additional health challenges for native wildlife through competition and disease introduction. The intricate relationship between urbanization, habitat fragmentation, and health ecology underscores a pressing need for integrated conservation strategies. Such approaches should focus on maintaining ecological connectivity to support wildlife resilience, monitoring zoonotic disease dynamics, and enhancing public health outcomes through sustainable urban planning.

Deforestation and Its Impact on Wildlife and Public Health

Deforestation significantly impacts biodiversity, wildlife health, and public health outcomes through various mechanisms, including habitat loss and the resultant ecological imbalances. These shifts often precipitate an increase in zoonotic diseases, i.e., pathogens that spill over from animals to humans, thereby posing serious health risks. A critical association has been established between deforestation and the prevalence of zoonotic diseases, primarily stemming from wildlife habitat alteration. For instance, deforestation can increase the density of long-tailed macaques, leading to crowding in remaining forest patches. This crowding can facilitate the transmission of pathogens such as Plasmodium knowlesi, which is responsible for malaria in humans. Similarly, deforestation-induced changes in vegetation promote the proliferation of vector species responsible for diseases such as scrub typhus, highlighting the relationship between altered landscapes and increased vector-borne disease risks. Habitat fragmentation compels wildlife to move into urban or agricultural areas, intensifying human-wildlife interactions. Areas with diminished biodiversity following habitat loss are more prone to higher incidences of infectious diseases. This idea is further substantiated by research indicating that biodiversity loss can disrupt ecological balance, leading to an increase in pathogen reservoirs and vectors in altered environments (Tajudeen et al., 2022). Maintaining biodiversity can mitigate the risk of infectious disease emergence by limiting the pathways through which pathogens can spill over into human populations. Furthermore,

declines in large wildlife populations can elevate disease prevalence among rodents, which often act as reservoirs for zoonotic diseases. This relationship indicates that as larger species disappear from ecosystems, the resulting ecological imbalance may favor the survival and proliferation of smaller wildlife, increasing the risk of disease spillover to humans. This can lead to increased transmission dynamics of various pathogens, posing heightened threats to public health. The implications of these dynamics elucidate the vital intersection of wildlife health, biodiversity, and human health within the One Health framework. As human activities resulting in habitat degradation escalate, they inadvertently increase the likelihood of interactions between humans and wildlife that harbor pathogens. The resultant health risks underscore the necessity for integrated conservation strategies that address both environmental and public health concerns simultaneously. The relationship between deforestation, biodiversity loss, and public health is multifaceted and critical. Deforestation exacerbates ecological imbalances that not only threaten wildlife and biodiversity but also heighten the risks of zoonotic diseases emerging. It is essential that conservation efforts prioritize habitat protection and restoration to mitigate these impacts and promote a healthier interface between humans and wildlife.

Ocean Acidification and Marine Food Security

Ocean acidification (OA), driven primarily by increased carbon dioxide emissions, is becoming a prominent threat to marine ecosystems, particularly impacting fisheries and food security. The shifts in ocean chemistry affect the physiological aspects of marine organisms and have cascading effects on nutrient profiles, which can influence human and animal diets. One of the most immediate impacts of ocean acidification is observed within marine fisheries. As OA progresses, the calcification processes of marine organisms, particularly shellfish and coral reefs, are adversely affected. Decreasing pH levels have been shown to lead to reduced growth rates in shellfish, which are critical to fisheries and human food sources. Furthermore, effective fisheries management is necessary to counteract the negative effects of climate change and ocean acidification, as these changes can alter community structures in fish populations and potentially reduce overall fish stocks available for capture. Loss of biodiversity due to OA can exacerbate food security issues, particularly in communities that rely heavily on marine proteins for their diets. The nutritional quality of fish and shellfish is also at risk from ocean acidification. Elevated CO2 can alter the lipid profiles of fish and may impact their nutritional value, particularly affecting species that traditionally provide essential omega-3 fatty acids (Queirolo & Sateler, 2023). Changes in their diets and metabolic responses to acidification can lead to altered levels of these important nutrients. Consequently, the health outcomes for both humans and animals consuming these marine resources may be significantly affected. Moreover, the socioeconomic implications for fisheries are profound. Effective fisheries management combined with adaptive measures to address climate change impacts, including ocean acidification, is crucial for sustaining fisheries and the livelihoods of coastal communities. Failure to mitigate the effects of OA could lead to reduced fishing revenues, food insecurity, and increased reliance on alternative, potentially less nutritious sources of food, further jeopardizing the health and nutritional profiles of diets on a global scale. Ocean acidification poses critical challenges not only for marine biodiversity but also for global food security. The shifts in nutrient profiles of marine life directly affect human diets, highlighting the need for robust management strategies that can adapt to changing ocean conditions. Stakeholders

must prioritize initiatives that address both environmental conservation and community resilience to ensure the sustainability and nutritional adequacy of marine food resources.

Waste Management Practices and Pathogen Proliferation

Improper waste management practices are a significant contributor to public health crises, leading to widespread outbreaks of diseases due to pathogen proliferation, rodent and insect infestations, as well as soil contamination. A multitude of studies highlights the dire consequences of inadequate waste management, revealing a complex interplay between environmental health and community well-being. One of the key issues associated with improper waste disposal is the proliferation of disease vectors such as rodents and insects, which thrive in unsanitary environments. As inadequate solid waste management in urban areas creates breeding grounds for vectors like mosquitoes and rodents, heightening the risk of disease outbreaks such as malaria and dengue fever. Similarly, poor domestic waste disposal practices in urban settings specifically create conditions conducive to the proliferation of disease-carrying insects, which can severely threaten public health. The link between waste management practices and soil contamination is critical as well. According to Omang et al., inadequate waste disposal can lead to both soil and water pollution, facilitating the transmission of infectious diseases, including cholera, via contaminated runoff. This illustrates how neglected waste can permeate ecosystems, leading to broader ecological consequences that affect both human and animal health. The environmental degradation resulting from poor waste management contributes to long-term health risks as pathogens entrenched in contaminated environments find their way into the human food chain. Furthermore, studies indicate that the public's lack of awareness and understanding of proper waste management protocols exacerbates these health issues. Despite positive attitudes towards waste management, there remains a disconnect between perception and practice among communities, leading to inefficient waste disposal methods that pose significant health risks. This gap underscores the critical need for improved educational initiatives surrounding waste management practices. The effects of such management failures extend beyond immediate health tragedies, affecting community morale and productivity. In the context of healthcare waste management, poor management practices not only pose health hazards but can also create an environment for the transmission of infectious diseases stemming from improperly disposed medical waste. This is particularly concerning as public health systems struggle to cope with the compounded threats of waste-related diseases. The relationship between waste management practices, pathogen proliferation, and public health is well-established. Improper disposal practices contribute to outbreaks of diseases through mechanisms such as vector proliferation and soil contamination. To mitigate these risks, comprehensive management strategies should focus on public education and adherence to waste management regulations, aiming to create healthier urban environments.

Antimicrobial Resistance in Agricultural and Urban sites

Antimicrobial resistance (AMR) in agricultural and urban sites is a growing global concern primarily driven by the overuse and misuse of antibiotics in both human and veterinary medicine. This phenomenon not only affects the efficacy of treatments but also facilitates the environmental spread of resistant pathogens through various pathways, significantly impacting public health. In

agricultural sites, the routine administration of antibiotics in livestock for growth promotion and disease prevention has been extensively documented. The intensified use of antimicrobials in food animals substantially contributes to AMR, as these practices create selective pressure that favors the survival of resistant bacteria. Agricultural sources of AMR pose significant risks to human health by entering the food chain and exposing consumers to resistant pathogens. The environmental consequences of this overuse are particularly alarming; research indicates that antibiotics from veterinary practices can leach into soil and water systems, promoting the proliferation of resistant strains that present direct threats to human and animal health. Moreover, the interconnectedness of urban and agricultural environments deepens the AMR crisis. In urban settings, individuals may come into contact with resistant bacteria through contaminated water and food systems. The antibiotic residues from agricultural runoff can reach drinking water supplies, fostering environments enabling the transmission of resistant bacteria to humans. Additionally, Nwobodo et al. highlight that direct exposure to contaminated food products contributes to the growing public health threat of AMR. The accumulation of antimicrobial agents in various environments underscores the importance of addressing AMR through a One Health approach, which recognizes the interconnectedness of human, animal, and environmental health (Cella et al., 2023).

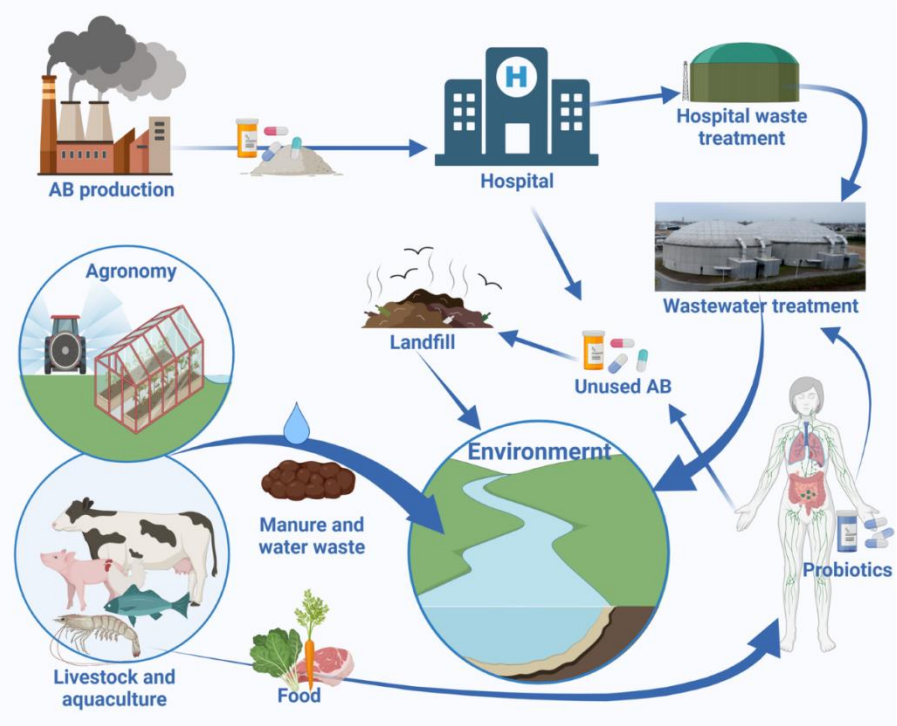


Figure 3. Emergence of Antimicrobial Resistance in Agricultural and Urban Landscapes (Stanley et al., 2022)

These factors, efforts to mitigate AMR must prioritize changes in both human and animal antibiotic use practices. Abbo et al. note that addressing overuse in hospitals and clinics through improved stewardship efforts can significantly impact the rates of AMR development in the community. Awareness campaigns targeting healthcare professionals and the general public can help promote responsible antibiotic use and reduce unnecessary prescriptions. Furthermore, alternatives to traditional antibiotic use in agriculture, such as probiotics and herbal supplements, are being

explored as strategies to maintain animal health without contributing to AMR. Research indicates that employing probiotics, for example, can provide health benefits to livestock while minimizing antibiotic use. The reduction of antibiotic use in livestock, coupled with stringent regulatory frameworks, is crucial for conserving the effectiveness of antibiotics and ensuring food safety. The pervasive overuse of antibiotics in agricultural and urban environments is a critical driver of antimicrobial resistance. As illustrated in *Figure 3*, agricultural practices involving excessive antibiotic use and urban wastewater discharge serve as major reservoirs and transmission routes for antimicrobial resistance, facilitating its spread across environmental and human health interfaces (Stanley et al., 2022). The spread of resistant pathogens poses significant risks to public health through environmental contamination and food systems. A multifaceted approach that includes improved antibiotic stewardship, public education, and the development of alternative treatments is essential for curbing the AMR crisis and safeguarding the effectiveness of antimicrobials for future generations.

Socioeconomic Disparities in Environmental Health Burdens

Socioeconomic disparities significantly shape environmental health burdens, leading to pronounced environmental injustice across rural versus urban settings and among different income classes. Health outcomes are influenced by a combination of environmental exposures, access to health services, and occupational hazards. Urban areas often face elevated environmental health risks due to industrial activities and higher population density. Residential segregation in U.S. metropolitan areas increases exposure to ambient air toxins, disproportionately affecting lower-income and minority groups. This highlights how urban locations, particularly those with significant low-income populations, encounter compounded health risks stemming from systemic inequities that maintain social disadvantage through environmental pollution. In contrast, rural populations experience unique challenges, particularly regarding access to health care services and exposure to agricultural pollutants. A cumulative impacts assessment tool was developed that illustrates how rural communities often endure poor environmental conditions without the necessary health resources to mitigate risks. This disparity in access contributes to broader health inequities, with rural inhabitants facing higher rates of occupational exposures linked to agricultural practices, which may not adhere to strict health and safety regulations. The intersection of socioeconomic status (SES) and environmental health is underscored by findings from health disparities in the U.S. are strongly correlated with social and economic inequalities, indicating that lower SES populations face higher environmental risks and poorer health outcomes. This reinforces the argument that disparities are not merely a product of individual choices but are deeply rooted in systemic inequities that privilege certain groups over others. Furthermore, socioeconomic factors, alongside contextual variables such as race and living conditions, profoundly impact health outcomes. Individuals in lower SES brackets often reside in neighborhoods with substandard living conditions, increasing their vulnerability to adverse health effects from environmental hazards. These findings stress the importance of addressing the social determinants of health by focusing on improving the living environments of disadvantaged populations. The implications of socioeconomic disparities extend to occupational exposures as well. Workers in low-income jobs, often characterized by hazardous conditions, encounter higher risks of occupational health issues. Populations display higher rates of exposure to toxic substances, contributing to a greater burden of disease and lower quality of life compared to their

higher-income counterparts. Effective policies must target these disparities, ensuring equitable access to protective measures and healthcare resources to mitigate occupational health risks. Socioeconomic disparities significantly shape environmental health outcomes, revealing stark contrasts between urban and rural settings, as well as among different income levels. Environmental injustice persists as a critical issue, demanding comprehensive strategies that integrate health equity into environmental policy. Addressing these disparities requires not only improved access to healthcare services but also systemic reforms aimed at reducing environmental hazards in disadvantaged communities.

Integrative One Health Approach to Environmental Health Threats

The One Health approach presents a holistic framework that integrates the disciplines of human health, animal health, and environmental health, recognizing their interdependencies and shared threats. The acknowledgment of this interconnectedness is especially critical in the context of environmental health threats, which can exacerbate issues of zoonoses, food security, and public health crises. The literature emphasizes the necessity for enhanced environmental representation within One Health implementations. Barrett and Bouley argue for greater collaboration with ecohealth practitioners, stressing that environmental drivers are often overlooked in favor of human and animal health concerns. Furthermore, the advancement of One Health is tied to addressing the environmental aspects that influence health outcomes. Intersectoral coordination can effectively address global health factors, including environmental determinants that influence infectious diseases. Such coordination is essential given that climate change is one of the most pressing challenges affecting health worldwide. The repercussions of climate-related changes on health and disease dynamics underscore the need for One Health initiatives to incorporate robust environmental science perspectives. The call for a One Health integration also resonates in discussions on antimicrobial resistance, as it highlights the need for comprehensive strategies that encompass human, livestock, and environmental health. These approaches aim to mitigate the spread of resistance mechanisms across sectors, thereby enhancing overall public health outcomes. The environmental factors contributing to global health crises are underscored frames climate and environmental change as "threat multipliers" that exacerbate the spread of diseases. He stresses that tackling these intertwined issues requires a fundamental shift in how health policies are designed and implemented acting on the collective knowledge that intersectoral cooperation can yield better health outcomes for all. Moreover, the role of education cannot be overstated. Educational programs incorporating a One Health perspective can significantly address sustainability challenges and improve interdisciplinary collaboration among health practitioners. By integrating environmental health literacy into curricula, future health professionals can be better prepared to manage the complexities of current and emerging health threats. The synthesis of these references demonstrates that an integrative One Health approach is vital in addressing environmental health threats effectively. By fostering collaborations across human, animal, and environmental health disciplines, and by placing significant emphasis on education and environmental literacy, stakeholders can develop more robust responses to the multifaceted health challenges faced globally.

References

Barguilla, I., Maguer-Satta, V., Guyot, B., Pastor, S., Marcos, R., & Hernández, A. (2023). In vitro approaches to determine the potential carcinogenic risk of environmental pollutants. International Journal of Molecular Sciences, 24(9), 7851. https://doi.org/10.3390/ijms24097851

Boctor, J., Hoyle, F.C., Farag, M.A. *et al.* Microplastics and nanoplastics: fate, transport, and governance from agricultural soil to food webs and humans. *Environ Sci Eur* **37**, 68 (2025). https://doi.org/10.1186/s12302-025-01104-x

Cella, E., Giovanetti, M., Benedetti, F., Scarpa, F., Johnston, C., Borsetti, A., … & Ciccozzi, M. (2023). Joining forces against antibiotic resistance: the one health solution. Pathogens, 12(9), 1074. https://doi.org/10.3390/pathogens12091074

Cioica, N., Tudora, C., Iuga, D., Deák, G., Matei, M., Nagy, E., … & György, Z. (2019). A review on phytoremediation as an ecological method for in situ clean up of heavy metals contaminated soils. E3s Web of Conferences, 112, 03024. https://doi.org/10.1051/e3sconf/201911203024

Dunnick, J. (2025). A review of the most frequent compounds, metals, and compound and metal mixtures found at u.s. superfund sites and their carcinogenic potential. Chemical Research in Toxicology, 38(6), 963-974. https://doi.org/10.1021/acs.chemrestox.4c00506

Hermosillo-Abundis, C., Ángulo-Molina, A., & Méndez-Rojas, M. (2024). Erythrocyte vulnerability to airborne nanopollutants. Toxics, 12(1), 92. https://doi.org/10.3390/toxics12010092

Hobson, S. (2025). Climate-sensitive zoonotic diseases transmissible by companion animals: a scoping review protocol. Plos One, 20(6), e0325568. https://doi.org/10.1371/journal.pone.0325568

Medeiros-Sousa, A., Oliveira-Christe, R., Ceretti-Júnior, W., Barrio-Nuevo, K., Evangelista, E., Wilk-da-Silva, R., … & Marrelli, M. (2025). The interplay of key abiotic factors with the occurrence and abundance of mosquito larvae in bromeliads.. https://doi.org/10.21203/rs.3.rs-6184497/v1

Menculini, G., Cirimbilli, F., Raspa, V., Scopetta, F., Cinesi, G., Chieppa, A., … & Tortorella, A. (2024). Insights into the effect of light pollution on mental health: focus on affective disorders a narrative review. Brain Sciences, 14(8), 802. https://doi.org/10.3390/brainsci14080802

Nabi M and Tabassum N (2022) Role of Environmental Toxicants on Neurodegenerative Disorders. *Front.Toxicol.* 4:837579. doi: 10.3389/ftox.2022.837579

Najmuldeen, H. and Razaq, C. (2023). Evaluation the impact of chlorination on development of chlorine and antibiotic resistant bacterial isolates in public swimming pools.. https://doi.org/10.21203/rs.3.rs-2628728/v1

Natterson-Horowitz, B., Boddy, A., & Zimmerman, D. (2022). Female health across the tree of life: insights at the intersection of women's health, one health and planetary health. Pnas Nexus, 1(2). https://doi.org/10.1093/pnasnexus/pgac044

Nicolella, H. and Assis, S. (2022). Epigenetic inheritance: intergenerational effects of pesticides and other endocrine disruptors on cancer development. International Journal of Molecular Sciences, 23(9), 4671. https://doi.org/10.3390/ijms23094671

Queirolo, D. and Sateler, J. (2023). Factors that affect the mean size of chilean hake (merluccius gayi gayi) caught with gillnets in 2001-2018 in central chile. Latin American Journal of Aquatic Research, 51(1), 67-78. https://doi.org/10.3856/vol51-issue1-fulltext-2904

Sanders, B. and Davis, M. (2023). Effects of climate change and air pollution on perinatal health. Journal of Midwifery & Women S Health, 68(3), 383-390. https://doi.org/10.1111/jmwh.13522

Sangchan, T., Koosakulchai, V., Sangsupawanich, P., Srisuk, B., & Yuenyongviwat, A. (2024). Trends of aeroallergen sensitization among children with respiratory allergy in southern thailand. Asia Pacific Allergy, 14(3), 103-107. https://doi.org/10.5415/apallergy.0000000000000142

Shah, B., Raj, R., Kaur, P., Karim, A., Bansari, R., Mehmoodi, A., … & Malik, J. (2024). Association of transportation noise with cardiovascular diseases. Clinical Cardiology, 47(5). https://doi.org/10.1002/clc.24275

Stanley, D., Batacan, R. & Bajagai, Y.S. Rapid growth of antimicrobial resistance: the role of agriculture in the problem and the solutions. *Appl Microbiol Biotechnol* **106**, 6953–6962 (2022). https://doi.org/10.1007/s00253-022-12193-6

Tajudeen, Y., Oladunjoye, I., Bajinka, O., & Oladipo, H. (2022). Zoonotic spillover in an era of rapid deforestation of tropical areas and unprecedented wildlife trafficking: into the wild. Challenges, 13(2), 41. https://doi.org/10.3390/challe13020041

Trebski, A., Gourlay, L., Gibb, R., Imirzian, N., & Redding, D. (2024). Sensitivity to climate change is widespread across zoonotic diseases.. https://doi.org/10.1101/2024.11.18.24317483

VanAcker, M., Hofmeester, T., Zhang-Sun, J., Goethert, H., & Diuk-Wasser, M. (2024). Urban greenspace connectivity drive shifts in host assemblages and tick-borne pathogen infection.. https://doi.org/10.1101/2024.11.12.623035

About The Authors

Muhammad Adnan Ul Hassan is an accomplished academic and researcher with a strong focus on complex system modeling and simulation. He earned his Ph.D. in Information and Communication Engineering from the prestigious North University of China. With over eight years of teaching experience as a Lecturer in Mathematics, Dr. Hassan has developed recognized expertise in mathematical biology, system dynamics, mathematical modeling, brucellosis transmission and control analysis, and environmental impact assessment. His research further extends to the application of mathematics in engineering and communication technologies. Adopting a distinctly interdisciplinary approach, his work bridges rigorous theoretical mathematics with practical, real-world applications, addressing pressing challenges in science, technology, and public health.

Email: malikadnanqaic@gmail.com **ORCID:** 0009-0000-9386-3953.

Khawar Ali is currently pursuing his Ph.D. at North University of China, following his M.S. in Molecular Biology. He has previously served as a Research Associate at the Virtual University of Pakistan and Cholistan University of Veterinary and Animal Sciences, Bahawalpur, where he gained extensive experience in Molecular Biology research and laboratory techniques. Additionally, he worked as a Molecular Biologist at IBN-E-SINA Clinical Laboratory & Research Center in Lahore, contributing to clinical diagnostics and research operations. His research interests include Oncology, Molecular Biology, Genomics, and Biotechnology, with a particular focus on Molecular Diagnostics and Cancer Biology. Through his academic and professional endeavors.

Email: khawarali1202@gmail.com **ORCID:** 0000-0002-0565-4535.

Prof. Dr. Mehmet OZASLAN received his PhD in 1995 Institute of Natural Sciences at Cukurova University, Turkiye. He is a Professor in Molecular Biology and Genetics. His research interests are included Cancer Genetics, Molecular Virology, Molecular Genetics, Microbiology, and Genetic mutations etc. He has published more than 200 research articles in national and international well reputed journals. He also has written many book chapters as well as edited books. He is an editor and editor in chief of many well reputed national and international journals.

E-mail: ozaslanmd@gantep.edu.tr, ORCID: 0000 0001 9380 4902

Similarity Index

The similarity index obtained from the plagiarism software for this book chapter is 3%.

To Cite This Chapter:

Hassan M et al., (2025). Impact of Environmental Factors on Human and Animal Health. In Current Studies in Life Sciences 2025 (pp.54-80). ISRES Publishing.

Unlocking AI's Potential in Food Safety and Public Health

Isha ARSHAD
Khadija ZAFAR
Mariam ZAFAR
Tasawar ABBAS
Sundas ASHRAF
Muhammad SAFDAR

Introduction

Global foodborne disease represents one of the significant public health concerns worldwide, with the World Health Organization (WHO) reporting that annually, around 600 million people suffer from illnesses caused by unsafe food, which worldwide results in nearly 420,000 deaths. They also set an economic impact, with foodborne diseases costing losses of more than $110 billion due to productivity loss and medical expenses (Scharff, 2012). The numbers stress the urgent need for a sound food safety system in terms of public health and consumer confidence. Ensuring food hygiene in contemporary food production systems faces increasing threats since raw materials and final products are obtained almost worldwide, making diagnosing and controlling microbial contamination more difficult. Food safety methods mainly rely on visual inspections and laboratory analysis after an incident. They are very slow in preventing large-scale contamination. At that point, the contamination may have already spread to other food systems, posing considerable risks to public health. Artificial Intelligence (AI) has emerged as a transformative technology that addresses these challenges. AI-powered food safety systems enable real-time tracking, predictive analytics, and the integration of diverse datasets, including environmental conditions, processing methods, and microbial genomics (Dhal & Kar, 2025). By shifting from reactive to preventive approaches, AI offers the ability to predict and address contamination risks before they impact consumers.

Contaminated food may expose a person to bacteria, viruses, parasites, and fungal toxins and cause severe health and economic consequences. The major *E. coli* outbreak in the United States in 2006, traced to spinach, cost the spinach industry over $350 million while disgusting many individuals (Calvin et al., 2009). Such occurrences emphasize that traditional food safety systems, which are mainly based on examinations and sampling for food safety, are not able to guarantee the protection against food contamination because these systems are slow, resource-intensive, and lack of continuous monitoring, which allows, at times, some links of the supply chain to become susceptible to contaminants. The emergence of AI in food safety, with practical risk management in mind, reinvented how integrated AI could help manage contamination. For instance, real-time data is analyzed by AI systems to anticipate possible contaminations and autonomously monitor environmental conditions, such as atmospheric parameters within food processing areas. In this kind of case, an AI could, for example, identify microbial growth conditions and temperature or humidity changes and take immediate corrective actions. It also leverages historical outbreak data, which is otherwise too difficult for traditional methods because

of their complexity, to model trends in outbreak occurrences and lead to increased preventive measures and early interventions (Adegoke et al., 2024).

This chapter explains how AI can enhance food safety by transforming global food supply chains. AI detects issues such as temperature changes during transportation by gathering real-time data from farms, processing plants, and retail outlets, and automatically stops distribution to prevent unsafe products. As AI technologies advance, the real-time prediction, detection, and prevention of contamination will become more precise, moving away from being solely reactive and toward a more proactive approach in food safety. If this change takes place, it would mitigate foodborne illnesses, thus enhancing public health and potentially improving food security around the globe.

Microbial Threats

Modern food supply chains allow higher chances for microbial contamination. Bacteria, viruses, and fungi can pose global food safety threats as raw materials and processed foods flow through various stages of production and distribution, often across borders. This work will focus on the most common and dangerous pathogens, how these enter the food supply chain, and their impact on public health and the economy.

1)Common Microbial Contaminants and Their Public Health Effects: Common microbial contaminants that cause significant foodborne illnesses across the globe include *Salmonella, E. coli O157, Listeria monocytogenes*, and *norovirus* (Lee & Yoon, 2021). They vary according to their sources of contamination and transmission routes, as well as public health effects. At the same time, *Salmonella* and *E. coli O157* are two agents that contribute to a large number of hospitalization and deaths, especially in vulnerable groups such as the elderly and young children. *Listeria monocytogenes* poses a unique risk because of its ability to grow at relatively low temperatures, and is particularly serious concerning pregnant women and newborns suffering from conditions such as miscarriage and neonatal infections. Though less severe in individual illness, norovirus leads to swift and thorough outbreaks that burden public health systems. Table 1 summarizes these pathogens regarding sources, transmission routes, symptoms, and public health outcomes.

Table 1. Key Foodborne Pathogens and Their Impact

Pathogen	Common Sources	Transmission Routes	Symptoms and Health Impact	Control Measures	References
Salmonella	Poultry, eggs, and contaminated produce	Cross-contamination, undercooked meats	Diarrhea, fever, abdominal cramps	Proper cooking, sanitation, and hygiene practices	(Mkangara, 2023)

E. coli O157	Undercooked beef, fresh produce	Contaminated food, undercooked meat	Bloody diarrhoea, kidney failure in severe cases	Thorough cooking, pasteurization, and food safety	(Abebe et al., 2023)
Listeria monocytogenes	Deli meats, dairy, and refrigerated foods	Growth at low temperatures, cross-contamination	Meningitis, miscarriage, neonatal infections	Refrigeration control, hygiene, and proper storage	(Osek et al., 2022)
Norovirus	Contaminated food, person-to-person	Person-to-person, contaminated food or water	Vomiting, diarrhea, and stomach pain	Hand hygiene, sanitation, and proper food handling	(Carlson et al., 2024)

2) Transmission Pathways in the Food Supply Chain: Microbial hazards can be introduced at any point along the food supply chain, from production through retail, each presenting its unique risks for disease spread. At the farming level, entry may be through contaminated water, soil, or animal waste, leading to outbreaks such as *E. coli* from irrigation or *Salmonella* from poorly practiced meat handling (Gartley et al., 2022). Contact occurs between raw and ready-to-eat foods at many processing stages and can lead to opportunities for cross-contamination if hygiene practices are insufficient. Temperature control breaches can encourage bacterial growth, thus increasing contamination during transport between multiple handling points. At the retail and household level, mishandling, such as hygienically unfit food displays, undercooking, or unsanitary practices, leads to misadventure and foodborne illnesses.

3) Economic and Public Health Impact.: Microbial contamination goes beyond these superficial public health effects and economic consequences. Foodborne outbreaks may lead to expensive product recalls and lawsuits, ruining consumer confidence in a given food entity. The United States experienced one of its largest recalls in history, worth over one billion dollars in 2008, when consumers were affected by an outbreak of *Salmonella* linked to peanut butter (Dhakal et al., 2024). According to World Bank estimates, unsafe food incurs an annual cost of $110 billion to poor and middle-income countries for healthcare and lost productivity. In terms of global facts, foodborne disease costs billions of dollars (Grace, 2023).

AI-Driven Risk Assessment in Food Safety

The food safety landscape is changing radically with AI enhancing risk assessment and compliance along the supply chain. Predictive analytics and real-time monitoring act on potential hazards before they become true threats. AI joins risk assessment, governance, and compliance into one smooth, efficient interface system, as shown in Figure 1.

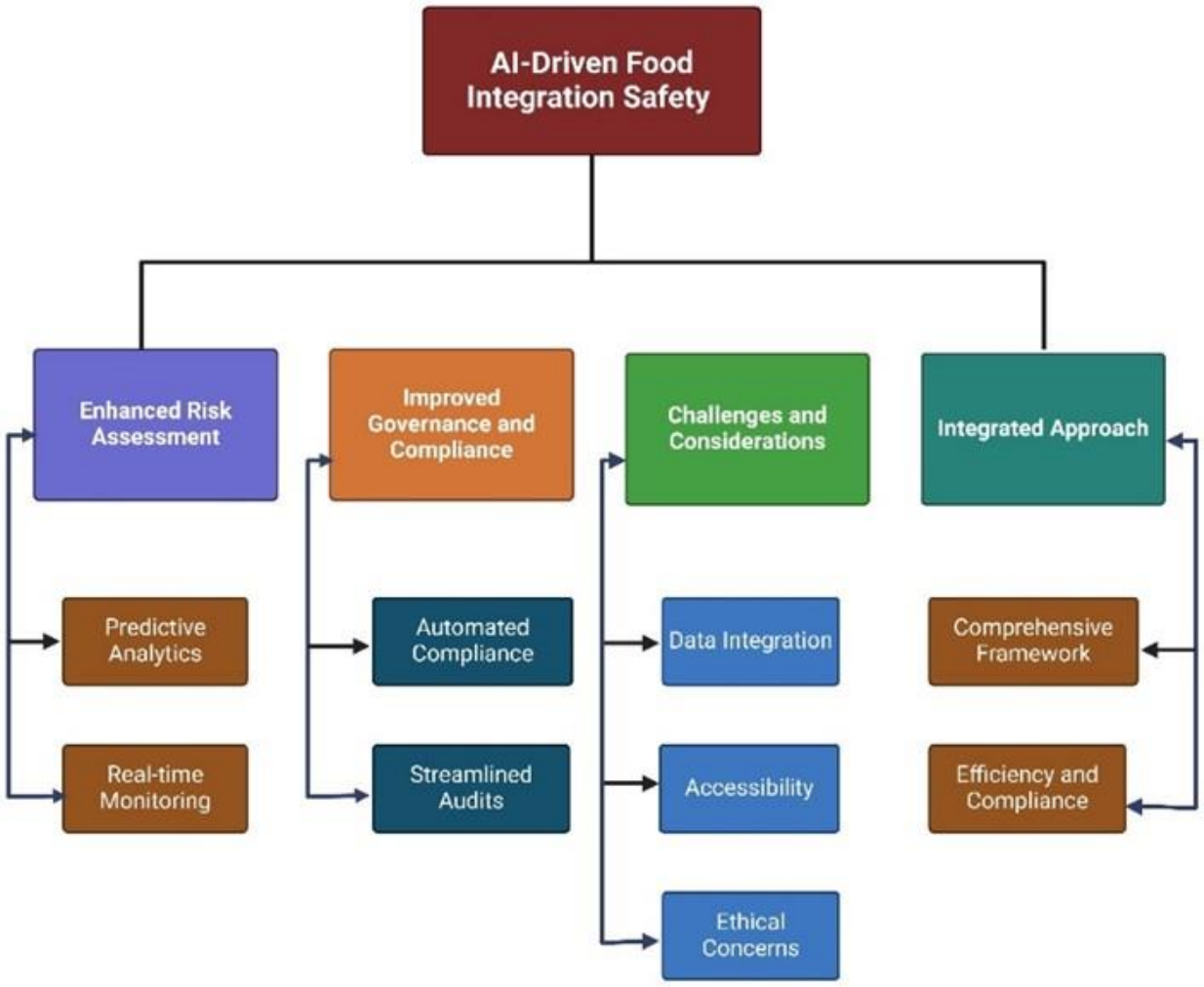


Figure 1. The Artificial Intelligence-Driven Food Safety Integration Framework

A predictive analytics model that AI follows identifies emerging risks by looking at environmental conditions and past contamination data through large datasets. It empowers the stakeholders to take preventive actions before contamination happens. For example, AI can model microbial growth under certain conditions to help prevent foodborne outbreaks. In real-time monitoring systems, AI monitors food production and distribution continuously. Sensors and IoT devices provide continuous data fed into AI algorithms, detecting abnormalities like temperature fluctuations and equipment breakdowns (da Costa et al., 2022). This allows for immediate action against preventing contamination while ensuring food safety. AI improves governance by automating compliance checks and report generation, reducing manual workload and boosting productivity. AI can also detect deviations in real time, enabling immediate remedial action to bridge any gaps in safety standards. Conversely, AI has quite a few challenges to handle: data harmonization, accessibility for small producers, and ethical issues like data privacy and transparency. This area needs special attention.

Genomic Surveillance: Up-to-date developments in food safety emphasize the increasing importance of rapid identification of sources of pathogenic bacteria in preventing an outbreak. A landmark progress in food safety is the real-time tracking and prevention of microbial threats

through genomic surveillance and AI technologies. AI assists in determining contamination hazards, predicting antimicrobial resistance, and risk stratification throughout the food supply chain. Figure 2 depicts the various avenues through which AI has been applied in genomic surveillance and how pathogen genomics and epidemiological modeling work hand-in-hand toward improving food safety management.

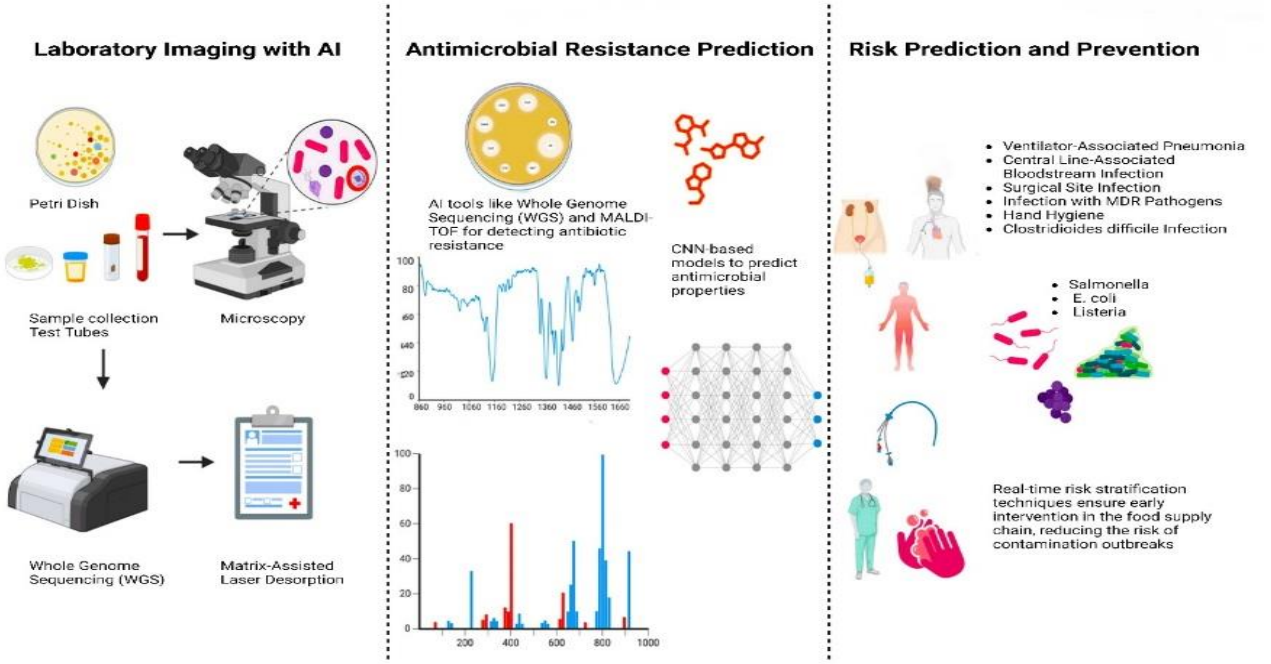


Figure 2. Applications of AI in Genomic Surveillance for Food Safety

AI's Role in Pathogen Genomics

The genetic study of microorganisms, microbial genomics, forms the backbone of modern food safety practice. Genomic sequencing of foodborne pathogens such as *Salmonella*, *E. coli*, and *Listeria* provides valuable insights into the evolution of these pathogens and their spread along supply chains. However, the total volume and complexity of the genomic data have hindered this endeavor, which makes traditional analysis methods boring and insufficient. Now that is where AI comes into play- it offers rapid interpretation of a large, expansive genomic dataset. AI's detection of tiny mutations within pathogens aids in assessing virulence, antibiotic resistance, and the potential for major outbreaks. Machine learning algorithms are particularly adept at capturing specific genomic patterns predictive of pathogenicity, allowing for early interruption before further dissemination (Rahman et al., 2023).

1)Improving Pathogen Identification and Sequencing: Traditional pathogen detection methods, including culturing and PCR, require days for results and thus can delay necessary intervention. AI genomic sequencing identifies pathogens within hours, providing a more rapid response. Therefore, AI accelerates and improves pathogen identification accuracy: deep learning algorithms identify mutations that indicate antibiotic-resistant bacteria. Furthermore, from a single sample of food, AI could completely sequence the genetic make-up of the pathogens, thus giving important information concerning their resistance patterns and possible sources of contamination. For

example, in the case of a new strain of *E. coli*, AI would help prevent its spread, especially since public health authorities can quickly intervene (Nafea et al., 2024).

2) Forecasting Pathogen Evolution and Mutation Rates: AI predictive capacity is one of the most significant advantages it has concerning pathogens' genomics. Pathogens adapt very quickly to their environment by taking on genetic changes. In contrast, others become drug-resistant or thrive under circumstances that would be extreme for humans or non-pathogenic conditions. In this way, AI will analyze historical genomic data to determine which recurring mutations are causing resistance, especially in bacteria like Salmonella and Listeria. Through such considerations, AI would forecast how fast a pathogen could develop resistance against specific antibiotics, enabling food producers and health authorities to act fast to prevent long-term effects of antibiotic-resistant organisms (Fisher, 2021).

3) Real-Time Tracking and Outbreak Management Using AI: RT pathogen tracking is very important in minimizing foodborne diseases. They have been designed specifically to monitor pathogens through the food supply chain, from production, transport, and hospitals. For example, in a meat processing plant, an AI could analyze continuously environmental conditions, sanitation practices, and other data from the plant itself to detect bacterial contamination. When there is contamination, the system can immediately alert the operators to be able to stop sales of those affected products. Moreover, AI may trace the contamination by analyzing farm genomic data to ensure that outbreaks are quickly contained. This proactive approach greatly minimizes public health risk and spreads fewer foodborne illness cases in the population (Salam, 2024).

4) Tailored AI Solutions for Small-Scale Producers: While the majority of AI systems are primarily concerned with applications on a large scale, the smaller producers do not often have the resources to adopt such technologies. The equitable improvement in food safety calls for the development of AI solutions that meet the specific needs of small producers. An example of this proposal comes from a South Asian poultry cooperative that rolled out an AI-supported mobile application that incorporates low-cost sensors for supply chain monitoring of storage temperatures and conditions of transit. Such cost-benefit analysis helps small-scale producers remain compliant with food safety standards without investing in expensive infrastructure. In such a way, scalable and cost-effective AI tools will allow even small producers to reap the benefits of advances in food safety that AI can bring about (Dhillon & Moncur, 2023).

5) Integrating Blockchain and AI for Enhanced Transparency: AI and blockchain integration is a transformative benefit for food supply chains in matters of transparency and traceability. While AI processes data to preempt risks and ensure compliance, blockchain secures that record as immutable. Each and every transaction is documented, making clear supply chains that could account for actions from production to consumption. The AI and blockchain merger thus allow effective tracking, while product recalls can be carried out almost instantly in events of contamination, as evidence in Figure 3.

Figure 3. Integration of Blockchain and AI for Transparency in Food Supply Chains

While this integration has tremendous potential, scaling these technologies can challenge small producers in low-income regions. Such systems usually require heavy investments in infrastructure, especially for reliable internet connectivity and logistical resources. Yet, trial studies in the less-developed areas have shown that lightweight blockchain, combined with AI technology, can give a cost-effective and scalable solution for small-scale producers (Talla, 2022).

The Future of Food Safety and Public Health with AI

However, the increasing complexity of food supply chains and changing public health concerns with scaling food production to meet growing demand led to the inevitable rise of AI in food safety. AI has all the available revolutionary tools in food safety risk mitigation, new foodborne pathogen response, and equity in access to safe food globally. The present section gives a holistic roadmap into innovations for AI in food safety and public health, with policy and research recommendations to maximize their impact (Dhal & Kar, 2025).

1)Advancements in AI for Food Safety: Incorporating artificial intelligence into emerging technologies continuously redefines food safety practices, while the future evolution will concentrate on automation, real-time monitoring, and increased prediction abilities. The following are significant areas in which AI is expected to make a remarkable difference:

2) Smart Sensors and Predictive Algorithms: AI-integrated intelligent sensors propel food safety by permanently cataloging environmental elements such as temperature, humidity, and microbial activity. When such sensors are amalgamated with some predictive algorithms, they can catch the preliminary signs of contamination, giving food safety managers a propelling advantage. For instance, these sensors can monitor some production units in dairy, detecting *Listeria* or sites favoring *E. coli* growth conditions, so that prevention interventions may occur before possible contamination (Ntuli et al., 2023). Henceforth, the forthcoming sensor developments will focus on developing sensors capable of analyzing multiple variables simultaneously, which is significant in

improving the precision and information reliability of contamination prediction (Lăzăroiu et al., 2022).

3) AI's Role in Strengthening Global Food Security: This artificial intelligence potentially addresses food security issues, especially for low-income and resource-poor regions. For example, in areas with poor food safety systems, AI-enabled mobile platforms could monitor crop and livestock health, predict bacterial threats, and deliver real-time updates to farmers using simple smartphone technology (Qazi et al., 2022). Such forms of empowerment enable farmers to identify problems and adopt preventative measures, reducing incidences of food-related illnesses. Future projects on AI have to be made more accessible by providing cheap offline solutions specifically for rural settings. An AI-enhanced blockchain solution could also help monitor traceability to keep up with food safety standards and avoid unwholesome products entering markets. Such integration of AI into the blockchain can assure accountability in the food supply chain while strengthening food safety across the chain (Bosona & Gebresenbet, 2023).

AI, in addition, can assist both the local level government and food producers in developing food safety governance systems more effectively. The effect of Automated monitoring and reporting in AI makes simple causes the risk of contamination identification for timely interventions and better regulatory enforcement in under-resourced areas. Innovations like these will ultimately change the food security scene, reducing foodborne illnesses, improving access to safe food for everyone, and robust public health systems (Unnevehr, 2022). The open-mindedness and affordability of AI-enabled technologies make them ideal for addressing the inequalities around those solutions on time and leading towards healthier, safer food systems at the global level.

4) Global Cooperation in Food Safety: AI can also be promoted to improve international collaboration in food safety concerning handling cross-border foodborne disease outbreaks. Advanced AI systems may provide real-time data sharing between governments, public health organizations, and food producers, enabling them to predict and respond to other emerging threats in the future (Mu et al., 2024). Meanwhile, data integration from hospitals, production facilities, and transportation networks should also model how pathogens spread and inform a coordinated global response. AI will even aid in formulating strategies for future threats, such as antibiotic-resistant bacteria or novel foodborne viruses. Priority should be given to policymakers for investment in AI-driven tools allowing early detection and preparedness for future outbreaks (Boatemaa et al., 2024). Figure 4 illustrates how AI contributes to global food safety cooperation by enabling advanced diagnostics, precision in treatment, and real-time decision-making. This would, in turn, model pathogen spread, help improve food safety systems, and support international public health efforts.

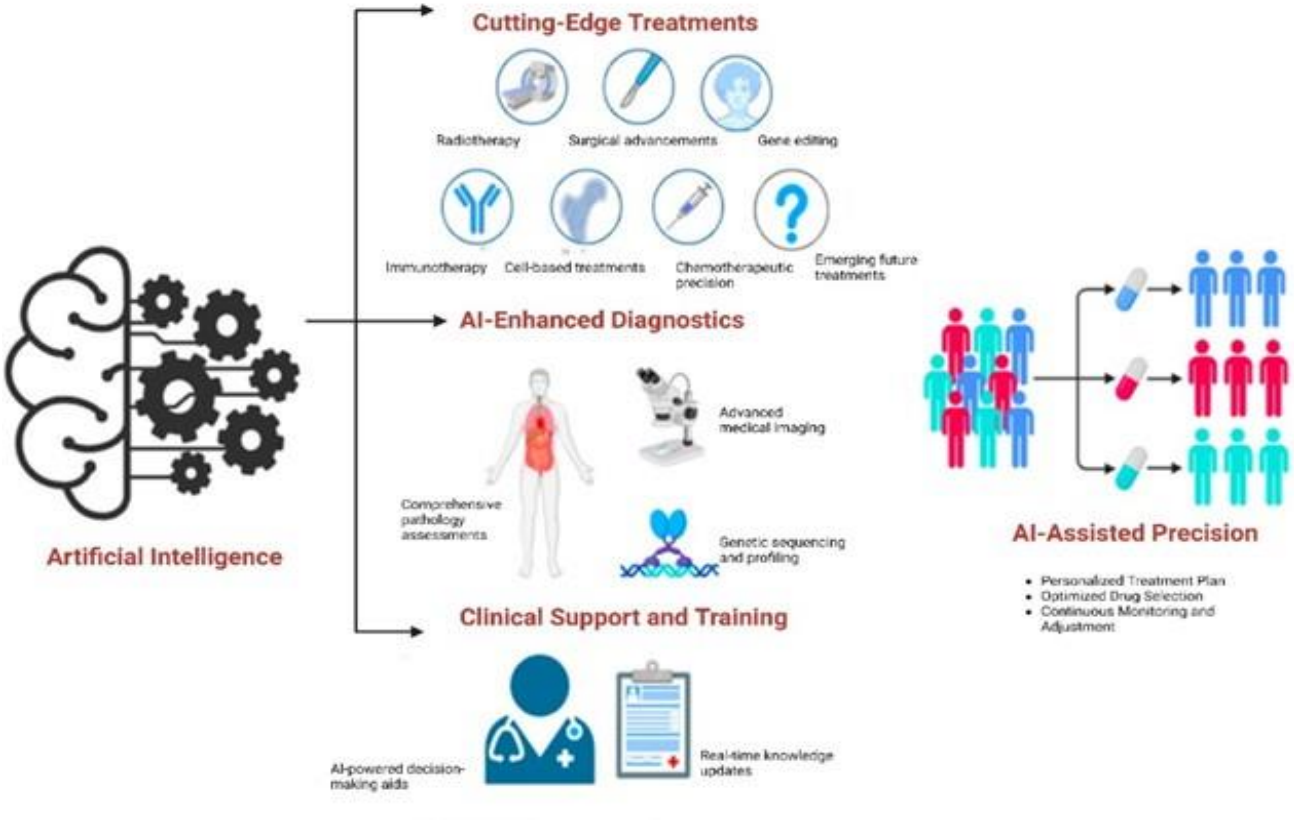


Figure 4. The Role of AI in Global Food Safety Cooperation

Summary

AI has contributed enormously to food safety, changing the paradigm from reaction to prevention. AI systems enhance the detection of pathogens, enable real-time monitoring, and provide predictive analytics for contamination risk management and public health outcomes. AI promotes efficiency and transparency in the food supply chain via blockchain, creating tamper-proof records. Furthermore, AI is critical in sustainability efforts since it reduces food waste and optimizes resource usage. To successfully integrate AI into food safety systems, there must be international collaboration between policymakers, technologists, and industry players to develop ethical, scalable, and affordable solutions that can contribute to a safer and sustainable food system.

References

Abebe, E., Gugsa, G., Ahmed, M., Awol, N., Tefera, Y., Abegaz, S., & Sisay, T. (2023). Occurrence and antimicrobial resistance pattern of E. coli O157: H7 isolated from foods of Bovine origin in Dessie and Kombolcha towns, Ethiopia. *PLoS neglected tropical diseases*, *17*(1), e0010706.

Adegoke, B. O., Odugbose, T., & Adeyemi, C. (2024). Data analytics for predicting disease outbreaks: A review of models and tools. *International journal of life science research updates [online]*, *2*(2), 1-9.

Boatemaa, R., Asare, S. O., Akabadin, S. C., Agyabeng, V., & Addy, A. (2024). The role of AI in Enhancing Disease Surveillance and Outbreak Response in Developing Countries. *Ghana Journal of Nursing and Midwifery*, *1*(4), 16-29.

Bosona, T., & Gebresenbet, G. (2023). The role of blockchain technology in promoting traceability systems in agri-food production and supply chains. *Sensors*, *23*(11), 5342.

Calvin, L., Jensen, H. H., & Liang, J. (2009). The economics of food safety: the 2006 foodborne illness outbreak linked to spinach. *Microbial safety of fresh produce*, 399-417.

Carlson, K. B., Dilley, A., O'Grady, T., Johnson, J. A., Lopman, B., & Viscidi, E. (2024). A narrative review of norovirus epidemiology, biology, and challenges to vaccine development. *npj Vaccines*, *9*(1), 94.

da Costa, T. P., Gillespie, J., Cama-Moncunill, X., Ward, S., Condell, J., Ramanathan, R., & Murphy, F. (2022). A systematic review of real-time monitoring technologies and its potential application to reduce food loss and waste: Key elements of food supply chains and IoT technologies. *Sustainability*, *15*(1), 614.

Dhakal, J., Cancio, L. P. M., Deliephan, A., Chaves, B. D., & Tubene, S. (2024). Salmonella Presence and Risk Mitigation in Pet Foods: A Growing Challenge with Implications for Human Health. *Comprehensive Reviews in Food Science and Food Safety*, *23*(6), e70060.

Dhal, S. B., & Kar, D. (2025). Leveraging artificial intelligence and advanced food processing techniques for enhanced food safety, quality, and security: a comprehensive review. *Discover Applied Sciences*, *7*(1), 1-46.

Dhillon, R., & Moncur, Q. (2023). Small-scale farming: A review of challenges and potential opportunities offered by technological advancements. *Sustainability*, *15*(21), 15478.

Fisher, A. M. (2021). The evolutionary impact of population size, mutation rate and virulence on pathogen niche width. *Journal of Evolutionary Biology*, *34*(8), 1256-1265.

Gartley, S., Anderson-Coughlin, B., Sharma, M., & Kniel, K. E. (2022). Listeria monocytogenes in irrigation water: An assessment of outbreaks, sources, prevalence, and persistence. *Microorganisms*, *10*(7), 1319.

Grace, D. (2023). Burden of foodborne disease in low-income and middle-income countries and opportunities for scaling food safety interventions. *Food security*, *15*(6), 1475-1488.

Lăzăroiu, G., Andronie, M., Iatagan, M., Geamănu, M., Ștefănescu, R., & Dijmărescu, I. (2022). Deep learning-assisted smart process planning, robotic wireless sensor networks, and geospatial big data management algorithms in the internet of manufacturing things. *ISPRS International Journal of Geo-Information*, *11*(5), 277.

Lee, H., & Yoon, Y. (2021). Etiological agents implicated in foodborne illness world wide. *Food science of animal resources*, *41*(1), 1.

Mkangara, M. (2023). Prevention and control of human Salmonella enterica infections: An implication in food safety. *International Journal of Food Science*, *2023*(1) , 8899596.

Mu, W., Kleter, G. A., Bouzembrak, Y., Dupouy, E., Frewer, L. J., Radwan Al Natour, F. N., & Marvin, H. (2024). Making food systems more resilient to food safety risks by including artificial intelligence, big data, and internet of things into food safety early warning and emerging risk identification tools. *Comprehensive Reviews in Food Science and Food Safety*, *23*(1), e13296.

Nafea, A. M., Wang, Y., Wang, D., Salama, A. M., Aziz, M. A., Xu, S., & Tong, Y. (2024). Application of next-generation sequencing to identify different pathogens. *Frontiers in microbiology*, *14*, 1329330.

Ntuli, V., Sibanda, T., Elegbeleye, J. A., Mugadza, D. T., Seifu, E., & Buys, E. M. (2023). Dairy production: microbial safety of raw milk and processed milk products. In *Present knowledge in food safety* (pp. 439-454). Elsevier.

Osek, J., Lachtara, B., & Wieczorek, K. (2022). Listeria monocytogenes–how this pathogen survives in food-production environments? *Frontiers in microbiology*, *13*, 866462.

Qazi, S., Khawaja, B. A., & Farooq, Q. U. (2022). IoT-equipped and AI-enabled next generation smart agriculture: A critical review, current challenges and future trends. *Ieee Access*, *10*, 21219-21235.

Rahman, S. Z., Senthil, R., Ramalingam, V., & Gopal, R. (2023). Predicting Infectious Disease Outbreaks with Machine Learning and Epidemiological Data. *Journal of Advanced Zoology*, *44*(S4), 110-121.

Salam, U. A. (2024). Real-Time Disease Surveillance: AI in Tracking and Controlling Outbreaks. *Emerging Trends in Medicine*, *1*(1), 9-13.

Scharff, R. L. (2012). Economic burden from health losses due to foodborne illness in the United States. *Journal of food protection*, *75*(1), 123-131.

Talla, R. R. (2022). Integrating Blockchain and AI to Enhance Supply Chain Transparency in Energy Sectors. *Asia Pacific Journal of Energy and Environment*, *9*(2), 109-118.

Unnevehr, L. J. (2022). Addressing food safety challenges in rapidly developing food systems. *Agricultural Economics*, *53*(4), 529-539.

About the Authors

Isha Arshad, I currently pursuing an MPhil in Microbiology at the University of Veterinary and Animal Sciences, Lahore. My research interests focus on unlocking the potential of artificial intelligence in enhancing food safety and public health. I aim to explore innovative AI-driven methodologies to improve the detection and management of foodborne pathogens. By integrating advanced technologies, I seek to contribute to developing more effective strategies for ensuring food safety and protecting public health. Ultimately, I aim to bridge the gap between microbiology and technological advancements for a safer food supply.

Email: ishaarshad.a@gmail.com ORCID: 0009-0009-5811-9227

Khadija Zafar completed a bachelors in nutritional sciences. from the University of Management and Technology. Currently doing Mphil from University of putra malaysia. My research interests are related to food sciences. I have worked in different hospital settings as a Dietitian.

Emails: khadijazaffar90@gmail.com OCID ID:0009-0006-6387-134X

Mariam Zafar completed a bachelors in dietetics and nutritional sciences. from the University of lahore. Currently doing Mphil from University of putra malaysia.

My research interest are related to food sciences. I have worked in different hospital settings as Dietitian

Email: mariamdj420@gmail.com OCID ID: 0009-0009-9859-9587

Mr. Tasawar Abbas received his M.Phill in Molecular Biology from National center for Excellence in Molecular Biology University of Punjab Lahore Pakistan. He has one review article. He has worked in Forensic Serology, He has excellent knowledge about Genetics, STRs, genetic polymorphism also on Stem cells, virology. His research interests include stem cell therapies, Immunology, Human genetics, and criminology.

Email: tasawarabbas33@yahoo.com ORCID: 0009-0002-7718-2634

Sundas Ashraf received her Bachelor degree in 2023 from Cholistan university of veterinary and Animal Sciences, Bahawalpur, Pakistan and is currently pursuing Her M.phil from University of Agriculture Faisalabad, Pakistan. Her Research interest Include, Epidemiology, public Health, Nanotechnology and Food safety.

Email: sundasashraf2001@gmail.com ORCID:0009-0002-8109-1209

Dr. Muhammad SAFDAR earned his PhD in molecular biology and genetics from Gaziantep University, Turkey. He is a lecturer in the Breeding and Genetics department at Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, Pakistan. His research interests are molecular genetics and genomics, nutrigenomics, nano-genomics, bioinformatics, biotechnology, and their applications. He has published more than 70 research articles in national and international journals. He has also written many book chapters as well as an edited book. He is an associate editor for international journals.

E-mail: drmuhammadsafdar8@gmail.com, ORCID: 0000 0002 3720 2090

Similarity Index

The similarity index obtained from the plagiarism software for this book chapter is 8%.

To Cite This Chapter:
Arshad, I. (2025). Unlocking AI's Potential in Food Safety and Public Health. , Current Studies in Life Sciences (pp. 81-93). ISRES Book Series. ISRES Publishing.

Soil Organic Matter, Characteristics and Environmental Function

Rabia CHERFOUH
Khaled OUALI
SI Ammar KADI
Azeddine MOUHOUS
Ali BOUZOURENE
Farid DJELLAL
Idir MOUALEK
Nacima ZIRMI-ZEMBRI
Zahia DORBANE
Houcine GUERMAH

1. Organic matter, the functional basis of soils

Organic matter is a ubiquitous component of terrestrial, aquatic and anthropogenic environments, including landfills. The origin and composition of organic matter are dependent on environmental characteristics, and its subsequent transformations influence the immediate environment, in particular soil pedogenesis. Soil Organic Matter (SOM) is considered a dynamic and essential component of terrestrial ecosystems. It plays a pivotal role in biogeochemical cycles, soil fertility, and climate regulation through carbon storage. In the context of global change, the importance of organic matter is increasing, due to its capacity to sequester carbon and maintain soil quality (Weil & Brady, 2016).

The primary source of soil organic matter is the accumulation of plant debris, including leaves, dead twigs, and bark, on the soil surface. These compounds undergo a series of transformations that ultimately result in the formation of humus, a more stable and degradation-resistant material.

The study of organic matter is of significant scientific interest due to its positive agronomic effects. These effects include:

•Enhancement of soil fertility, structure and erosion resistance; •Limitation of pesticide and organic pollutant activity through complexation and copolymerisation; •Reduction in the mobility of both organic and inorganic pollutants; •Decreased bioavailability of heavy metals and other potentially toxic elements.

2. Nature and Composition of Soil Organic Matter

2.1. Living Organic Matter

Living Organic Matter (LOM) is defined as the totality of active biomass, encompassing both soil fauna and flora. This compartment, representing the dynamic component, contains a wide variety of living organisms, including but not limited to bacteria, fungi, algae, protozoa,

etc. Indeed, according to Gobat & al., (2010), soil fauna can reach several hundred million individuals per square metre, while several hundred invertebrate species may be present, as highlighted by Cluzeau & al. (2005). The Table 1, provides order of magnitude estimates of abundance and biomass for certain soil fauna taxa.

Table 1. Soil microbial biomass (Cluzeau & *al*., 2005).

Groupe	Individus per m^2	Biomasse ($g.m^{-2}$)
Protozoa	10^5 to 10^{11}	6 to +30
Nematodes	10 to 30 million	1 to 30
Earthworms	50 to 400	20 to 400
Mites	2 $x10^4$ to 40$x10^4$	0,2 to 4
Springtails	2 $x10^4$ to 40$x10^4$	0,2 to 4
Insect larvae	Up to 500	4,5
Millipedes	20 to 70	0,5 to 12,5
Centipedes	100 to 400	1 to 10
Isopods	Up to 18000	Up to 4

Animals, whether vertebrates or invertebrates such as earthworms, play an active role in organic matter decomposition, soil renewal, and modification of soil structure and permeability through their burrowing activities. The galleries and channels created by these organisms enhance soil aeration, root penetration, the formation of preferential water flow pathways, and contribute to the distribution of organic and mineral compounds throughout different soil horizons.

2.2. Fresh Organic Matter

This particular fraction of soil organic matter is constituted of plant-derived debris (i.e. plant residues and exudates), animal-derived matter (e.g. faeces and carcasses), as well as fungal and microbial remains (e.g. carcasses and exudates) that accumulate on the soil surface and undergo transformation at varying rates. Fresh organic matter, also referred to as litter, comprises two types of constituents:

(i) Soluble components (carbohydrates, tannins, peptides and amino acids); and (ii) insoluble components (lignin, cellulose and hemicellulose compounds) which decompose progressively, giving rise to both soluble neo-formed compounds and other slowly-decomposing

insoluble compounds. The insoluble fraction typically resides on the surface, though, in certain instances, it undergoes gradual incorporation into the soil through mechanical processes facilitated by earthworms.

2.3. Stabilised Organic Compounds

The decomposition of litter, a fundamentally biological process, leads to the formation of stable humic compounds. The stabilised organic matter fraction, which constitutes humus, is composed of humic substances including fulvic acids, humic acids and humins. It is estimated that these substances account for 70-90% of soil organic matter, and they play an essential role in maintaining soil fertility over the medium and long term (Hayes & Swift, 2020). The organic matter content measured during soil analyses primarily corresponds to this stabilised form, which directly influences the soil's chemical properties, structure and structural stability.

3. Decomposition Processes: From Biomass to Humus

3.1. Role of Decomposer Organisms

The process of decomposition of soil organic matter (SOM) is driven by distinct groups of living organisms, whose roles are contingent on the nature and extent of degradation of the organic material. Collectively, these organisms engage in pivotal biochemical reactions that propel the comprehensive senescence process of all living organisms.

The term "pedofauna" refers to the fauna inhabiting the soil, and within this category, the mesofauna comprises earthworms (lumbricids), gastropods, crustaceans, arachnids and mites. These organisms play a vital role in the transport and burial of bacteria and fungi, either through their external surfaces or via their excreta (Bonneau & Souchier, 1994).

The decomposition of both soluble and insoluble organic fractions is primarily mediated by bacterial groups. While the taxonomic diversity of soil microbial communities and their capacity to degrade various substrates is remarkable, this does not preclude the existence of substrate-specific specialisation or differential activity based on degradation state Gobat & al., (2010). For instance, Gram-negative bacteria have been observed to show a preference for recent plant tissues, whereas carbon derived from decomposed soil organic matter is primarily assimilated by Gram-positive bacteria (Kramer & Gleixner, 2006). In addition, it has been demonstrated that bacterial diversity plays a crucial role in maintaining microbial community stability and resilience against disturbances such as pollution or environmental changes (Girvan & al., 2005).

Eumycota fungi, including Armillariella mellea and Laccaria laccata, are responsible for brown and soft rot, which is optimised in neutral environments where cellulose is primarily degraded, while lignin is relatively left unmodified.

The relationship between microbial community diversity and organic matter (OM) decomposition dynamics is interpreted differently among researchers. Don & al., 2017 and Nannipieri & al., (2003) describe functional redundancy, whereby multiple species perform

analogous ecological functions. Conversely, Guigue & al., (2015) have demonstrated that a reduction in bacterial diversity results in diminished decomposition rates.

4. Formation of humic substances: key biochemical mechanisms

4.1. Transformation pathways

Humic substances can be considered as the synthetic product resulting from the transformation of simple organic compounds produced by microbial decomposition of the initial substrate. A number of pathways have been proposed by scientists to explain the formation of these substances from the decomposition of fresh organic matter brought to the soil surface.

Waksman's Theory (Pathway 1)

Waksman (1936) attributes the formation of humic substances to lignin transformations. The validity of this ligno-protein pathway is substantiated by the substantial similarity between lignin and humic acids, which are characterised by low biodegradability, solubility and the presence of methoxyl groups. Consequently, this hypothesis asserts that lignin, a predominant constituent of plant tissues, plays a pivotal role in the process of humus formation.

To form humic acids, lignin undergoes several transformations including:

- Oxidation of side chains
- Introduction of carboxyl groups (-COOH)
- Loss of methoxyl groups (-OCH3)
- Increase in phenolic hydroxide groups (-OH)

In soils, lignin is only partially degraded by microorganisms, and the undecomposed residues become a major component of soil humus (Entery & Backman, 1995; Wu & al., 2017).

Lignin serves as a precursor for phenols and polyphenols, consisting of an assembly of three phenylpropanoid units: Coniferyl alcohol, Hydroxycinnamyl alcohol and Sinapyl alcohol. These units are interconnected through C-C bonds and ether oxides.

Quinone Mechanism (Pathways 2 and 3)

This mechanism involves the polymerisation of quinones derived from the degradation of phenolic compounds to form complex humic structures. Phenolic compounds are oxidised to quinones by enzymes secreted by bacteria and actinomycetes, which then react with amino acids and nitrogenous compounds to form condensed humic substances.

Based on the origin of the quinones, two different pathways can be identified (Figure1)

1. Lignin-derived pathway: Phenolic acids and aldehydes (polyphenols) produced by microbial lignin degradation, particularly by myxobacterial activity, are converted to quinones by specific enzymes (Huang & Hardie, 2009). These quinones then form humic macromolecules either by autopolymerisation or by combination with nitrogenous compounds.

2. Microbial pathway: Quinones are derived from polyphenols synthesised by microorganisms using non-lignin organic compounds, mainly of cellulosic origin (Wu & al., 2017).

Sugar-Amine Condensation (Pathway 4)

This theory proposes that sugars and amino acids interact to form humic precursors. The pathway involves non-enzymatic polymerisation of reduced sugars and microbial metabolic amines to form nitrogenous brown polymers (Wu & al., 2017; Huang & Hardie, 2009). Globally, in natural systems, these proposed pathways are not mutually exclusive and may operate simultaneously, with their relative importance depending on soil conditions and organic matter properties.

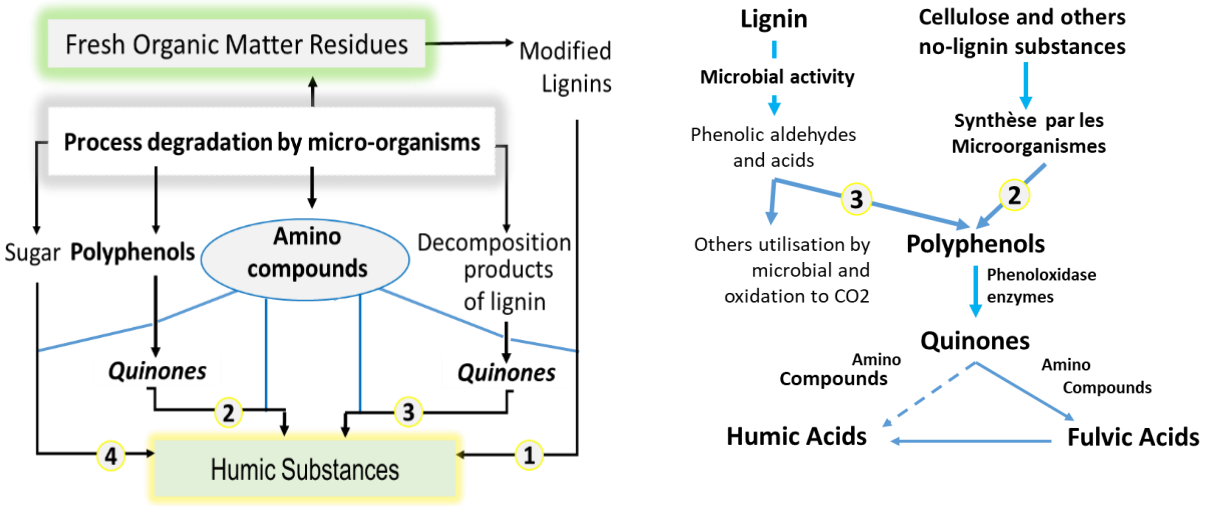


Figure 1. Formation mechanisms and pathways of humic substances (Stevenson, 1982)

4.2 Resulting fractions.

Humus, the end product of the decomposition and transformation of organic matter in the soil, is composed of several different molecular fractions. These humic substances consist of different aromatic cores (phenolic or quinonic) linked by acidic functional groups and aliphatic side chains (including saccharides, peptides, etc.). The heterogeneous and complex chemical structure (supramolecular or macromolecular) of humic fractions results from: - Diversity of organic substrates - Composition of microbial communities - Environmental factors influencing humification processes (Piccolo, 2001; Swift, 1979). The primary fractions - fulvic acids, humic acids and humin - differ in their: Solubility characteristics; Chemical structure; Functional role in soil properties (Stevenson, 1994; Lehmann & Kleber, 2015).

Fulvic acids

Fulvic acids are the most soluble and least polymerised fraction of humus (González-Vila & al., 2001). Characterised by low molecular weight (1,000-10,000 Da) and abundant carboxyl (-COOH) and hydroxyl (-OH) functional groups, they exhibit high chemical reactivity. Their pH-independent solubility facilitates vertical mobility within soil profiles (Wang & al., 2024; McColl & Pohlman 1986), where they perform essential functions such as:

- Metal chelation (Al, Cu, Zn) - Enhancement of plant-available micronutrients - Translocation of mineral elements to deeper soil horizons

Table 2. Classification and general characteristics of humic substances (Stevenson & Cole, 1999).

Fulvic acids		Humic acids	
Crenic Acids	Apocrenic Acids	A H Bruns	AH Grays
Light Yellow	Yellow Brown	Dark Brawn	Blac k-Gray
(-) ---------- Degree of Polymerization ---------- (+)			
(2000) (-) ---------- Molar Mass (g/mol) ---------- (+) (300 000)			
(45%) (-) ---------- Carbon Content ---------- (+) (62%)			
(48%) (+) ---------- Oxygen Content ---------- (-) (30%)			
(1400) (+) ---------- Acidity (mmoles/g) ---------- (-) (500)			

Humic acids.

Humic acids precipitate at pH less than 2. These dark coloured (brown to black) polymers have high molecular weights (10,000-300,000 Da) and complex aromatic structures which confer soil stabilising properties (Piccolo, 2001). Formed by oxidative condensation of phenolic compounds, they are typically associated with - Amino acids - Peptides and - Polysaccharides. Their main ecological functions are: formation of clay-humic complexes, critical for soil aggregate stability (Tisdall & Oades, 1982; Chenu & al., 2000) and medium-term carbon sequestration, enhancing soil fertility (Schmidt & al., 2011).

Humin

Humin represents the most stable and least soluble fraction of soil organic matter (across all pH levels), being intimately associated with soil minerals. Its highly polymerised structure and resistance to degradation make it a crucial long-term carbon reservoir (Tisdall & Oades, 1982;

Chenu & al., 2000; Schmidt & al., 2011). This fraction consists of large, complex organic macrostructures that are tightly bound to the soil's mineral fraction. Key characteristics include:

- Exceptional resistance to biodegradation
- Contribution to soil physical cohesion
- Protection against climatic erosion factors

The persistence of organic matter in soils, the significance of the residence time of organic matter in soils varies considerably, ranging from a few days for labile fractions (Chenu & al., 2000) to thousands of years for recalcitrant fractions (Lehmann & al., 2020).

The resistance to microbial decomposition has been attributed to four main mechanisms: (i) Robust molecular architecture, (ii) Physical isolation in disconnected pore spaces (iii) Intercalation within clay lattices, and (iv) Presence of decomposition inhibitors

6. Organo-mineral interactions: Fundamental drivers of soil properties.

Soil organic matter (SOM) not only modulates soil properties but also enhances their resilience to environmental perturbations. The functions resulting from these complex interactions between SOM and mineral particles improve edaphic conditions for plant growth, while also having significant implications for ecosystem services and sustainable soil management.

6.1. Functions of soil organic matter (SOM).

Soil organic matter (SOM) is a fundamental component of agro-ecosystems, playing a pivotal role in sustaining agricultural productivity and the global environment (Lal, 2020). As demonstrated in the following figure, SOM serves as a crucial metric for evaluating soil health, enhancing soil properties across a range of dimensions, including physical, chemical, and biological (figure 2). These enhancements contribute to enhanced soil fertility, augmented nutrient cycling, and elevated plant resilience. Furthermore, SOM's ability to enhance water retention ensures the stability of crops during periods of drought. Concurrently, the pivotal role of SOM in carbon sequestration is instrumental in mitigating the effects of climate change, while concurrently enhancing air quality by reducing atmospheric CO_2 and minimising dust emissions from erosion. Beyond the agricultural advantages, SOM provides essential ecosystem services, including stabilising soil aggregates to prevent erosion, filtering pollutants to protect water quality, and storing carbon to reduce greenhouse gas emissions, thereby safeguarding air quality.

Figure 2. The functions performed by soil organic matter.

Soil structure.

Soil organic matter (SOM) plays a pivotal role in the formation and stability of soil aggregates. Polysaccharides and proteins derived from transformation processes act as binding 'cements' for mineral particles (Chenu & Cosentino, 2011). Exopolysaccharides secreted by soil bacteria increase particle cohesion, thereby improving structural stability (Colica & al., 2014). This structuring also influences soil porosity, facilitating water and air circulation. SOM-derived polysaccharides and proteins act as binding agents, while clay minerals provide reactive surfaces for adsorption of organic compounds.

Water retention.

Soil organic matter (SOM) significantly increases the water-holding capacity of soils (Lal, 2020). Hydrophilic organic compounds such as humic acids can hold up to 20 times their weight in water (Rawls & al., 2003). This property is particularly important in drought-prone regions where SOM plays a vital role in maintaining soil moisture and improving plant water use efficiency. The formation of stable aggregates further contributes by creating pore networks that retain plant-available water.

Soil fertility

Mineralisation of organic compounds is an important pathway for replenishing plant and microbial nutrient reserves (Tiessen & al., 1994). In agricultural soils, crop residue inputs stimulate microbial activity and promote the release of bioavailable nutrients, including carbon, nitrogen, phosphorus and potassium. In addition, Roots interact with soil microorganisms, particularly mycorrhizal fungi and non-symbiotic rhizobacteria, to promote nutrient availability (phosphorus and nitrogen) and plant growth. (Richardson & al., 2009). Root exudates rich in organic compounds enhance the mobilisation of nutrients.

6.2. Organo-Mineral Interaction Mechanisms

Two primary mechanisms govern the binding between soil mineral particles and organic matter: adsorption and complexation. These mechanisms are critical for SOM stabilisation and the formation of organo-mineral complexes, particularly clay-humic associations.

Functional groups in SOM (e.g. carboxyl, phenolic) form electrostatic bonds with mineral surfaces, often mediated by polyvalent cations (Kleber & al., 2007). These interactions stabilise SOM and reduce microbial degradation. In clay-rich soils, SOM is strongly adsorbed to mineral surfaces, increasing its persistence and maintaining soil carbon stocks.

Cation Bridging.

Polyvalent cations (e.g. Ca2+, Fe2+, Al3+) play an important role in the formation of organo-mineral complexes by acting as bridges between negatively charged organic functional groups and mineral surfaces (Rowley et al., 2018). For example, in calcium-rich calcareous soils, the formation of Ca2+-SOM complexes is an important mechanism for stabilising organic matter.

Carboxyl (-COOH), phenolic (-OH) and amino ($-NH_2$) groups from organic matter bind to mineral surfaces (clays, iron/aluminium oxides) via cationic bridges. This interaction protects organic matter from microbial decomposition and enhances soil carbon sequestration. In addition, these bonds immobilise nutrients, regulating their bioavailability to plants and influencing the pH of the soil solution.

Complexation with iron and aluminium oxides.

Iron oxides (e.g. hematite [Fe_2O_3], goethite [FeO(OH)]) and aluminium oxides (e.g. gibbsite [$Al(OH)_3$]) are highly reactive soil minerals. Their surfaces contain hydroxyl (-OH) groups which interact with organic compounds and contribute to - stabilisation of organic matter, - protection against microbial degradation, - regulation of nutrient availability.

These oxides form stable complexes with humic and fulvic acids, limiting the access of microbial enzymes to SOM. In highly weathered tropical soils, aluminium oxides are particularly dominant in SOM protection.

Iron and aluminium oxides complex with organic molecules via:

1. Electrostatic interactions: Under acidic conditions, carboxylate (-COO-) and phenolate (-O-) groups are attracted to positively charged oxide surfaces.

2. Coordination bonding: Oxygen or nitrogen atoms from organic functional groups (-COOH, -OH, $-NH_2$) form coordination bonds with surface Fe^{3+} or Al^{3+} ions

Clay-humic complexes.

The complexation mechanisms between soil organic matter (SOM) and clay minerals are critical processes governing both organic matter stabilisation and pedogenesis. Kleber & al. (2007) and Kleber & al. (2015) have shown that these interactions take place primarily through the following mechanisms:

Electrostatic adsorption : Clay minerals possess permanent negative charges generated by (i) isomorphous substitution (e.g. Al3+ replacing Si4+ in silicate lattices) and (ii) dissociation of hydroxyl groups (-OH) on clay surfaces Negatively charged organic compounds (e.g. humic acids) interact with adsorbed cations (Ca2+, Mg2+, Al3+) on clay surfaces, forming cation bridges. In smectites, interlayer cations (e.g. Ca2+) can form bridges between clay layers and organic functional groups (-COOH).

Hydrogen bonding : Hydroxyl (-OH), carboxyl (-COOH) and carbonyl (C=O) functional groups can form hydrogen bonds with oxygen atoms or hydroxyl groups on the surface of illites rich in -OH groups.

Complexation with metal cations: Metal cations Al3+ and Fe3+ in the form of oxides and hydroxides form organo-minerals by acting as bridges between MOS and clay minerals.

Metal cation complexation: Metal cations (Al^{3+}, Fe^{3+}) in oxide and hydroxide forms facilitate organo-mineral associations by bridging SOM and clay minerals.

Intercalation in clay sheets: Smectites exhibit expandable layered structures and high cation exchange capacity (CEC), allowing humic acid intercalation into interlayer spaces to form stable organo-mineral complexes. In contrast, illites have a lower negative charge and limited expandability due to potassium (K^{+}) fixation in interlayers, and primarily adsorb polysaccharides via hydrogen bonding.

In smectite-rich soils, organo-clay interactions are enhanced: - SOM stabilisation (reducing microbial degradation), - carbon sequestration, - pesticide immobilisation (via hydrophobic interactions or cation bridging), thereby limiting soil mobility. In addition, SOM-clay complexes promote the formation of stable aggregates, improving soil structure and erosion resistance.

6.3 Implications for Ecosystem Services

Soil organic matter (SOM)-mineral particle interactions are fundamental to the functioning of terrestrial ecosystems and have a direct impact on soil-based ecosystem services. These interactions lead to the formation of organo-mineral complexes that stabilise SOM and improve soil structure. Improved soil structure promotes water infiltration, nutrient retention and erosion resistance, thereby supporting key ecosystem services such as water cycle regulation, soil fertility and carbon sequestration (Chenu & Cosentino, 2011; Lehmann & al., 2020; Chenu & al., 2000).

SOM stabilisation processes slow microbial decomposition kinetics, thereby prolonging the persistence of organic matter in soils. This mechanism is critical for climate regulation services,

enabling long-term carbon storage and mitigation of greenhouse gas emissions (Lal, 2020) . It also maintains soil fertility by preserving organic and mineral nutrients essential for plant growth.

SOM-mineral interactions also influence soil biodiversity. Well-aggregated soil structures provide favourable habitats for microorganisms, fungi and soil fauna. These organisms play a central role in organic matter decomposition, nitrogen fixation, and biogeochemical cycling. Mycorrhizal fungi, which form symbiotic associations with plant roots, particularly benefit from structured soils, facilitating the expansion of hyphal networks, improving nutrient uptake by plants and protecting the soil against erosion. (Rillig & al., 2002; Miller & Jastrow, 1992) .

Overall, SOM-mineral interactions provide critical ecosystem services, including:

- Climate regulation through carbon sequestration
- Soil fertility maintenance
- Biodiversity support

These processes highlight the importance of sustainable soil management in maintaining ecological functions. Current research shows that understanding these mechanisms is essential for developing agricultural and land management practices that optimise resource use while minimising environmental impacts (Candan-Helvacı, 2021).

7. Interaction organic matter-metal species

The interaction between organic matter and metals in agricultural soil is crucial for understanding soil fertility, heavy metal mobility and environmental consequences (Savci, 2012). Organic matter, mainly derived from plant and animal residues, interacts with metals such as copper, zinc, lead and cadmium to form organo-metallic complexes. The role of humic substances, as the stable form of organic matter in soils, is particularly important in shaping the behaviour of metals through complexation and immobilisation processes.

7.1. Humic Substances- metals complexation

Humic substances, such as humic acids and fulvic acids, are highly reactive with metals, allowing them to form stable organo-metallic complexes. These substances form thermodynamically stable organo-metallic complexes through:

1. Chelation via polydentate ligand formation
2. Electrostatic interactions with deprotonated functional groups

Complexation of metals with humic substances can temporarily immobilise metals within the clay-humic complex, thereby reducing their bioavailability and limiting their mobility in the soil.

This effect can be particularly beneficial in soils contaminated with toxic metals, preventing the metals from migrating to deeper layers or entering the water table. However, environmental perturbations (e.g., pH changes, microbial activity) can destabilise these complexes, potentially remobilising metals into soil solution and increasing their phytoavailability.

7.2. Mechanisms of Complexation

The interaction between metals and organic matter in soil involves various mechanisms including electrostatic attraction, hydrogen bonding and coordination with functional groups such as carboxyl (R-COOH), phenolic (R-OH) and thiol (R-SH) groups. These functional groups provide specific binding sites for metals, enhancing their retention in the soil and reducing leaching potential. Complex stability depends on three key factors:

1. Soil pH
2. Organic matter concentration
3. Metal chemical properties (e.g., ionic radius, charge density).

Research demonstrates that organic matter substantially influences metal speciation, modifying both solubility and mobility patterns (Merdy & al., 2024; Cherfouh & al., 2022).

The table below shows the affinity of metals for various soil fractions, highlighting variations in complexation capacity based on the nature of organic and inorganic components, as well as the specific properties of the metals.

In contaminated soils, Parvin et al (2002) and Rosse (1994) observed a strong correlation between copper mobility and humic acids, suggesting that humic substances play an important role in regulating the movement of metals in soil (table 3). Similarly, Cherfouh & al., (2018); cherfouh & al., (2021) and Merdy & al., (2024) showed that the application of sewage sludge to soils increased the proportion of metals such as cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) associated with organic matter.

Table 3. Empirical affinity series of metals for soil constituents (Ross, 1994).

Soil fraction Material	Affinity sequence
Amorphous aluminium oxide (Al2O3)	Cu > Pb > Zn > Ni > Co > Cd
Amorphous iron oxide (Fe2O3)	Pb > Cu > Zn > Ni > Cd > Co
Goethite (αFeOOH)	Cu > Pb > Zn > Cd
Hematite (αFe2O3)	Pb > Cu > Zn > Co > Ni > Cd > Mn
Manganese oxide (MnO2)	Cu > Pb > Mn= Co > Zn > Ni

Fulvic acid (pH5)	Cu > Pb > Zn
Humic acid (pH4-6)	Cu > Pb >> Cd > Zn

7.3. Environmental consequences

The influence of organic matter on metal speciation holds particular significance in contaminated soil systems. Dissolved organic matter (DOM), comprising both humic substances and low molecular weight organic compounds, forms soluble organo-metallic complexes that fundamentally alter metal behaviour in soil-water systems. These properties are critical for sustainable agricultural productivity, particularly in the face of climate change and land-use intensification (Savci, 2012).

These complexes can change the speciation of metals, making them more or less toxic, depending on the stability of the complex and the affinity of the metal for the organic ligands. Tadini & al., (2020) demonstrated DOM's capacity to transform metal speciation, with direct implications for bioavailability and ecotoxicological risk assessment. The ability of organic matter to influence the behaviour of metals in soil solution is critical to understanding the risks of contamination to plants, animals and the wider ecosystem.

Advanced analytical techniques elucidate metal-organic interactions:

1. **Fluorescence spectroscopy:** Tracks fluorescence quenching upon metal binding to organic ligands (Tadini & al., (2020; Merdy & al., 2009)
2. **Sequential extraction:** Quantifies metal fractionation and organic matter's role in metal sequestration (Impellitteri & al., 2002)

These approaches enable determination of the labile metal fraction - often organically-complexed species that may become mobile under environmental perturbations. Such analyses prove indispensable for assessing environmental risk and predicting contaminant transfer into food chains (Cherfouh et al., 2021; Savci, 2012; Merdy et al., 2002). Notably, metal-organic complexation typically reduces phytoavailability, potentially mitigating heavy metal toxicity in agricultural systems.

8. Conclusion

Soil organic matter (SOM) is defined as a dynamic and multifunctional component of terrestrial ecosystems, governing critical biogeochemical processes that underpin soil fertility, ecosystem resilience, and environmental quality. It is widely regarded as a cornerstone of soil health, integrating ecological functions and anthropogenic needs. Consequently, its conservation and enhancement are vital for global sustainability. Its central role is evidenced by its capacity to sequester carbon in stable organo-mineral complexes and to mitigate greenhouse gas emissions. Furthermore,

SOM enhances water and nutrient cycling by improving soil aggregation, which increases water infiltration, retention, and nutrient bioavailability.

In addition to its physical and chemical benefits, soil organic matter (SOM) directly supports soil fertility through the mineralisation of organic compounds, thereby releasing essential plant nutrients. Furthermore, SOM sustains soil biodiversity by providing habitats for microbial and faunal communities that drive decomposition, nitrogen fixation, and other key ecological processes. The ability of SOM to form stable complexes with clay minerals and metal oxides, through mechanisms such as cation bridging and chelation, enhances its persistence in soils. It has been demonstrated that SOM can mitigate contamination by immobilising toxic metals and binding organic pollutants. However, dissolved organic matter (DOM) can also facilitate metal transport, highlighting SOM's dual role in contaminant dynamics.

Given its multifaceted contributions, the strategic management of soil organic matter (SOM) is imperative for achieving global sustainability goals, ranging from carbon neutrality to food security. The protection and restoration of SOM stocks must be prioritised in agricultural, environmental, and climate policies to ensure the resilience of ecosystems in the long term.

References

Bonneau, M., & Souchier, B. (1994). Pédologie. 2. Constituants et propriétés du sol. Ed. Masson, Paris. 665p.

Candan-Helvacı, S. (2021). Environmental Knowledge. In S. Erten (Ed.) , Different Perspectives on Environmental Education (pp. 255–282). ISRES Publishing

Chenu C & Cosentino D.J. (2011) Microbial Regulation of Soil Structure Dynamics. Chap in book (37-70): The architecture and biology of soils: life in inner space, Publisher: CABI,

Chenu, C., Le Bissonnais, Y., & Arrouays, D. (2000). Organic matter influence on clay wettability and soil aggregate stability. Soil Science Society of America Journal, 64(4) , 1479-1486.

Cherfouh R, Lucas Y, Derridj A, & Merdy P (2018) Long-term, low technicality sewage sludgeamendment and irrigation with treated wastewater under Mediterranean climate: impact on agronomical soil quality. Environ Sci Pollut Res 25:35571–35581.

Cherfouh, R., Lucas, Y., Derridj, A., & Merdy, P. (2022). Metal speciation in sludges: A tool to evaluate risks of land application and to track heavy metal contamination in sewage network. Environmental Science and Pollution Research, 29(46) , 70396-70407.

Cluzeau D., Garnier-Zarly E., Lavelle P., Blanchart E., Peres G., Ablain F., Cuendet G., Fayolle L., 2005. Chapitre 17 Faune du sol et Lombriciens dans les sols tempérés agricoles. In Girard M.C., Walter C., Remy J.C., Berthelin J., Morel J.L., 2005. Sols et Environnement. Dunod (Ed.) 81 6p.

Colica, G., Li, H., Rossi, F., Li, D., Liu, Y., & De Philippis, R. (2014). Microbial secreted exopolysaccharides affect the hydrological behavior of induced biological soil crusts in desert sandy soils. Soil Biology and Biochemistry, 68, 62-70.

Don, A., Böhme, I. H., Dohrmann, A. B., Poeplau, C., & Tebbe, C. C. (2017). Microbial community composition affects soil organic carbon turnover in mineral soils. Biology and fertility of soils, 53(4) , 445-456.

Entry, J. A., & Backman, C. B. (1995). Influence of carbon and nitrogen on cellulose and lignin degradation in forest soils. Canadian Journal of Forest Research, 25(8) , 1231-1236.

Girvan, M.S., Campbell, C.D., & Killham K.J.I. (2005) Bacterial diversity promotes community stability and functional resilience after perturbation. Volume7, Issue3 March 2005 Pages 301-313.

Gobat, J.M., Aragno, M., & Matthey. (2010) Le sol vivant: bases de pédologie, biologie des sols. EPFL Press, 817 pages

González-Vila, F. J., Tinoco, P., Almendros, G., & Martin, F. (2001). Pyrolysis− GC− MS analysis of the formation and degradation stages of charred residues from lignocellulosic biomass. Journal of Agricultural and Food Chemistry, 49(3) , 1128-1131.

Guigue J, Lévèque J, Mathieu O, Schmitt-Kopplin P, Lucio M, Arrouays D, Jolivet C, Dequiedt S, Chemidlin Prévost-Bouré N, Ranjard L (2015) Water-extractable organic matter linked to soil physico-chemistry and microbiology at the regional scale. Soil Biol Biochem 84:158–167.

Hayes Michael, H.B. & Swift, R.S. (2020) Vindication of humic substances as a key component of organic matter in soil and water. Advances in Agronomy. Volume 163.

Huang, P. M., & Hardie, A. G. (2009). Formation mechanisms of humic substances in the environment. Biophysico-chemical processes involving natural nonliving organic matter in environmental systems, 1, 41-109

Impellitteri, C. A., Lu, Y., Saxe, J. K., Allen, H. E., & Peijnenburg, W. J. (2002). Correlation of the partitioning of dissolved organic matter fractions with the desorption of Cd, Cu, Ni, Pb and Zn from 18 Dutch soils. Environment International, 28(5) , 401-410.

Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R., & Nico, P. S. (2015). Mineral–organic associations: formation, properties, and relevance in soil environments. Advances in agronomy, 130, 1-140.

Kleber, M., Sollins, P., & Sutton, R. (2007). A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. Biogeochemistry, 85, 9-24.

Kramer C., & Gleixner G., Variable use of plant- and soil-derived carbon by microorganisms in agricultural soils, Soil Biol. Biochem., 38, 3267-3278. November 2006.

Lal, R. (2020). Soil organic matter and water retention. Agronomy Journal, 112(5), 3265-3277.

Lehmann, J., & Kleber, M. (2015). The contentious nature of soil organic matter. Nature 528:60–68.

Lehmann, J., Hansel, C. M., Kaiser, C., Kleber, M., Maher, K., Manzoni, S., ... & Kögel-Knabner, I. (2020). Persistence of soil organic carbon caused by functional complexity. Nature Geoscience, 13(8), 529-534.

McColl, J. G., & Pohlman, A. A. (1986). Soluble organic acids and their chelating influence on Al and other metal dissolution from forest soils. Water, Air, and Soil Pollution, 31, 917-927.

Merdy, P., Cherfouh, R., & Lucas, Y. (2024). Long-term agricultural reuse of treated wastewater and sewage sludge: developing a Time to Critical Content Index for metal species. Environmental Monitoring and Assessment, 196(9) , 836. 10.1007/s10661-024-12999-z

Miller, R. M., & Jastrow, J. D. (1992). The role of mycorrhizal fungi in soil conservation. Mycorrhizae in sustainable agriculture, 54, 29-44.

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003) Microbial diversity & soil functions. Europ J Soil Sci 54: 655–670.

Parvin, A., Moniruzzaman, M., Hossain, M. K., Saha, B., Parvin, A., Suchi, P. D., & Hoque, S. (2022). Chemical speciation and potential mobility of heavy metals in organic matter amended soil. Applied and Environmental Soil Science, 2022(1) , 2028860.

Piccolo, A. 2001. The supramolecular structure of humic substances. Soil Sci. 166:810–832.

Rawls, W. J., Pachepsky, Y. A., Ritchie, J. C., Sobecki, T. M., & Bloodworth, H. (2003). Effect of soil organic carbon on soil water retention. Geoderma, 116(1-2), 61-76.

Richardson, A. E., Barea, J. M., McNeill, A. M., & Prigent-Combaret, C. (2009). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms.

Rillig, M. C., Wright, S. F., & Eviner, V. T. (2002). The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant and soil, 238, 325-333.

Ross, S.M., 1994. Toxic Metals in Soil–Plant Systems. John Wiley & Sons Ltd, Chichester.

Savci, S. (2012). Investigation of effect of chemical fertilizers on environment. Apcbee Procedia, 1, 287-292.

Schmidt, M. W., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., ... & Trumbore, S. E. (2011). Persistence of soil organic matter as an ecosystem property. Nature, 478(7367), 49-56.

Stevenson, F. J., & Cole, M. A. (1999). Cycles of soils: carbon, nitrogen, phosphorus, sulfur, micronutrients. John Wiley & Sons. Publishers, Hoboken, 427 p.

Swift, R. S. (1999) Macromolecular properties of soil humic substances: Fact, fiction, and opinion. Soil Sci. 1999, 164, 790-802.

Tadini, A. M., Mounier, S., & Milori, D. M. B. P. (2020). Modeling the quenching of fluorescence from organic matter in Amazonian soils. Science of the total environment, 698, 134067.

Tiessen, H., Cuevas, E., & Chacon, P. (1994). T he role of soil organic matter in sustaining soil fertility. Nature, 371(6500), 783 -785.

Tisdall, J. M., & Oades, J. M. (1982). Organic Matter and Water-Stable Aggregates in Soils. European Journal of Soil Science, 33, 141-163.

Waksman, S.A. 1936. Humus: Origin, chemical composition and importance in nature. Williams and Wilkins, Baltimore, MD.

Wang, G., Cissé, G., & Staunton, S. (2024). Changes in chemical fractionation of copper and zinc in soil as a function of incubation moisture content and organic matter amendments. Chemosphere, 351, 141198.

Weil, R., & N.C. Brady. 2016. The nature and properties of soils. 15th ed. Pearson Education, Harlow, UK.

Wu, J., Zhao, Y., Zhao, W., Yang, T., Zhang, X., Xie, X., ... & Wei, Z. (2017) . Effect of precursors combined with bacteria communities on the formation of humic substances during different materials composting. Bioresource technology, 226, 191-199

About The Authors

Dr. Rabia CHERFOUH

Affiliation: Faculty of Biological and Agronomical Sciences. Mouloud MAMMERI University of Tizi-Ouzou, Algeria.

E-mail: **rabia.cherfouh@ummto.dz** ORCID: 0000-0001-7275-6286

Mr. OUALI Khaled

Affiliation: Faculty of Biological and Agronomical Sciences. Mouloud MAMMERI University of Tizi-Ouzou, Algeria.

E-mail: **oualikhaled2025@gmail.com**

Pr. Si Ammar KADI.

Affiliation: Faculty of Biological and Agronomical Sciences. Mouloud MAMMERI University of Tizi-Ouzou, Algeria.

E-mail: **siammar.kadi@ummto.dz** ORCID: 0000-0002-1048-2280

Dr. Azeddine MOUHOUS.

Affiliation: Faculty of Biological and Agronomical Sciences. Mouloud MAMMERI University of Tizi-Ouzou, Algeria.

E-mail: **azeddine.mouhous@ummto.dz** ORCID: 0000-0003-0558-1919

Mr Ali BOUZOURENE

Affiliation: Faculty of Biological and Agronomical Sciences. Mouloud MAMMERI University of Tizi-Ouzou, Algeria.

E-mail: **ali.bouzourene@ummto.dz**

Dr. Farid DJELLAL

Affiliation: Department of Agronomical Sciences, Ferhat ABBAS University of Sétif 1, Algeria.

E-mail: **fariddjellal@univ-setif.dz** ORCID: 0000-0002-8066-2455

Dr Idir MOUALEK

Affiliation: Faculty of Biological and Agronomical Sciences. Mouloud MAMMERI University of Tizi-Ouzou, Algeria.

E-mail: **idir.moualek@ummto.dz** ORCID: 0000-0002-4455-5556

Dr. Nacima ZIRMI-ZEMBRI

Affiliation: Faculty of Biological and Agronomical Sciences. Mouloud MAMMERI University of Tizi-Ouzou, Algeria.

E-mail: **nacima.zembri@ummto.dz** ORCID: 0009-0009-3527-1357

Dr. Zahia DORBANE

Affiliation: Faculty of Biological and Agronomical Sciences. Mouloud MAMMERI University of Tizi-Ouzou, Algeria.

E-mail: **zahia.dorbane@ummto.dz** ORCID: 0000-0003-3093-4763

Dr Hocine GUERMAH

Affiliation: Department of Agronomical Sciences, Mohamed Boudiaf University, M'sila, Algeria

E-mail: **hocine.guermah@univ-msila.dz** ORCID: 0000-0003-2378-1420

To Cite This Chapter

Cherfouh, R., Ouali K., Kadi, S.A., Mouhous, A., Bouzourene, A., Djellal, F., Moualek, I., Zirmi-Zembri, N., Dorbane, Z., Guermah, H. (2025). Soil Organic Matter, Characteristics and Environmental Function. In Current Studies in Life Sciences 2025 (pp. 95-112. ISRES Publishing.

The role of Microbial Communities in Health and Disease

Khawar ALI
Nosheen MAJEED
Farhat Ameer CHUGHTAI
Mehmet OZASLAN

Introduction

The human microbiome, comprising trillions of microorganisms residing predominantly in the gastrointestinal tract, plays a vital role in maintaining health and influencing disease outcomes. Understanding the composition and function of microbial communities within the human body, along with their symbiotic roles, is crucial for advancing medical science and enhancing overall health. This intricate ecosystem acts not merely as a passive resident but as a dynamic participant in metabolic processes, immunity, and even mental well-being. One foundational aspect of the microbiome is its significant role in metabolism. The gut microbiota is involved in the fermentation of indigestible carbohydrates, producing short-chain fatty acids (SCFAs) like butyrate, which have anti-inflammatory properties and other metabolic benefits. This productive interaction underscores the microbiome's influence on host metabolism and its link to chronic conditions such as obesity and type 2 diabetes. Dysbiosis, or an imbalance of microbial communities, has been implicated in the pathogenesis of these metabolic disorders, emphasizing the necessity of a healthy microbiome for metabolic homeostasis. Studies has shown that maternal and neonatal microbiotas play critical roles in shaping the infant's developing immune system. The process of microbial colonization begins at birth, where factors such as the mode of delivery and antibiotic exposure can profoundly affect the microbiome's composition during this crucial window (Jeyaraman et al., 2024). Dysbiosis during early life has been correlated with various health outcomes, including allergies and autoimmune diseases, highlighting the long-term repercussions of microbial communities established during infancy. The dietary influences on the microbiome are reflective of its complex interactions with the host. Particular foods, especially fermented products, have been shown to enhance gut health by promoting microbial diversity and supporting the abundance of beneficial species. Fermented foods have garnered attention for their potential to modulate the gut microbiome in favorable ways, potentially ameliorating conditions such as metabolic syndrome and mental health issues. This relationship demonstrates the vital role of nutrition not only in providing substrates for microbial metabolism but also in guiding the composition of the microbiome to favor health-promoting bacteria. In addition to metabolic and nutritional links, emerging evidence suggests that the microbiome plays a significant role in the gut-skin axis a pathway where gut health influences skin conditions such as acne and psoriasis. Research indicates that a balanced microbiome in the gut may prevent or alleviate skin disorders by modulating systemic inflammation and immune responses. prebiotics targeted toward enhancing gut health are increasingly employed as therapeutic strategies to influence skin conditions, illustrating the interconnectedness of gut health and skin integrity. Moreover, the microbiome's interaction with the immune system is another focal area of research. The gut microbiota plays a crucial role in educating the immune cells, thus preparing the body to respond effectively to pathogens while maintaining tolerance to non-

harmful entities, including food and commensal microorganisms. Dysbiosis has been linked to autoimmune diseases, where the failure of the immune system to distinguish between self and non-self can lead to damage to host tissues. This relationship emphasizes the microbiome's fundamental influence on immunomodulation, potentially offering insights into novel therapeutic approaches for autoimmune conditions. The interaction of our environment with the microbiome must also be acknowledged. Outdoor microbiomes, shaped by environmental factors, can influence the composition of human-associated microbiomes, leading to varying health outcomes for individuals based on their living conditions. For instance, urban living often exposes individuals to a less diverse microbiome as opposed to rural settings, which might offer more varied microbial exposure. This highlights the need for comprehensive studies examining how lifestyle and socio-economic factors contribute to microbiome diversity and health disparities. As the field of microbiome research progresses, the complexities and untapped potential of microbial treatments are coming to light. Innovations such as microbiome therapy and personalized probiotic regimens are emerging as promising avenues for managing various health conditions. Despite the challenges of translating microbiome research into clinical practice partly due to the vast diversity of the microbiota and the unfamiliarity of many symbiotic relationships an interdisciplinary approach encompassing microbiology, nutrition, immunology, and genomics could herald new strategies for disease prevention and health optimization. Moreover, the implications of microbiome research extend beyond individual health to broader systemic issues. Microbiome studies increasingly address societal challenges, including aging, nutrition, and mental health, necessitating a holistic understanding of microbial interactions and their socio-economic contexts. For example, insights into how dietary habits in disadvantaged communities shape microbial health may inform interventions aimed at reducing disease prevalence linked to microbiome dysbiosis. The continual investigation into the complexities of the human microbiome shows promise for a future in which personalized medicine becomes the norm, targeting interventions based on an individual's unique microbial composition. Such personalized approaches could revolutionize not just treatment but also prevention strategies across diverse populations, improving health outcomes on a global scale. Harnessing the power of the microbiome in human health holds incredible potential. As we continue to decipher the roles these microorganisms play in our lives, integrating microbiome science into everyday health practices will pave the way for enhanced wellness and sustained disease resistance.

Diversity and Ecology of the Human Microbiome

The human microbiome is a complex and diverse assemblage of microorganisms that inhabit various niches in the human body. Understanding the diversity and ecology of these microbial communities is essential for elucidating their roles in health, disease, and human evolution. Microbial habitats within the human body are multifaceted, comprising distinct environments such as the gut, oral cavity, skin, and female reproductive tract, each with unique microbial compositions and adaptations. Studies has revealed that the gut microbiome is particularly significant due to its vast diversity and the varying functions it serves. Studies indicate that human gut microbiota exhibit a remarkable level of species richness, with phylogenetic analyses revealing complex communities that differ substantially across individuals. For instance, a comprehensive metagenomic study identified over 150,000 genomes across

diverse populations, highlighting extensive unexplored diversity within the microbiome. This richness allows for functional redundancy, which is vital for the stability and resilience of the microbiome against environmental changes and perturbations. Human microbiome diversity has undergone noticeable shifts due to changes in diet and lifestyle, particularly the transition from ancestral populations to modern urban settings. Compared to their wild counterparts, humans exhibit a reduction in microbial diversity that correlates with higher rates of metabolic disorders. The loss of dietary fibers in contemporary diets has also led to a diminished microbial diversity in housed primates, paralleling observations in human populations, and reinforcing the significance of diet in shaping the microbiome. This underscores the impact of ecological context and dietary habits in sustaining microbial diversity and the associated health benefits. Microbial communities in specific habitats also demonstrate niche-specific interactions and adaptations. For example, the oral microbiome reflects a unique assembly with core species varying from those found in the gut; the differentiation highlights how ecological factors shape community structures. In the vaginal microbiome, a dominant presence of specific Lactobacillus species is crucial for preventing infections and maintaining reproductive health. The distinct microbiota in the female reproductive tract is influenced by ecological pressures such as hormonal changes and microbial interactions during pregnancy, as well as genetic predispositions. Furthermore, the interaction between host genetics and microbial ecology plays a crucial role in microbiome diversity. Populations experiencing genetic bottlenecks tend to exhibit reduced microbiome diversity and poorer health outcomes, pointing to a relationship between host genetics and microbial ecosystem stability (Ørsted et al., 2022). The concept of functional redundancy among the microbiome underscores the importance of having a diverse set of species within these communities to maintain host health and provide resilience against environmental insults. In broader terms, ecological models such as the Diversity-Area Relationship (DAR) have been suggested to help understand how microbial diversity relates to health outcomes at the population level. While human microbiome diversity varies greatly among individuals, patterns in microbial community assembly and location provide insights into how lifestyle, ecology, and evolutionary history shape our microbial partners. The human microbiome represents a dynamic ecosystem that reflects evolutionary adaptations alongside ecological and anthropogenic changes. The diversity of microbial species in various habitats contributes to functional capabilities that are integral to human health, making it imperative to further explore how these communities affect and are affected by host physiology.

Development of the Microbiome Across the Human Lifespan

The human microbiome undergoes significant changes throughout the lifespan, beginning with colonization at birth and continuing through childhood, adulthood, and into aging. This dynamic process is influenced by various factors, including maternal health, mode of delivery, feeding practices, and environmental exposures. At birth, the infant microbiome is primarily shaped by maternal contributions. Studies have demonstrated that maternal vaginal and fecal microbiota can be transferred to the newborn during delivery, establishing the first microbial communities in the infant's gut. For instance, Ferretti et al. reported how microbial transmission occurs from different maternal body sites, influencing the infant's gut microbiome. Similarly, Ward et al. indicated that infant microbiota develops rapidly during the early days of life, with an increase in diversity over the first month. The mode of delivery plays a crucial role; infants born

via cesarean section often exhibit distinct microbial profiles compared to those delivered vaginally, receiving fewer maternal microbes, as noted by several studies (Foessleitner et al., 2024). Additionally, feeding practices, particularly breastfeeding, significantly affect the composition of the infant microbiome since breast milk contains specific oligosaccharides that encourage the growth of beneficial bacteria, such as Bifidobacteria. As children grow, their microbiome continues to evolve, becoming more complex and diverse, particularly around the transition to solid foods and the cessation of breastfeeding. Research has shown that early introduction of solid foods can lead to a shift in microbiome composition toward an adult-like state, especially when breastfeeding is reduced. The microbial environment of children is also affected by genetic background and socio-economic factors, which can further shape their microbial communities. Health outcomes in childhood have been associated with microbial diversity present during these formative years, suggesting a link to chronic conditions later in life. During adulthood, the gut microbiome stabilizes but remains susceptible to changes due to factors such as diet, lifestyle, antibiotic use, and environmental exposure. Some studies have reported that adult microbial communities exhibit significant variations based on chronic health conditions and antibiotic use, which can diminish microbial diversity and disrupt the balance of gut bacteria. The recognition of the microbiome as an ancillary organ underscores its importance in host health, with implications for digestion, immune function, and mental health via the gut-brain axis. In aging, changes in the microbiome often manifest as a decrease in diversity and beneficial microorganisms. Elderly individuals may experience microbiome shifts that predispose them to various health issues, such as inflammation and increased susceptibility to infections. Literature suggests that the gut microbiome of older adults is less stable than that of younger individuals, which may be linked to factors such as decreased dietary diversity and increased medication use. This instability may lead to dysbiosis, which has been associated with a range of age-related conditions. The development of the microbiome across the human lifespan is a complex interplay of maternal influences, dietary transitions, and external factors, shaping individual health trajectories. The initial colonization of the microbiome at birth and its subsequent maturation during early childhood are critical for establishing a foundation that influences health throughout life.

Technological Advances in Microbiome Research

The evolution of microbiome research has significantly accelerated with technological advances, particularly with the development of culture-independent methods that allow for in-depth analysis of microbial communities. This evolution began with traditional culturomics, which focused on culturing microorganisms, but has now shifted toward comprehensive omics approaches such as metagenomics, meta-transcriptomics, and metabolomics. Each of these methodologies provides a unique view into microbial communities, enhancing our understanding of their diversity, functionality, and interactions within ecosystems. Metagenomics is a fundamental technique that involves the sequencing of genetic material directly from environmental samples, thereby bypassing the need for cultivating microbes in laboratory settings. This method allows researchers to capture the full diversity of microbial communities, revealing previously unrecognized taxa and functional capabilities. For instance, Manor and Borenstein highlight how metagenomic processing can uncover biologically significant functional variation

within the human microbiome, which could serve as a unique signature for individuals over time. Furthermore, Asnicar et al. demonstrated the relationship between the gut microbiome and host metabolism, providing insights into how specific microbial profiles correlate with dietary habits. These insights underscore the potential of metagenomics not only to identify microbial diversity but also to correlate it with physiological states or conditions. Moreover, the use of shotgun sequencing in metagenomics has been shown to outperform traditional methods by identifying a broader array of microbial genera. Shotgun metagenomic sequencing of salivary samples revealed more complex microbial compositions compared to targeted amplicon sequencing approaches. Such findings emphasize the importance of employing diverse sequencing methodologies to gain deeper insights into microbiome complexities. Meta-transcriptomics, involving the analysis of RNA transcripts from microbial communities, allows researchers to investigate microbial activity and functional expression in situ. This method addresses the dynamic nature of microbial communities by providing insights into which genes are actively being expressed under specific environmental conditions. For example, the work by Li et al. elaborated on the application of meta-transcriptomics in ruminants, illustrating how microbial metabolic functions are linked to host physiology. The combination of RNA sequencing with metagenomic data creates a more nuanced understanding of how microbial communities respond to various stimuli. Furthermore, the integration of metabolomics complements metagenomic and meta-transcriptomic analyses by allowing researchers to characterize the metabolites produced by microbial communities. This multi-omics strategy captures a holistic view of microbiomes, linking genetic potential and functional output to the metabolic profiles observed in different health or disease states. For instance, studies have indicated that metabolomic profiling in conjunction with genomic data can reveal biomarkers for various diseases, including obesity and inflammatory bowel diseases. Overall, the advancement of these culture-independent methods has transformed microbiome research, enabling the exploration of complex interactions within microbial ecosystems and their hosts. The transition from culture-dependent methods to these sophisticated omics approaches signifies a pivotal shift towards a more integrated understanding of microbiomes, heralding new opportunities for discovering disease markers and therapeutic targets while unraveling the intricacies of microbial ecology.

Host–Microbe Interactions

The intricate dynamics underlying host-microbe interactions encompass a multitude of communication mechanisms facilitated by signaling pathways, microbial metabolites, and immune modulation. This analysis synthesizes relevant literature to explore how these factors contribute to the regulation and communication within host-microbe systems. Host-microbe interactions are primarily mediated through complex signaling pathways that the host and microbes employ to communicate and regulate physiological functions. Various plant-associated microbes utilize hormone signaling pathways, such as ethylene and auxin, to optimize plant growth. Symbiotic interactions among plants, bacteria, and fungi can modulate plant hormonal statuses, enhance nutrient uptake and alter metabolic pathways, culminating in increased biomass and productivity in plants (Rawat et al., 2025). These modifications in hormonal signaling not only bolster growth but also establish a communication network between the host and the microbial community that influences the health and resilience of the plant (Rawat et al., 2025).

This reciprocal signaling framework extends to animal models as well, where the intestinal microbiota influences the host's immune responses through similar mechanisms, underpinning the regulatory mechanisms essential for maintaining homeostasis. Microbial metabolites serve as critical components in the communication and regulatory dialogues between hosts and their associated microbiomes. Studies indicate that microbial metabolites like short-chain fatty acids (SCFAs) produced by gut microbiota contribute significantly to host metabolic processes. These metabolites can influence host gene expression, modulate immune responses and potentially offer protective effects against various diseases. The role of these metabolites in immunological modulation is emphasized by the work of Kamada et al., who illustrate how SCFAs enhance intestinal barrier function and promote anti-inflammatory responses, demonstrating a link between microbe-derived metabolites and the host immune landscape. Additionally, these interactions are complex; metabolites can also have detrimental effects under certain conditions, illustrating the dual nature of these metabolic communications. Epigenetic modifications represent another layer of interaction, where microbial influences can induce changes affecting host gene expression without altering the DNA sequence. Rajeev et al. discuss how epigenetic interactions between microbes and mammalian hosts result in substantial modulations of host genes, which can lead to altered cellular functions and influence disease susceptibility. This epigenetic cross-talk embodies a vast network of interactions where shifts in microbial composition affect the host's gene regulatory fields, modulating responses to external stressors, including pathogenic challenges. The evolutionary implications of such interactions reiterate the necessity for a nuanced understanding of host-microbe relationships. The immune system of the host is intricately intertwined with microbial interactions, playing a pivotal role in both disease resistance and susceptibility. Research by Bolnick et al. delineates how variations in Major Histocompatibility Complex (MHC) class II molecules can dictate the composition and diversity of gut microbiota, influencing immune responses. Innovations in understanding these immunological interactions reveal that innate immune mechanisms, particularly those involving pattern recognition receptors like Toll-like receptors (TLRs), engage with microbial communities to initiate protective responses. This immune engagement shapes the microbial community structure, promoting beneficial microbial taxa while suppressing potential pathogens. Moreover, O‘Keeffe et al. highlight those infections with co-occurring parasites can elicit immune responses that modulate interaction dynamics among microbial partners, leading to complex outcomes in disease progression. Hosts and microbes engage in a complex molecular dialogue mediated by biochemical signals and regulatory networks, shaping outcomes ranging from mutualistic coexistence to disease as illustrated in Figure 1 (Chisholm et al., 2006).

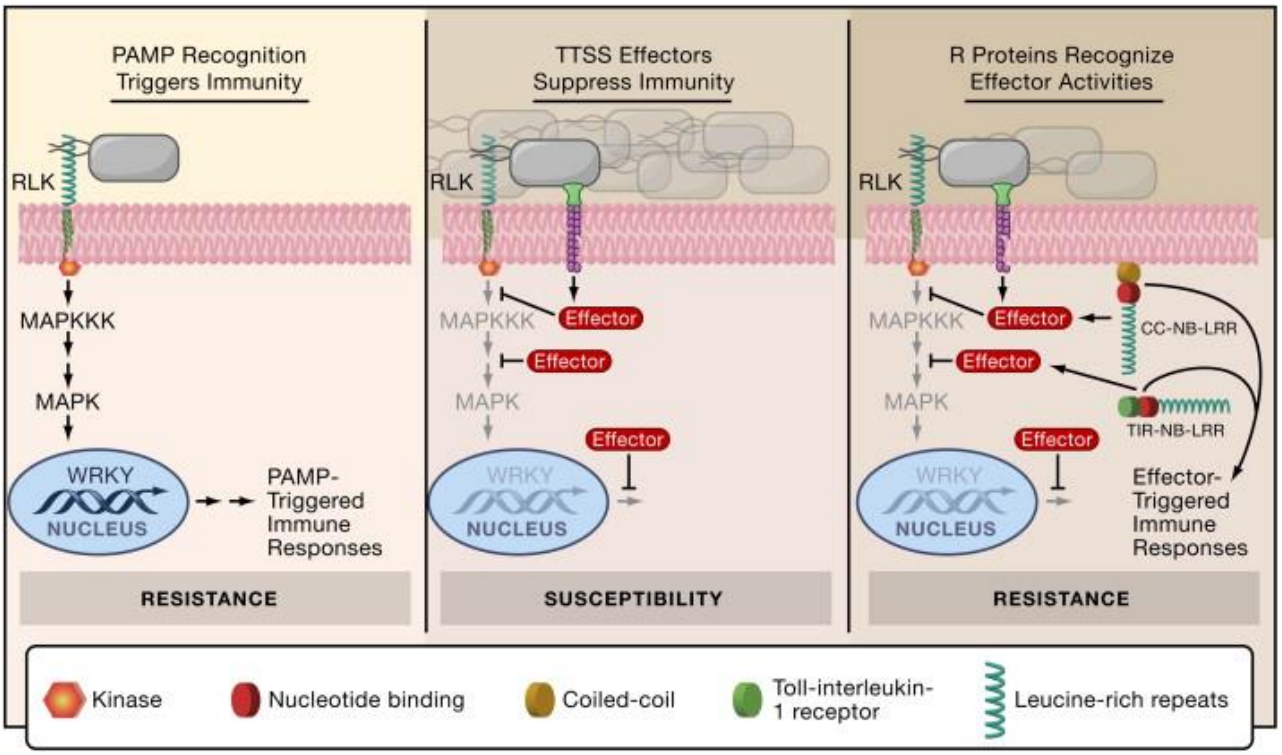


Figure 1. Molecular Dialogue Between Hosts and Microbes: Communication and Regulation Mechanisms (Chisholm et al., 2006)

Within these host-microbe ecosystems, a rich network of microbial interactions, including intra-species and inter-species associations, influences health and disease states. Baishya et al. present compelling evidence that both beneficial and harmful bacterial interactions contribute to host health by determining community characteristics and functionalities. These microbiota communities are dynamic, driven by competitive and cooperative interactions that can alter disease outcomes significantly. The work of Poudel et al. further illustrates how establishing synthetic bacterial communities can enhance plant health, suggesting that engineered microbial consortia can optimize beneficial interactions while disfavoring pathogens. The interplay between microbial communication and host defenses highlights the dual role that microbes play in promoting health and exacerbating disease. Fischbach and Segre review how interspecies signaling within microbial communities can dictate health outcomes, emphasizing the need for understanding these interactions in the context of developing therapies to restore microbial balance. Additionally, the potential for microbial metabolites to modulate host defense mechanisms opens avenues for therapeutic interventions aimed at exploiting these interactions to enhance disease resistance. Moreover, the evolutionary aspects of host-microbe interactions are critical for understanding their long-term consequences on microbial and host fitness, as suggested by King and Bonsall, where defensive microbes may influence the coevolution of host-parasite systems, underscoring the adaptive significance of these interactions. Such insights into the evolutionary trajectories of host-microbe relationships emphasize that microbes are not merely passengers but integral players in the health and evolutionary strategies of their hosts. The multifaceted interactions between hosts and microbes, characterized by signaling pathways, metabolic exchanges, and immune modulation, represent a cornerstone of biological functionality

across diverse ecosystems. The literature illustrates how microbes profoundly influence host physiology and how these interactions are finely tuned through evolutionary processes to facilitate resilience and adaptability. Future research should focus on unraveling the complexities of these interactions, particularly the implications of synthetic communities and their applications in therapeutic contexts.

The Gut Microbiome and Gastrointestinal Health

The human gut microbiome plays a crucial role in digestion, nutrient absorption, and the development of gastrointestinal (GI) diseases. Understanding the interactions between the microbiome and the host is vital for maintaining gastrointestinal health, as dysbiosis may contribute to various disorders. The gut microbiome, which consists of trillions of microorganisms, significantly affects the digestion and metabolism of nutrients. Comess and Abad-Jorge provide an overview of the gut microbiome's composition and its substantial impact on health and disease processes, highlighting that the gut, particularly the colon, serves as the primary habitat for these microbial communities influenced by dietary choices, environmental factors, and lifestyle (Comess & Abad-Jorge, 2023). One well-documented function of the gut microbiome is its role in the metabolism of carbohydrates and lipids. How specific gut bacteria can alter energy extraction from dietary sources, influencing obesity and metabolic health. They emphasize that microbial compounds can modify bile acid signaling, leading to unique metabolic profiles that affect fat storage and insulin resistance. These interactions demonstrate the gut microbiome's role in regulating host metabolism and indicate its potential impact on treatment approaches for obesity and diabetes. Additionally, the gut microbiome is critical for maintaining the integrity of the gastrointestinal barrier, which is essential for preventing pathogen translocation and supporting nutrient absorption. The gut microbiota provides protective benefits against pathogens by enhancing the structure and functionality of the gut barrier. Dysfunction in this system, referred to as dysbiosis, can lead to increased intestinal permeability, which contributes to GI disorders such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) (Shang et al., 2024). The relationship between the gut microbiome and the immune system is also vital for gastrointestinal health. Studies indicate that dysbiosis is frequently linked to increased inflammation and a higher prevalence of conditions like IBD and other GI disorders (Shang et al., 2024). Moreover, the gut microbiome modulates immune functions through metabolites, such as short-chain fatty acids (SCFAs), which help regulate inflammatory pathways and maintain mucosal integrity. The gut-brain axis exemplifies the complexity of host-microbe interactions concerning gastrointestinal health. Mayer et al. explore how fluctuations in gut microbiota are connected to brain functions and behaviors, affecting conditions such as IBS. Perturbations in the gut microbiome not only correlate with gastrointestinal symptoms but may also have psychological implications, highlighting the importance of holistic approaches in treating disorders linked to both GI and mental health. Research emphasizes the influence of age and lifestyle factors on gut microbiome composition. Microbial diversity often decreases with age, which may affect susceptibility to gastroenterological diseases (Shang et al., 2024). This observation underscores the dynamic nature of the gut microbiome and the necessity for ongoing evaluation of how microbial communities adapt to physiological and environmental changes throughout life. Overall, the gut microbiome significantly contributes to digestion, nutrient

absorption, and the prevention of gastrointestinal diseases. By maintaining a balanced microbial community, the gut microbiome supports metabolic functions, intestinal health, immune responses, and overall wellness. Figure 2, illustrates the gut microbiome exerts a profound influence on gastrointestinal physiology, facilitating the enzymatic breakdown of complex carbohydrates, enhancing nutrient absorption, modulating mucosal and systemic immune responses, and maintaining intestinal barrier integrity (Afzaal et al., 2022).

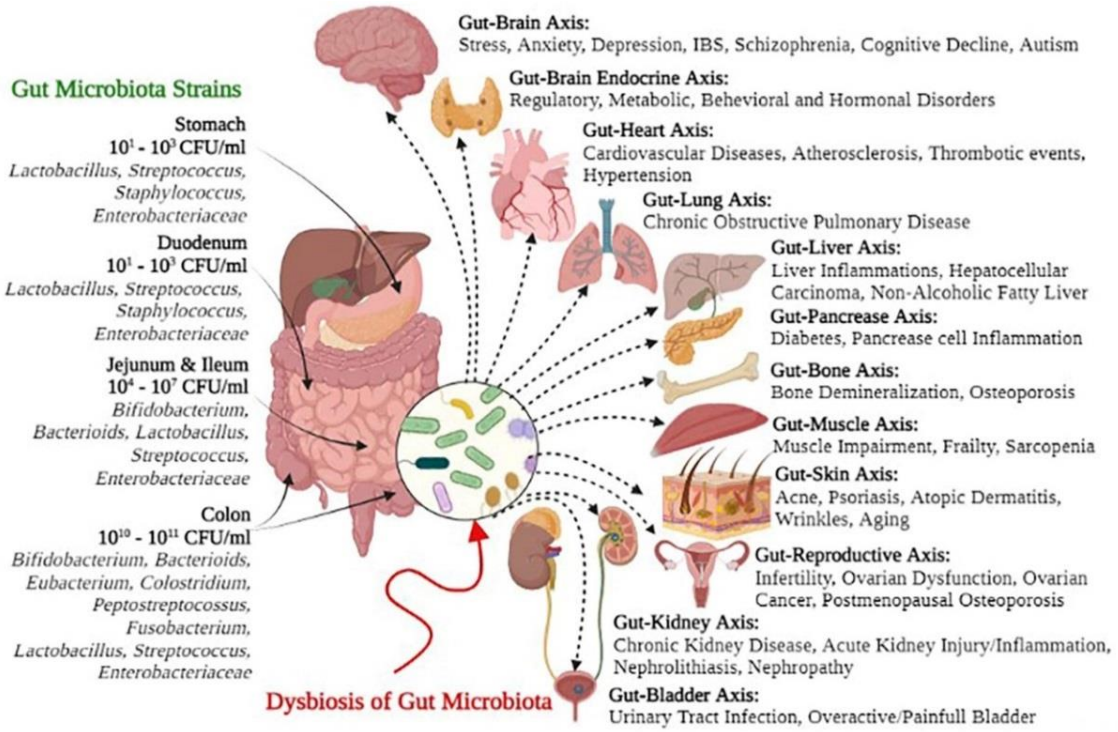


Figure 2. The Gut Microbiome and Its Impact on Gastrointestinal Functions (Afzaal et al., 2022)

Microbiome and the Immune System

The human microbiome plays a vital role in educating and regulating immune responses, establishing a complex interplay with the host's immune system that significantly influences health and disease. Numerous studies have highlighted how the microbiome fosters immune development and homeostasis, underscoring its importance as a modulator of immune responses. First and foremost, the composition and balance of the gut microbiota are critical for maintaining immune homeostasis. The gut microbiome comprises a diverse array of microorganisms that interact cooperatively with the immune system, facilitating the development of both innate and adaptive immunity. These microorganisms contribute to the production of secondary metabolites, such as short-chain fatty acids (SCFAs), which have been shown to promote regulatory T cell differentiation and enhance anti-inflammatory responses. In particular, SCFAs foster the integrity of the intestinal barrier, which plays a crucial role in preventing systemic inflammation. Moreover, dysbiosis, characterized by an imbalance in microbial populations, has significant repercussions for immune regulation. It has been associated with various inflammatory diseases, including inflammatory bowel diseases (IBD) and autoimmune disorders. In these contexts, dysbiosis can lead to heightened immune responses and chronic inflammation as a result of

dysregulation of the gut epithelial barrier, which may precipitate microbial translocation and undermine mucosal immune function. Specifically, the microbiome‘s composition has a pivotal role in modulating inflammatory responses; for instance, the presence of certain microbial derivatives can either escalate or mitigate inflammatory pathways. Recent research has demonstrated that the microbiome's influence extends beyond the gastrointestinal tract, affecting systemic immunity and even neural functions through what is termed the microbiota-gut-brain axis. Microbial metabolites, such as those produced by specific gut bacteria, can affect neuroimmune interactions, modulating the activation states of microglia and influencing the risk of neurodegenerative conditions (Warren et al., 2024). This broader context highlights the microbiome‘s potential as a therapeutic target, not only for gastrointestinal diseases but for neuroimmune conditions as well. Furthermore, studies have indicated that immune responses to specific vaccines may be contingent upon the status of the gut microbiome, suggesting that tailored microbiome-centered interventions could enhance vaccine efficacy. For instance, infants with a diverse and resilient gut microbiota demonstrated enhanced immune responses to the rotavirus vaccine, illustrating the critical link between microbiome composition and vaccine-induced immunity. The microbiome plays a pivotal role in educating and regulating host immune responses through multiple mechanisms, including the production of immunoregulatory metabolites, maintaining intestinal barrier integrity, and influencing systemic immune behaviors. The interplay between the microbiome and the immune system is complex and crucial for understanding various pathologies, paving the way for innovative therapeutic approaches centered on microbiome modulation.

The Microbiome–Brain Axis

The microbiome-brain axis represents a significant area of research illustrating the complex relationship between gut microbiota and brain function, with implications for mental and neurological health. This connection is bidirectional: changes in the microbiome can significantly influence neural activity, mood, and cognitive functions, and vice versa. Research has particularly focused on how gut microbiota may contribute to various mental disorders, including depression, anxiety, and autism spectrum disorders (ASD). Gut microbiota communicates with the central nervous system (CNS) through multiple pathways, including immune responses and neuroendocrine signaling. The impact of microbiome composition on mood and behavior can be attributed to metabolites produced by gut bacteria, such as short-chain fatty acids (SCFAs), which have been shown to affect brain function. For instance, a study by Bravo et al. indicated that probiotics like *Lactobacillus rhamnosus* can modulate emotional behavior in mice via the vagus nerve, suggesting a physiological mechanism for how gut microbiota influences mood and anxiety. Furthermore, evidence suggests that dysbiosis, or changes in gut microbiota composition, is associated with an increased prevalence of psychiatric conditions like depression and anxiety, highlighting its potential as a target for therapeutic interventions. Specific disorders, such as ASD, have drawn attention as some subpopulations exhibit gastrointestinal disturbances alongside neurological symptoms. Research indicates that alterations in gut microbiota can directly affect social behavior and synaptic plasticity in animal models, underscoring the potential for microbiome modulation as an intervention strategy. Moreover, the interaction between the gut and brain, via the vagus nerve and immune signaling pathways, contributes not only to behavioral

adaptations but also to cognitive development and function throughout the lifespan. Prenatal and early life factors also play a crucial role in establishing the microbiota and its subsequent influence on brain development, further emphasizing the importance of this axis in psychiatric health from an early age. Clinical trials exploring probiotic supplementation have shown promise in enhancing mental well-being among various patient populations, including postpartum women, where *Lactobacillus rhamnosus* supplementation has been associated with reduced symptoms of postpartum depression. Additionally, the impact of dietary components on the microbiome and subsequent mood regulation is important, as fermented foods known to enhance gut health have also been linked to improved mood and cognitive functioning in adults (Kargbo, 2023). The microbiome-brain axis is a complex system with significant implications for understanding and treating mental health disorders. The interplay between gut bacteria, the CNS, and behaviors underscores the important role of microbiota in regulating emotional states and cognitive processes, presenting a novel frontier for therapeutic strategies in psychiatry.

Skin and Oral Microbiomes in Health and Disease

The skin and oral microbiomes are essential components of human health, influencing both local and systemic diseases. The microbial communities in these regions demonstrate diverse compositions, which can be altered in various pathological conditions. The skin microbiome is predominantly composed of phyla such as Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes, which have been consistently documented across studies examining healthy and diseased states, particularly psoriasis. Psoriasis is marked by significant dysbiosis characterized by reduced microbial diversity and an elevated presence of specific bacterial taxa. These alterations have been linked to immune system responses, particularly the polarization of CD4+ T helper cells towards a Th17 phenotype, which exacerbates inflammation. In contrast, the oral microbiome is marked by a high degree of diversity but similar patterns of dysbiosis can be observed in various systemic conditions, including alcohol use disorder. Research has noted that treatments affecting the microbiome, such as systemic antibiotics, can lead to significant alterations in both the skin and oral microbiomes, potentially leading to increased antimicrobial resistance. This phenomenon underscores the delicate balance of microbial communities and how systemic medications may disrupt this homeostasis. Moreover, the interaction between the skin and gut microbiomes plays a critical role in skin health and disease. The "gut-skin axis" posits that dysbiosis in the gut can lead to systemic inflammation that adversely affects skin homeostasis (Wagner et al., 2024). Studies show that interventions targeting the gut microbiome, such as the administration of probiotics, can positively influence skin health by restoring a balanced microbiome state. Conversely, conditions such as atopic dermatitis illustrate how microbial diversity is closely linked to disease severity, where a compromised skin microbiome correlates with higher disease severity scores. Recent investigations have further revealed the implications of environmental factors, including skincare routines and topical applications, on the skin microbiome. For instance, specific skincare regimens have been shown to foster favorable shifts in microbial communities that enhance skin health. In infants, factors such as delivery mode and early topical interventions can significantly shape the initial establishment of the skin microbiome, reflecting its adaptable nature. The intricate dynamics of the skin and oral microbiomes highlight their profound impact on human health. Alterations in these microbial

communities can have local effects, such as skin diseases, and systemic repercussions linked to various metabolic and inflammatory conditions. Ongoing research is crucial to comprehend these interactions and develop targeted therapies that leverage microbiome modulation for improved health outcomes.

The Urogenital Microbiomes

The vaginal and urogenital microbiomes are increasingly recognized for their crucial roles in reproductive health, particularly in aspects such as fertility, pregnancy outcomes, and susceptibility to infections. A growing body of research indicates that these microbiomes significantly influence reproductive processes. One of the primary factors in fertility is the composition of the vaginal microbiome. Lactobacilli, particularly Lactobacillus crispatus and Lactobacillus gasseri, are dominant in healthy vaginal microbiota and have been identified as protective agents against infections. Studies have demonstrated that the presence of these beneficial bacteria can create an optimal environment for sperm survival and motility, thereby increasing the chances of conception (Wang et al., 2024). Conversely, a dysbiotic microbiome characterized by a higher diversity of bacterial species, which may include pathogens, is frequently associated with decreased fertility and adverse reproductive outcomes (Wang et al., 2024). Inflammatory cytokines, which are upregulated in cases of dysbiosis, have been implicated in infertility by creating an inhospitable environment for embryo implantation (Hong et al., 2022). During pregnancy, the vaginal microbiome undergoes notable changes, becoming less diverse and predominantly dominated by Lactobacilli. This shift is believed to play a protective role against infections that could compromise fetal health. The endometrial microbiome, although less studied, also appears to influence pregnancy success. Research suggests that the endometrial environment, informed by its microbiome, is significant during embryo implantation, which can potentially affect the success rates of assisted reproductive technologies (ART) such as in vitro fertilization (IVF). Moreover, maintaining a balanced bacterial population is essential for preserving the integrity of pregnancy and preventing complications such as preterm birth. A diverse microbial community in the vaginal tract has been linked to higher rates of preterm labor and delivery. Thus, the type and quantity of microorganisms present play a pivotal role in mediating immune responses that can either protect or harm the pregnancy. The interaction between reproductive health and the vaginal microbiome also extends to susceptibility to various infections. A healthy vaginal microbiome serves as the first line of defense against pathogens, maintaining local immunity through the production of substances that inhibit pathogen colonization. Dysbiosis is associated not only with infertility and poor pregnancy outcomes but also with higher susceptibility to urogenital infections such as bacterial vaginosis and urinary tract infections. Research has illustrated that women with lower Lactobacillus dominance may experience higher rates of these infections, suggesting a direct link between microbiota composition and overall reproductive health. Emerging evidence underscores the importance of personalized approaches that consider the vaginal microbiome's role in fertility treatments and reproductive healthcare. Monitoring and potentially correcting dysbiosis before and during pregnancy could enhance reproductive outcomes and reduce the risk of infectious complications. Overall, an integrated perspective on the vaginal microbiome as a dynamic participant in reproductive health can provide novel insights into future clinical practices and treatments.

Microbiome and Metabolic Disorders

The interplay between the gut microbiome and metabolic disorders such as obesity, diabetes, and non-alcoholic fatty liver disease (NAFLD) has garnered significant attention in recent years. As an integral component of host metabolism, the gut microbiome's composition can profoundly influence metabolic health through mechanisms including immune modulation, energy extraction from foods, and the production of metabolites that affect metabolic pathways.

Dysbiosis, characterized by an imbalance in microbial communities, is increasingly recognized as a major contributor to metabolic syndromes. Studies indicate that specific microbial populations and their metabolites can serve as biomarkers for conditions like obesity and type 2 diabetes mellitus (T2DM). For instance, the presence of certain bacteria from the genus Clostridium has been positively correlated with obesity, while a reduction in diversity such as an increase in the Firmicutes/Bacteroidetes ratio has been reported in obese individuals. Additionally, high-caloric diets, particularly those rich in fats and sugars, exacerbate dysbiosis, leading to impaired metabolic function and increased inflammation. Moreover, microbial metabolites play crucial roles in regulating metabolism. Short-chain fatty acids (SCFAs), produced during the fermentation of dietary fibers by gut bacteria, have been shown to enhance insulin sensitivity and exert anti-inflammatory effects. Conversely, microbial alterations associated with obesity and T2DM can affect bile acid metabolism, further contributing to metabolic dysfunction. Notably, interventions targeting the gut microbiome, such as pre- and probiotics, have shown promise in ameliorating these conditions, highlighting the potential for microbiome-targeted therapies in managing metabolic disorders (Stadlbauer, 2023). The connection between microbiome dysbiosis and NAFLD is particularly compelling. Alterations in gut microbiota have been implicated in the pathogenesis of NAFLD, which is often regarded as the hepatic manifestation of metabolic syndrome. Evidence suggests that certain gut bacteria may influence hepatic lipid metabolism, thereby contributing to the development of fatty liver disease. For example, increased abundance of certain microbes has been linked to heightened blood alcohol levels in NAFLD patients, implicating a novel mechanism through which gut bacteria might exacerbate liver inflammation and damage. Additionally, maternal obesity and gut microbiota composition can have transgenerational effects on offspring metabolism, indicating a complex interplay that merits further investigation. Thus, targeting the microbiome may offer novel strategies for preventing or treating metabolic disorders not only in the existing population but also in future generations. The gut microbiome serves as a crucial mediator between diet, metabolic health, and disease pathology. As research progresses, understanding these mechanisms will be key to developing effective preventive and therapeutic measures for obesity, diabetes, and NAFLD.

Microbiome and Autoimmune and Inflammatory Diseases

The gut microbiome has become recognized as a significant contributor to the pathogenesis of autoimmune and inflammatory diseases, including Inflammatory Bowel Disease (IBD), rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE). Dysbiosis, or microbial imbalance in the gut flora, is consistently linked to the onset, progression, and severity

of these conditions, indicating that microbiome composition may influence disease outcomes through various immunological and metabolic pathways. In the context of IBD, research indicates notable alterations in gut microbiome composition. Studies have shown that patients with Crohn‘s Disease (CD) and ulcerative colitis exhibit decreased diversity of gut microbes alongside an increase in pathogenic bacterial populations such as certain strains of Enterobacteriaceae (Andoh & Nishida, 2022). These changes may exacerbate intestinal inflammation by elevating the production of pro-inflammatory cytokines. Specifically, an increase in aerobic and facultative anaerobes is observed in inflamed tissues, while beneficial anaerobes are reduced, resulting in a feedback loop that perpetuates inflammation. Additionally, the presence of specific microbial communities has been associated with the production of metabolites that can modulate immune responses, thus influencing clinical manifestations of IBD. These microbiome-related mechanisms also apply to systemic autoimmune conditions such as RA and SLE. In RA, alterations in gut microbiota have been linked to the promotion of systemic inflammation, potentially due to the gut‘s role in shaping immune responses. Specific bacterial populations are found to be more prevalent in RA patients, with these changes correlated to the presence of certain autoantibodies. Similarly, dysbiosis in the gut microbiome of individuals with SLE has been suggested to affect systemic inflammation and immune dysregulation, emphasizing potential therapeutic targets for disease management. Moreover, genetic predispositions, especially those related to specific human leukocyte antigen (HLA) genotypes, affect an individual‘s gut microbiome composition, which is intertwined with the development of autoimmune diseases. Certain HLA haplotypes may shape microbial communities in a way that creates an environment favorable for autoimmunity prior to the manifestation of clinical symptoms. This illustrates the importance of the interplay between genetic risk factors and microbial environments in determining susceptibility to these diseases. Notably, findings indicate that the early establishment of gut microbiota can influence the likelihood of developing autoimmune conditions. For example, dysbiosis has been detected prior to the onset of autoantibodies in children at risk for developing autoimmune diseases, suggesting a potential window for preventive interventions. The gut microbiome plays a critical role in the pathogenesis of autoimmune and inflammatory diseases through mechanisms related to immune modulation, metabolic alterations, and genetic predisposition. This intricate interaction suggests that therapeutic strategies aimed at manipulating the gut microbiome could offer promising avenues for managing conditions such as IBD, rheumatoid arthritis, and systemic lupus erythematosus.

Microbiome and Cancer

The emerging role of the microbiome in cancer is a burgeoning field of research, providing critical insights into oncogenesis, tumor progression, and therapeutic outcomes. Recent studies indicate that the composition of the microbiome not only influences cancer initiation and development but also affects the efficacy of treatments such as immunotherapy and chemotherapy. A prominent aspect highlighted in recent literature is the association between the tumor microbiome and host immune responses. For example, investigations into the tumor microbiome have revealed its potential impact on cancer prognosis. Specifically, a study utilizing weighted gene co-expression network analysis showed how variations in the tumor-associated microbiome can be correlated with mutations in oncogenes, suggesting that certain microbiome

profiles may predispose tumors to specific immune evasion mechanisms (Guan et al., 2023). This correlation is especially noted in colorectal cancer, where microbial signatures varied according to KRAS mutation status, emphasizing the need to consider microbial influences in tumor biology (Guan et al., 2023). Moreover, the microbiome's modulation of the immune environment plays a critical role in therapeutic outcomes. For instance, specific microbes in the tumor microenvironment can influence the behavior of immune cells, such as T cells and myeloid-derived suppressor cells. This modulation affects how tumors respond to immunotherapies like PD-L1 and CTLA-4 inhibitors. The presence of beneficial microbiota can enhance the immune response against tumors, while dysbiosis may contribute to a more immunosuppressive microenvironment, diminishing treatment efficacy.

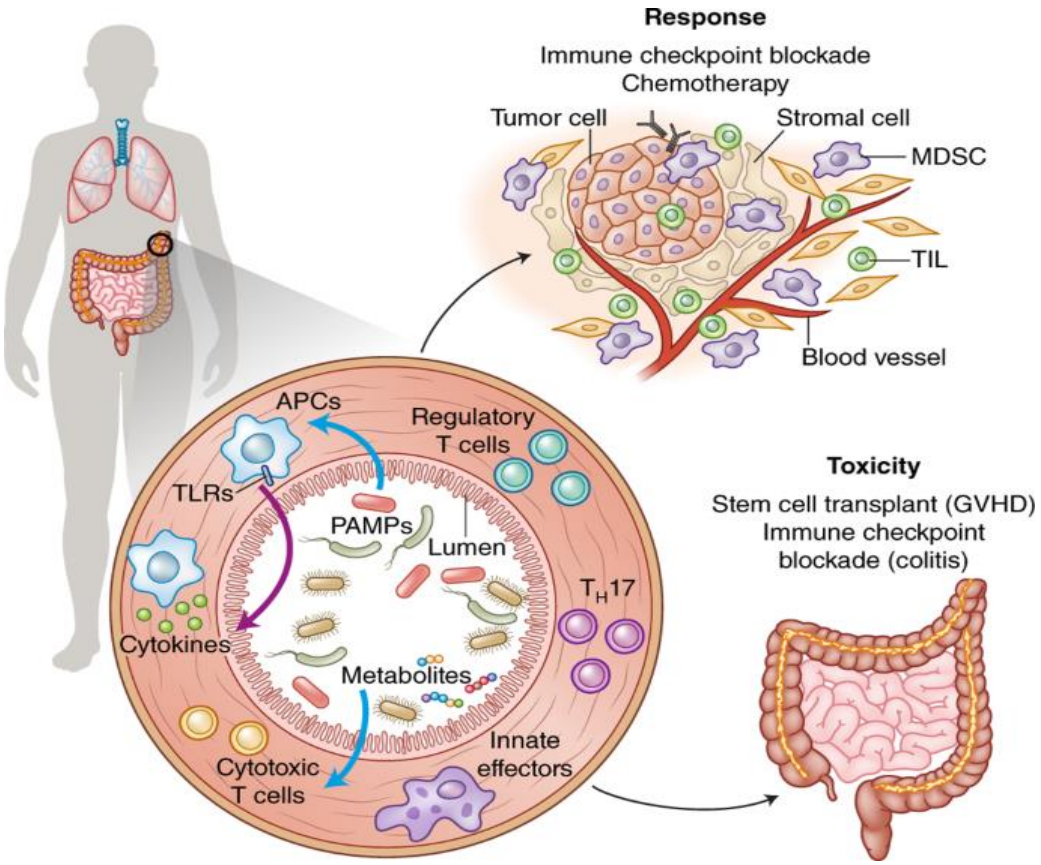


Figure 3. Role of the Microbiome in Cancer: Pathogenesis, Progression, and Therapy (Helmink et al., 2019)

Chemotherapy outcomes have also been linked to the gut microbiome's composition. Studies have shown that diverse gut microbiota can enhance the effectiveness of chemotherapy by combating the adverse effects commonly associated with treatment, such as gastrointestinal toxicity and systemic inflammation. Notably, a metagenome association study focused on lung cancer patients found specific gut microbial features that could predict responses to chemotherapy, underscoring the microbiome's potential as a biomarker for treatment outcomes. Similarly, cervicovaginal microbiome diversity has been associated with improved survival rates in patients undergoing chemoradiation, suggesting that targeting the microbiome may provide additional therapeutic strategies. Investigations into the oral microbiome have also revealed

significant associations with gastrointestinal and other cancer risks. Emerging evidence suggests that oral bacteria may play a role in carcinogenesis, particularly in colorectal cancer. Identifying microbial profiles associated with cancer could lead to innovative screening methods and preventative strategies. Figure 3, depicts the microbiome can influence cancer development and progression by modulating inflammatory pathways, shaping antitumor immunity, altering host metabolism, and affecting responses to immunotherapy and chemotherapy (Helmink et al., 2019). The microbiome is an intricate factor influencing the etiology, progression, and therapeutic responses in cancer. Its role in modulating immune responses, affecting tumor microenvironments, and contributing to treatment outcomes represents a promising avenue for future research and clinical application. Efforts to understand these relationships further could pave the way for novel therapeutic interventions targeting the microbiome in cancer care.

Environmental, Dietary, and Lifestyle Influences on the Microbiome

The human microbiome is profoundly influenced by environmental, dietary, and lifestyle factors, which can shape microbial communities over time in various ways. Research demonstrates that these external influences can affect not only the microbial composition but also the functions these microbes perform, which ultimately impacts human health. One of the most significant environmental factors shaping the gut microbiome is exposure to diverse microbiota throughout life, commonly referred to as the exposome. Studies indicate that factors such as rural living, pet ownership, green space exposure, and socioeconomic status contribute positively to microbiome diversity and health. These observations align with the microbiome diversity hypothesis, which proposes that reduced exposure to microbiota correlates with an increased incidence of autoimmune diseases and allergies. In contrast, modern urban lifestyles often involve limited exposure to diverse microbial environments, potentially leading to reduced microbial diversity. Diet is another critical determinant of microbiome composition. High-fiber diets rich in fruits, vegetables, and fermented foods promote the growth of beneficial microbes such as Bifidobacteria and Lactobacilli, as evidenced by numerous studies. In contrast, diets high in saturated fats, sugars, and processed foods are associated with dysbiosis, characterized by decreased diversity and an increase in potentially harmful bacteria. Notably, the association between diet and microbiome composition is influenced by dietary preferences that can have heritable components, indicating that genetic factors may also play a role in dietary impacts on the microbiome. Lifestyle choices, including physical activity and medication usage (especially antibiotics), significantly affect microbial communities. Regular physical activity has been linked to a more diverse gut microbiome, which is conducive to better health outcomes. Antibiotics are known to disrupt microbiome balance, leading to decreased biodiversity and the potential overgrowth of resistant or pathogenic species. Furthermore, studies have shown that external abiotic factors, including seasonal variations and environmental conditions, can significantly influence the gut microbiome (Liukkonen et al., 2024). Additionally, migration and acculturation significantly influence microbiome composition, particularly in immigrant populations. Those who adopt a Western lifestyle, characterized by processed diets and urban environments, often experience a decline in microbial diversity. This exemplifies how lifestyle transitions can result in discernible shifts in the microbiome, affecting the overall health of individuals. The shaping of microbial communities within the human microbiome is a dynamic process influenced by a

myriad of environmental, dietary, and lifestyle factors. Understanding these influences not only elucidates the complexities of human health and disease but also opens avenues for targeted interventions aimed at modulating the microbiome for better health outcomes.

Antibiotic Use, Dysbiosis, and Antimicrobial Resistance

The overuse of antibiotics profoundly impacts human health by perturbing the microbiome and contributing to the growing concern of antimicrobial resistance (AMR). Antibiotics are often prescribed excessively, leading to alterations in microbial communities that can have both immediate and long-term consequences. One of the most significant effects of antibiotic overuse is dysbiosis, a condition characterized by an imbalanced gut microbiome. Antibiotics indiscriminately target a wide range of bacteria, including beneficial commensals that play crucial roles in digestion, immunity, and overall health. Research indicates that antibiotic-induced dysbiosis can lead to gastrointestinal issues such as antibiotic-associated diarrhea and can facilitate opportunistic infections due to the overgrowth of pathogenic organisms like *Clostridium difficile*. Long-term studies have shown that even after the cessation of antibiotic therapy, certain beneficial microbial populations may be permanently diminished, leading to a dysbiotic state that predisposes individuals to chronic diseases such as obesity, type 2 diabetes, and inflammatory bowel disease. Moreover, the relationship between antibiotic use and antimicrobial resistance is increasingly concerning. The emergence of resistant bacteria is directly linked to the inappropriate and excessive use of antibiotics in both clinical and agricultural settings. For instance, broad-spectrum antibiotics, which are frequently prescribed for common infections, can diminish microbial diversity and promote the development of resistant pathogens that pose a significant public health challenge. The potential for resistant strains to spread through communities and clinical settings highlights the importance of implementing effective antibiotic stewardship programs, aimed at optimizing antibiotic prescribing and minimizing unnecessary use. The consequences of antibiotic overuse extend beyond individual health to encompass societal implications as well. The economic burden associated with treating infections caused by resistant bacteria, alongside the increased length of hospital stays and higher treatment costs, underscores the urgency for public health initiatives aimed at curbing antibiotic misuse. Additionally, communication strategies to raise public awareness about the consequences of antibiotic overuse are critical for driving changes in prescribing behavior among healthcare providers. The antibiotic overuse presents a dual challenge: it disrupts the microbiome and accelerates the development of antimicrobial resistance. Addressing this issue requires comprehensive strategies that involve healthcare providers, public health officials, and the general public to ensure responsible antibiotic use, promote microbiome health, and mitigate the spread of resistance.

Probiotics, Prebiotics and Symbiotic

Probiotics, prebiotics, and symbiotic represent promising strategies for modulating the microbiome to improve health outcomes. These microbial-based therapies provide benefits by restoring or enhancing gut microbiota composition and activity, with applications in various clinical contexts. However, there are important limitations and challenges that must be addressed to optimize their use. Probiotics are live microorganisms that confer health benefits to the host

when administered in adequate amounts. Evidence suggests that specific probiotic strains can help manage gastrointestinal disorders, obesity, and metabolic conditions like type 2 diabetes. For example, studies have shown that supplementation with probiotics such as *Lactobacillus rhamnosus* can help prevent dysbiosis and bolster gut health in infants. Furthermore, the impact of the gut microbiome on the pharmacokinetics of certain medications, including potential improvements in drug absorption and efficacy through microbial modulation, is an area of ongoing research. Prebiotics, defined as non-digestible food ingredients that selectively stimulate beneficial bacteria, play a significant role in shaping the gut microbiome. They enhance the growth of probiotics and other beneficial microbial populations, thereby contributing to improved gut health and metabolic outcomes. Research indicates that dietary fibers classified as prebiotics can reduce markers of insulin resistance and aid in weight management, implicating their role in preventing obesity-related diseases. Additionally, prebiotics may lead to the production of short-chain fatty acids (SCFAs) through fermentation, which are known to have anti-inflammatory effects and support gut barrier integrity. Synbiotics, which combine probiotics and prebiotics, represent a synergistic approach that leverages the benefits of both to promote gut health more effectively. They can enhance gut microbiota diversity and stability, making them applicable in managing various health conditions such as irritable bowel syndrome (IBS) and metabolic disorders. The synergy between prebiotics and probiotics allows for improved survival of beneficial bacteria as they traverse the gastrointestinal tract, potentially enhancing therapeutic efficacy. Despite their potential, several limitations impede the widespread clinical application of probiotics, prebiotics, and synbiotics. One major challenge is the variability in individual responses to these therapies, which can depend on existing gut microbiome composition, genetic predisposition, diet, and health status. Additionally, the impact of the delivery method (e.g., food vs. supplements) and formulation (viability of probiotics, concentration of prebiotics) can influence therapeutic outcomes. Furthermore, regulatory approvals for these products can vary greatly, leading to inconsistent quality, labeling, and efficacy claims that can mislead consumers. As the field continues to expand, there is a need for more rigorous clinical trials to establish clear guidelines for dosage, duration, and strain-specific applications for optimal effectiveness. prebiotics, and synbiotics offer promising avenues for microbiome modulation with potential health benefits. While the clinical applications of these therapies are expanding, ongoing research is vital to overcome existing limitations and optimize their use for individualized patient care.

Fecal Microbiota Transplantation (FMT)

Fecal Microbiota Transplantation (FMT) has emerged as a promising therapeutic strategy for various gastrointestinal disorders, particularly in conditions characterized by dysbiosis, such as recurrent Clostridium difficile infection (CDI) and inflammatory bowel diseases (IBD). The process involves transferring gut microbiota from healthy donors to patients, aiming to restore the microbial balance in the gastrointestinal tract, which can alleviate disease symptoms. The mechanisms underlying FMT's efficacy largely relate to the restoration of a healthy gut microbiome. Successful FMT has been associated with significant increases in beneficial microbial genera, such as Lactobacillus, and decreases in pathogenic strains, including Bacteroides and Clostridium (Lauko et al., 2023). In particular, FMT has demonstrated remarkable efficacy in treating recurrent CDI, with clinical resolution rates reported to be between

70% and 90%. The role of gut microbiota in regulating the immune response is crucial; the restoration of microbial diversity can enhance immune function, thus reducing the severity of conditions like ulcerative colitis. Clinical applications of FMT have expanded beyond CDI to include steroid-refractory intestinal acute graft-versus-host disease (GvHD) and IBD. Pilot studies have suggested positive outcomes in these areas, indicating FMT's potential as a treatment option when conventional therapies fail. Evidence suggests that FMT for IBD may induce clinical remission in some patients; however, variability in responses indicates that further optimization of donor selection and transplantation protocols is necessary. Despite its potential benefits, concerns about the safety of FMT have emerged. While adverse events are generally classified as minor and infrequent, there is a risk of introducing pathogenic organisms and triggering gastrointestinal complications. Thus, rigorous donor screening and proper administration techniques are essential to minimize these risks. Moreover, studies highlight the importance of standardized protocols for FMT, as variations in the composition of fecal material can significantly impact treatment outcomes. FMT represents a significant advancement in managing dysbiosis-associated conditions by restoring gut microbiota balance and interacting with the immune system. While current evidence supports its efficacy and relative safety, ongoing research is warranted to refine its application and confirm long-term outcomes across various clinical contexts.

Microbiome-Based Diagnostics and Biomarker Discovery

Microbiome-based diagnostics are paving the way for innovative approaches in disease prediction and precision health, leveraging intricate microbial ecosystems as potential biomarkers. The human microbiome, comprising trillions of microorganisms, communicates with host physiology in multifaceted ways, influencing health outcomes and disease mechanisms. This intricate relationship offers valuable insights into potential biomarkers for various diseases, including neurodegenerative disorders and metabolic syndromes. One primary avenue for microbiome-based diagnostics is the integration of microbial profiles with traditional biomarker strategies. For example, Varesi et al. emphasized the necessity for large-cohort studies that consider multiple biomarkers, including both bacterial composition and microbiota-derived metabolites, to enhance diagnostic accuracy for diseases like Alzheimer‘s. Similarly, Han et al. noted that metabolic profiling through advanced sequencing techniques, such as PacBio's full-length 16S rRNA sequencing, can optimize the identification of biomarkers, enhance our understanding of disease mechanisms and aid in predictive modeling. The exploration of human metabolites alongside microbiome data is equally critical. The utility of urine and plasma, termed "liquid biopsies," as they provide accessible means to uncover microbiome-derived metabolites that serve as indicators of health or disease. This integrated approach illustrates the potential of metabolomics in identifying unique biological signatures that correlate with specific health conditions. Moreover, applying advanced computational techniques, including machine learning (ML) and deep learning (DL), can expedite biomarker discovery by revealing complex patterns within microbiome data. Research has combined traditional bioinformatics with ML methods, demonstrating that these tools are vital in refining biomarker identification and tailoring personalized therapeutic strategies based on individual microbiome profiles (Dakal et al., 2025). This synergy between computational advancements and microbiomic research could significantly enhance diagnostic capabilities, enabling healthcare practitioners to predict disease progression

and treatment responses more accurately. In addition to utilizing microbiome profiles for biomarker identification, investigations into specific disease contexts have shown promising results. For instance, Wan et al. identified a fecal microbial marker panel that aids in diagnosing autism spectrum disorders, underscoring how differential abundance analysis can pinpoint microbial signatures associated with specific medical conditions. The ability to distinguish microbial patterns linked to diseases like autism suggests broader applications for microbiome-based diagnostics in pediatric health. Additionally, studies on the oral and gut microbiomes' interactions showcase potential diagnostic applications. The distinctive oral microbiota profiles in colorectal cancer patients exhibit diagnostic efficacy, which could be integrated with non-invasive tests for more comprehensive risk assessments and disease screening. This highlights the value of multidisciplinary approaches that harness various microbiota sources in clinical diagnostics. Microbiome-based diagnostics represent a significant frontier in personalized medicine, offering the potential to predict disease outcomes and tailor therapies. The integration of microbiome profiles with advanced analytical techniques and traditional biomarker measures can enhance our ability to detect and manage health conditions effectively, paving the way for precision health initiatives.

Personalized Microbiome Interventions and the Era of Precision Medicine

Personalized microbiome interventions are at the forefront of precision medicine, with the potential to tailor diets, drugs, and therapies based on individual microbiome signatures. These interventions aim to optimize health outcomes by considering the unique microbial composition of each individual, leading to more effective management of various health conditions. One prominent approach is the customization of dietary interventions designed to promote beneficial microbial communities. Personalized diets can significantly alter gut microbiota composition, which is crucial for managing diseases such as inflammatory bowel disease and metabolic disorders. By individualizing dietary recommendations, healthcare practitioners can enhance therapeutic efficacy for conditions that are influenced by dietary factors. Furthermore, the use of specific dietary patterns, such as low-FODMAP or fiber-rich diets, has been shown to enhance the growth of beneficial commensals like *Faecalibacterium prausnitzii*, which produces short-chain fatty acids (SCFAs) and exhibits anti-inflammatory properties. These adaptations highlight the importance of considering both dietary ingredients and the individual microbiome profile in designing effective treatments. Additionally, there is growing interest in harnessing artificial intelligence to refine dietary strategies based on microbiome responses. Microbiome-based AI-assisted personalized diets could more effectively address specific conditions like irritable bowel syndrome (IBS) by targeting unique symptom patterns and underlying pathophysiologies. This tailored approach offers the potential for personalized symptom relief compared to generic diets. Personalized dietary interventions could regulate glycemic responses, illustrating the potential of customizing nutrition to control diabetes and related metabolic disorders. The role of probiotics in personalized medicine is also gaining attention. Research indicates that probiotic efficacy varies considerably among individuals due to differences in gut microbiome composition. The indigenous gut microbiota markedly influences the success of probiotic interventions, evidencing the necessity of tailoring probiotics to individual microbiome profiles to enhance colonization resistance and therapeutic outcomes. This finding underscores the potential for developing

personalized probiotic strategies that align more closely with individual health and dietary needs. Emerging evidence also supports the integration of microbiome profiling into interventions for metabolic disorders such as obesity and prediabetes. Personalized dietary approaches could modify the oral and gut microbiome, revealing significant metabolic changes among prediabetic individuals resulting from tailored dietary plans. Moreover, this growing field opens pathways for utilizing non-invasive microbiome analysis to guide interventions, potentially improving adherence and long-term health outcomes. Looking forward, multitiered approaches that combine dietary, probiotic, and potentially pharmacological interventions informed by microbiome data are critical to advancing personalized health. The successful implementation of these strategies necessitates further research, particularly in understanding the complex interactions between diet, microbiome, and health status. Targeted interventions, particularly through real-time metabolic and microbiome monitoring, hold promise for shaping the future of healthcare based on precision medicine paradigms. Personalized microbiome interventions provide a multifaceted approach to optimizing health outcomes in the context of precision medicine. By integrating dietary modifications, probiotics, and advanced technology, clinicians can develop tailored treatments that resonate with individual microbiome signatures, thereby enhancing the efficacy of therapeutic strategies.

Future Directions in Microbiome Research

Future directions in microbiome research are increasingly being shaped by innovative technologies and interdisciplinary approaches, including artificial intelligence (AI), systems biology, and synthetic ecology. These advancements are poised to enhance our understanding of microbial communities and their intricate functions within both natural and engineered environments. AI is playing a transformative role in microbiome research through advancements in data analytics and machine learning. For example, Wang et al. introduced a deep learning approach to identify keystone species within microbial communities, which is crucial for understanding ecosystem stability and health. By leveraging large datasets, AI can uncover complex patterns and interactions that would otherwise be missed through traditional analytical methods. This capability enhances our ability to predict community responses to various environmental changes or interventions. Systems biology approaches are also vital for elucidating the dynamic interactions within microbial communities. Research by Shetty et al. highlighted the importance of modeling minimal microbiomes that exhibit ecological properties, allowing for the study of metabolic interactions and community behavior under different perturbative conditions (Shetty et al., 2022). Such modeling provides insights into how specific species interact, including their trophic roles and collective responses to environmental stressors. This foundational knowledge is necessary for designing effective interventions aimed at manipulating microbiome compositions for health benefits. Additionally, innovative methods, such as those discussed by Xu et al., emphasize the intersection of microbiome research with environmental biology by assessing microbial communities affected by contaminants like atrazine, using a range of techniques including metagenomics and mass spectrometry. This multifaceted approach enables the identification of relationships between microbial composition, environmental pollutants, and ecosystem health, underscoring the relevance of microbiomes in bioremediation efforts. Synthetic ecology represents another promising frontier, where researchers are exploring how engineering microbiomes can

achieve specific outcomes. The application of synthetic biology, involves creating engineered microbial populations designed to perform desired functions that can extend to applications such as terraforming. As understanding of microbial interactions and community dynamics deepens, future research may enable the creation of tailored microbiomes that can enhance ecosystem services or agricultural productivity. Moreover, the integration of ecological theories into microbiome research is becoming increasingly important. For instance, applying evolutionary principles to better understand microbiome dynamics, emphasizing the roles of ecological interactions and evolutionary history in shaping microbial communities. This perspective can elucidate how microbial diversity and stability develop over time and under various environmental pressures. Another essential area of research is the industrial and biotechnological applications of synthetic microbiomes. The necessity of designing microbiomes that can address societal challenges, including health and environmental sustainability. By employing a design-build-test-learn framework, scientists can investigate how engineered microbiomes can be harnessed for beneficial outcomes, such as improving human health or enhancing agricultural yields through symbiotic relationships with host organisms. The future of microbiome research is marked by the integration of AI, systems biology, and synthetic ecology. These interdisciplinary approaches not only enhance our understanding of microbial interactions and their implications but also facilitate the development of innovative solutions for health and environmental challenges. As research continues to evolve, the potential for tailored microbiome interventions will expand, reshaping how we approach individual and planetary health.

References

Afzaal M, Saeed F, Shah YA, Hussain M, Rabail R, Socol CT, Hassoun A, Pateiro M, Lorenzo JM, Rusu AV and Aadil RM (2022) Human gut microbiota in health and disease: Unveiling the relationship. *Front. Microbiol.* 13:999001. doi: 10.3389/fmicb.2022.999001

Andoh, A. and Nishida, A. (2022). Alteration of the gut microbiome in inflammatory bowel disease. Digestion, 104(1), 16-23. https://doi.org/10.1159/000525925

Chisholm, S. T., Coaker, G., Day, B., & Staskawicz, B. J. (2006). Host-microbe interactions: shaping the evolution of the plant immune response. *Cell*, *124*(4), 803-814.

Comess, J. and Abad-Jorge, A. (2023). Introduction to the gut microbiome and its impact on health and disease. Topics in Clinical Nutrition, 38(3), 183-195. https://doi.org/10.1097/tin.0000000000000324

Dakal, T., Xu, C., & Kumar, A. (2025). Advanced computational tools, artificial intelligence and machine-learning approaches in gut microbiota and biomarker identification. Frontiers in Medical Technology, 6. https://doi.org/10.3389/fmedt.2024.1434799

Foessleitner, P., Pjevac, P., Granser, S., Wisgrill, L., Pummer, L., Eckel, F., … & Farr, A. (2024). The maternal microbiome in pregnancy, delivery, and early-stage development of neonatal microbiome after cesarean section: a prospective longitudinal study. Acta Obstetricia Et Gynecologica Scandinavica, 103(5), 832-841. https://doi.org/10.1111/aogs.14773

Guan, S., Lin, Q., Wu, X., & Yu, H. (2023). Weighted gene coexpression network analysis and machine learning reveal oncogenome associated microbiome plays an important role in tumor immunity and prognosis in pan-cancer. Journal of Translational Medicine, 21(1). https://doi.org/10.1186/s12967-023-04411-0

Helmink, B.A., Khan, M.A.W., Hermann, A. *et al.* The microbiome, cancer, and cancer therapy. *Nat Med* **25**, 377–388 (2019). https://doi.org/10.1038/s41591-019-0377-7

Jeyaraman, N., Jeyaraman, M., Dhanpal, P., Ramasubramanian, S., Ragavanandam, L., Muthu, S., … & Lana, J. (2024). Gut microbiome and orthopaedic health: bridging the divide between digestion and bone integrity. World Journal of Orthopedics, 15(12), 1135-1145. https://doi.org/10.5312/wjo.v15.i12.1135

Kargbo, R. (2023). Microbiome–gut–brain axis modulation: new approaches in treatment of neuropsychological and gastrointestinal functional disorders. Acs Medicinal Chemistry Letters, 14(6), 692-695. https://doi.org/10.1021/acsmedchemlett.3c00168

Lauko, S., Gancarčíková, S., Hrčková, G., Hajdučková, V., Andrejčáková, Z., Fecskeová, L., … & Sopková, D. (2023). Beneficial effect of faecal microbiota transplantation on mild, moderate and severe dextran sodium sulphate-induced ulcerative colitis in a pseudo germ-free animal model. Biomedicines, 12(1), 43. https://doi.org/10.3390/biomedicines12010043

Liukkonen, M., Muriel, J., Martínez-Padilla, J., Nord, A., Pakanen, V., Rosivall, B., … & Ruuskanen, S. (2024). Seasonal and environmental factors contribute to the variation in the gut microbiome: a large-scale study of a small bird. Journal of Animal Ecology, 93(10), 1475-1492. https://doi.org/10.1111/1365-2656.14153

Ørsted, M., Yashiro, E., Hoffmann, A., & Kristensen, T. (2022). Population bottlenecks constrain host microbiome diversity and genetic variation impeding fitness. Plos Genetics, 18(5), e1010206. https://doi.org/10.1371/journal.pgen.1010206

Rawat, A., Han, B., Patel, N., Allehaibi, H., Rosado, A., & Hirt, H. (2025). Symbiotic plant–bacterial–fungal interaction orchestrates ethylene and auxin signaling for optimized plant growth. The Plant Journal, 122(2). https://doi.org/10.1111/tpj.70174

Shang, Z., Pai, L., & Patil, S. (2024). Unveiling the dynamics of gut microbial interactions: a review of dietary impact and precision nutrition in gastrointestinal health. Frontiers in Nutrition, 11. https://doi.org/10.3389/fnut.2024.1395664

Shetty, S., Kostopoulos, I., Geerlings, S., Smidt, H., Vos, W., & Belzer, C. (2022). Dynamic metabolic interactions and trophic roles of human gut microbes identified using a minimal microbiome exhibiting ecological properties. The Isme Journal, 16(9), 2144-2159. https://doi.org/10.1038/s41396-022-01255-2

Stadlbauer, V. (2023). Liver-gut-interaction: role of microbiome transplantation in the future treatment of metabolic disease. Journal of Personalized Medicine, 13(2), 220. https://doi.org/10.3390/jpm13020220

Wagner, N., Valeriano, V., Diou-Hirtz, S., Björninen, E., Åkerström, U., Engstrand, L., … & Gillbro, J. (2024). Microbial dynamics: assessing skincare regimens‘ impact on the facial skin microbiome and skin health parameters. Microorganisms, 12(12), 2655. https://doi.org/10.3390/microorganisms12122655

Wang, T., Li, P., Xue, B., Tian, S., Yang, M., Leng, D., … & Li, D. (2024) . Vaginal microbiota are associated with in vitro fertilization during female infertility. Imeta, 3(3). https://doi.org/10.1002/imt2.185

Warren, A., Nyavor, Y., Beguelin, A., & Frame, L. (2024). Dangers of the chronic stress response in the context of the microbiota-gut-immune-brain axis and mental health: a narrative review. Frontiers in Immunology, 15. https://doi.org/10.3389/fimmu.2024.1365871

About The Authors

Khawar Ali is currently pursuing his Ph.D. at North University of China, following his M.S. in Molecular Biology. He has previously served as a Research Associate at the Virtual University of Pakistan and Cholistan University of Veterinary and Animal Sciences, Bahawalpur, where he gained extensive experience in Molecular Biology research and laboratory techniques. Additionally, he worked as a Molecular Biologist at IBN-E-SINA Clinical Laboratory & Research Center in Lahore, contributing to clinical diagnostics and research operations. Khawar Ali has published several research articles in reputable peer-reviewed scientific journals. His research interests include Oncology, Molecular Biology, Genomics, Biochemistry and Biotechnology, with a particular focus on Molecular Diagnostics and Cancer Biology. Through his academic and professional endeavors, he is dedicated to advancing scientific knowledge and contributing to innovations in biomedical research.

Email: khawarali1202@gmail.com **ORCID:** 0000-0002-0565-4535.

Nosheen Majeed, an M.Phil. Molecular Biology scholar at the University of Okara, with academic background in Biotechnology from the Virtual University of Pakistan. Her research focuses on the body‘s defense against infectious agents and NRAMP-1 gene, a key immune-related gene, and its genetic variations that may significantly impact the susceptibility to various diseases. She is deeply interested in exploring the molecular mechanisms underlying host-pathogen interactions. Through her research, she aims to contribute to a better understanding of genetic resistance to infections, potentially informing the development of improved diagnostic tools and therapeutic strategies. Her work reflects a strong commitment for the advancement of knowledge in Biotechnology, Genetics, Molecular Biology and infectious disease control.

E-mail: nosheenmbuo23@gmail.com **ORCID:** 0009-0005-3360-0322.

Dr. Farhat Ameer Chughtai is a Senior Demonstrator in the Department of Pharmacology at Nishtar Medical University, Multan, Pakistan. Her research interests encompass medicine, pharmacology, toxicology, neurology, and neurological disorders. With a strong commitment to both education and research, Dr. Chughtai focuses on exploring innovative therapeutic approaches for neurological conditions as well as the pharmacological and toxicological mechanisms underlying disease processes. Her growing contributions to these fields reflect her dedication to advancing clinical knowledge and improving patient outcomes, establishing her as an emerging scholar in the medical sciences.

Email: drfarhatkhurram@gmail.com **ORCID:** 0009-0004-4979-6473

Prof. Dr. Mehmet ÖZASLAN received his PhD in 1995 Institute of Natural Sciences at Cukurova University, Turkiye. He is a Professor in Molecular Biology and Genetics. His research interests are included Cancer Genetics, Molecular Virology, Molecular Genetics, Microbiology, and Genetic mutations etc. He has published more than 200 research articles in national and international well reputed journals. He also has written many book chapters as well as edited books. He is an editor and editor in chief of many well reputed national and international journals.

E-mail: ozaslanmd@gantep.edu.tr, ORCID: 0000 0001 9380 4902

Similarity Index

The similarity index obtained from the plagiarism software for this book chapter is 15 %.

To Cite This Chapter:

Ali K et al., (2025). Precision Medicine Personalized Approaches for Diagnosis, Treatment, and Prevention. In Current Studies in Life Sciences 2025 (pp.114-139). ISRES Publishing.

AI-Powered Molecular Diagnostics: Revolutionizing Point-of-Care Testing

Maria NAZIR
Samra MAQSOOD
Syed Sohail AHMAD
Nimra SHAHZADI
Ghanwa MARYAM
Muhammad Usman QAMAR
Muhammad SAFDAR

Introduction

Molecular diagnostics has historically been said to be at the heart of healthcare transformation, using genetic and molecular aspects to achieve swifter diagnosis and, more importantly, personalized treatment plans (Haleem et al., 2022). Point-of-care testing (POCT), coupled with next-generation sequencing (NGS) and artificial intelligence (AI) algorithms, has brought a new outlook to diagnostics, not only eliminating the wait but also extending diagnostic capacities beyond the rudiments of the traditional clinical setting. The very mention of making timely decisions in emergencies, such as in stroke or trauma, will bring out the importance of these technologies in improving the capacity to improve patient outcomes. There were important milestones in the flying journey of molecular diagnostics. The polymerase chain reaction (PCR) was first introduced to the molecular testing landscape in 1983, which allowed DNA amplification for highly accurate detection of pathogens and genetic mutations. The affordability and efficiency of fully sequencing an entire genome were revolutionized by NGS technology, particularly around the mid-2000s, opening the way to personalized medicine (Sahoo et al., 2024).

The gates of accurate gene editing and modern diagnostic assays were also opened by its introduction in 2015. Table 1 summarizes these major events in chronological order, outlining their importance in the development of healthcare. In 1983, PCR emerged as a revolutionary tool and stronghold for DNA-based testing. For the first time, Fluorescent in Situ Hybridization (FISH) was developed in 1990, allowing visualization of DNA material, which delays cancer diagnosis. NGS revolutionized genetic research in 2005 due to its ability to provide fast and detailed sequencing of DNA. Modern medicine is now much closer to bringing the potential for personalized treatments. CRISPR-Cas9 changed the game of gene editing while offering new horizons for precision diagnostics in 2015 (Chanchal et al., 2024).

Rapid point-of-care testing stands out in its use for rapid diagnostics directly from the patient's site of care, and it is crucial outside the hospital setting in cases of an acute emergency, such as a heart attack, that can save lives by providing early treatment. While traditional laboratory methods require sample processing in a central laboratory, POCT removes the delays built into these steps. It is, therefore, particularly relevant for under-resourced areas without advanced laboratory testing facilities. Also, POCT saves costs through less infrastructure and staffing needed and enables timely diagnosis and treatment (Land et al., 2019). AI boosts the impact of POCT. AI-enabled devices analyze real-time datasets, improve the reliability of results, reduce errors, and learn continuously for the population. AI-implemented tools were invaluable in tracking outbreaks and informing treatment decisions during the COVID-19 pandemic (Adnan et al., 2024). By

quickly testing and analyzing advanced data, AI-powered POCT is advancing precision medicine; it ensures efficient health service provision while being patient-centric and accessible globally.

AI and Machine Learning in Molecular Diagnostics

Artificial intelligence (AI) and machine learning (ML) are crucial to molecular diagnostics. They can analyze vast amounts of data, detect numerous indicators, make predictions, and provide more precise results. This section examines how AI and ML are deeply integrated into molecular diagnostics to enhance precision, accuracy, and efficiency.

1) AI in Healthcare: Artificial Intelligence in healthcare mimics human intelligence to perform data analysis, task automation, and diagnostic enhancement. The vast volume of images, such as MRI and CT scans, is interpreted rapidly, with increased accuracy in disease detection, e.g., tumors. In drug development, it predicts the compound's efficacy, thus shortening the development timeline and relying less on clinical trials. AI provides personalized treatment by analyzing patient data and offering customized therapies. In molecular diagnostics, AI interprets complex data from next-generation sequencing (NGS) to identify biomarkers, thus improving diagnostic accuracy (Chandrashekar et al., 2024).

2) Enhancing Diagnostic Performance: AI impacts molecular diagnostics to improve their precision, accuracy, and speed in detecting the most subtle genetic mutations and molecular changes and discovering targeted therapeutics. AI algorithms trained on vast datasets may significantly reduce human error and produce fewer false positives and negatives for more assured results. In addition, real-time AI processing of molecular data enables the diagnostics work, which may only take hours or even days to complete within the minutest moments. This phenomenon will be remarkably useful in testing infectious diseases, where rapid result generation can help in the timely decisions of treatments (Chan et al., 2013).

Supervised and Unsupervised Learning: Artificial intelligence (AI) primarily relies on machine learning techniques to process large amounts of data and improve diagnostic performance. AI systems can be powered by two primary fronts: supervised learning and unsupervised learning.

Supervised Learning: Learning with Labels: Training an AI through a supervised learning approach includes subjecting it to labeled data, where the input data, like medical images and lab results, are associated with the appropriate diagnosis. Thus, a model trained on chest X-rays labeled pneumonia will predict pneumonia on any new, unlabeled X-ray. This works excellently for conditions in well-established diagnosis, like breast cancer: AI has identified malignant tumors from labeled mammograms, thereby increasing the speed and accuracy of the decisions made by the doctors (Sharafaddini et al., 2024).

3) Unsupervised Learning: Finding Hidden Patterns: In unsupervised learning, in contrast, you offer an AI a box of jigsaw pieces without dating the person. The AI does the remaining work by analyzing data autonomously, looking for patterns or clusters. This technique is valuable for finding emerging disease subtypes that no human can identify. For instance, unsupervised learning has clustered patients using their expression profiles. For example, scientists do not know much about the reason behind aggressive behavior in a specific type of cancer. AI can analyze genetic

data from such patients and identify clusters of very similar cases, thus discovering new subtypes of the disease and opening new routes for personalized treatment (Johansson et al., 2023).

4) Real-Life Impact: AI can develop new diagnostics by combining both methods. Supervised learning is accurate and reliable in known environments and allows one to determine new insights through new and creative patterns in care. For example, during COVID-19 supervision in the learning system, the supervised trained and supervised learning algorithm helped identify the virus from chest CT scans. The unsupervised and supervised learning algorithms recognized the mutation of an individual's genes to develop a treatment targeted toward curing the virus infection based on that mutation (Hunt et al., 2022).

5) AI-Powered POCT Platforms: By integrating artificial intelligence with many modern technologies like biosensors, nanotechnology, and microfluidics, POCT can provide rapid and accurate diagnoses. In this case, the biosensors help enhance sensitivity and specificity for reliable biomarker detection using AI, delivering intelligent, real-time data analysis. The disease marker detection through nanotechnology is achieved by using gold nanoparticles to resolve the disease markers at the molecular level, hence facilitating early diagnosis. Microfluidics has a significant impact on POCT by precisely manipulating small volumes of fluids on a single chip, while AI optimizes fluid flow and sample handling to allow for an accurate diagnosis in very remote places (Zare Harofte et al., 2022). These innovations affect AI-enabled POCT platforms, transforming molecular diagnostics into unprecedented speed and accuracy.

Revolutionizing Patient Care with AI-Powered POCT

POCT empowered by artificial intelligence serves various critical healthcare needs, diagnosing quickly, accurately, and personally, making it all possible without any hassle (Aminizadeh et al., 2024). Objective point-of-care testing systems would be developed to resolve clinical issues like time delays in diagnosis, limited access to advanced testing, and high demand for personalized medicine, especially in rural and underserved areas. AI-based point-of-care testing integrates advanced diagnostic technologies with intelligent data processing to streamline the entire workflow, as reflected in Figure 1.

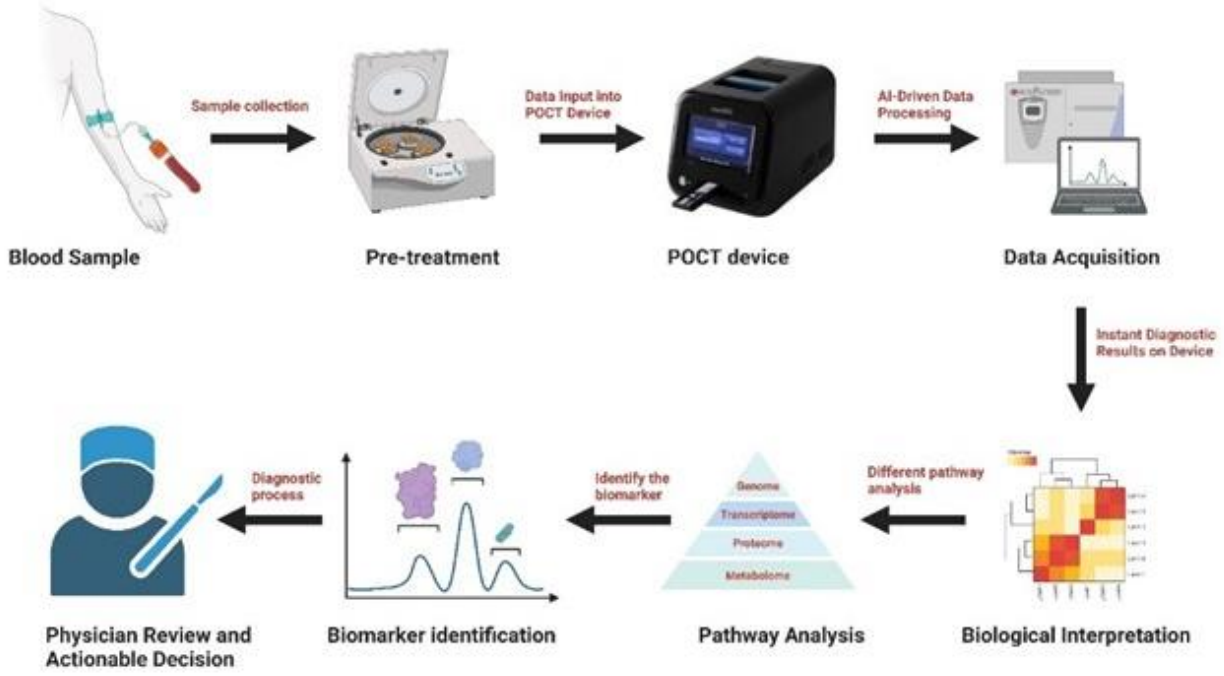


Figure 1. Real-Time Diagnostics and Point-of-Care Testing (POCT)

Transforming patient care has significantly enhanced onsite testing and shortened diagnostic timelines. Such patient care is especially pertinent in remote and resource-limited settings. Now, real-time interpretation of information supports the newfound demand for individualized diagnostic information that underlies precision medicine. In this case, patient outcomes are advanced while healthcare delivery models change for the better (Vinchurkar et al., 2024).

1) Speed and Accuracy: Another significant advantage of applying AI technology in POCT is the reduced time required to deliver a diagnosis. Conventional laboratory tests often have a turnaround time of several hours or even days. Conventional tests may not support timely clinical management, especially in myocardial infarction or suspected infectious diseases. In contrast, AI-integrated POCT provides prompt, real-time diagnosis, delivering results with remarkable speed, often within minutes, regardless of the severity of the condition. This speed is crucial for early identification in cases with signs of infection, as the window for effective treatment intervention can be very short (Chen et al., 2022). In addition to speed, AI enhances the accuracy of diagnostic results. This leads to more precise detection of biomarker levels, reducing the occurrence of false positives and false negatives commonly seen in manual diagnoses. AI algorithms continuously improve by integrating new disease data, making them increasingly accurate.

2) AI in Cancer Treatment: AI has emerged as the biggest boon in personalized medicine, particularly oncology. Molecular diagnosis involving AI could discover specific genetic mutations in tumors, such as the BRCA1 and BRCA2 genes concerning breast and ovarian cancers. For example, in non-small cell lung cancers (NSCLC), AI algorithms utilized to analyze tumor genetics can reveal the existence of mutations in EGFR. Targeted therapy, such as tyrosine kinase inhibitors (TKIs), has widely improved patient survival by prescribing based on those findings (Attwood et

al., 2021). AI does not just tell an actionable mutation but also ensures the most favorable therapies for patients on which the most promising results can be expected, relieving therapists of the burden of guesswork in their conventional treatments.

3) AI in Pharmacogenomics: AI has also revolutionized the entire field of pharmacogenomics, studying how gene variations modify a patient‘s response to drugs. For example, AI-enabled platforms predict drug metabolism using genetic biomarkers. A classical case is warfarin, an anticoagulant with a narrow therapeutic range. AI systems scan genetic variants in an enzyme, such as CYP2C9 and VKORC1, to tailor the dose administered to that patient, thus reducing the risk of complications such as bleeding or clotting (Henderson, 2019). Artificial intelligence-oriented POCT has also been central to genetic conditions, including cystic fibrosis. AI identifies likely beneficiaries of modulators like ivacaftor through genotypic tests on mutations in the CFTR gene to find patients likely to respond to the specific precision therapy that targets their genetic profile. Hence, in addition to improved patient responses, it has also reduced costs associated with unnecessary treatments.

AI Workflow in Personalized Medicine

Incorporating AI into next-generation sequencing and correlating that to a patient's clinical history generates a systematic workflow of personalized treatments. As shown in Figure 2, AI analyzes the molecular markers and corresponding treatment recommendations based on the input data in the genetic sequences and clinical and lifestyle factors to ensure precision therapy for each patient's drug selection, dosage, and lifestyle modifications.

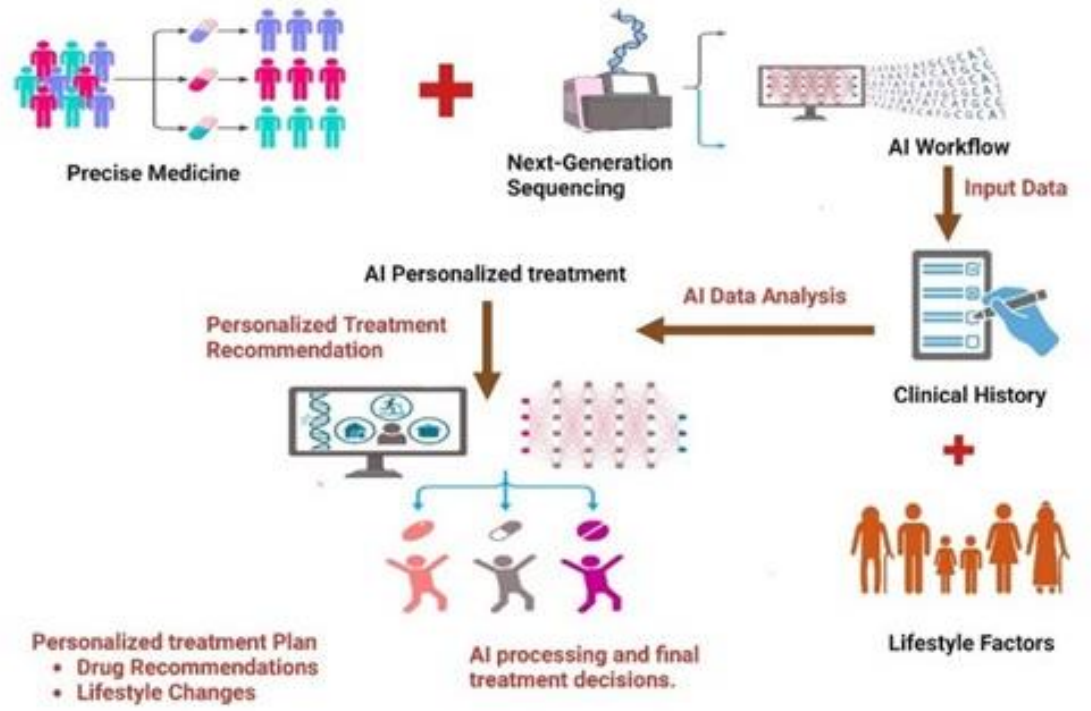


Figure 2. AI workflow in Personalized Medicine

1) Real-Time Treatment Adjustments: AI-powered POCT systems allow real-time modification of therapeutic strategies. For instance, AI examines biomarker information such as C-reactive protein levels from patients with rheumatoid arthritis when making predictions concerning disease progression. Treatment can then be adjusted using biological therapies optimally to manage the disease while minimizing the consumption of unnecessary medications. In the same way, adaptive cancer immunotherapy has the potential to use AI. Identifying neoantigens means using tumor-specific mutations, and that helps prepare individualized immunotherapy protocols because they can be tracked in the tumors, thus increasing efficacy and reducing adverse effects (Gupta et al., 2021).

2) Developing Remote and Low-Resource Locations: AI integration into POCTs transforms diagnostic capabilities in remote and resource-limited regions where poor infrastructure and a lack of trained medical personnel render traditional diagnostics economically unfeasible and inaccessible. Most conventional tests depend on some centralized facility with specialized equipment, which poses serious challenges for rural and underserved communities. AI POCT could provide a cost-effective solution: onsite, portable AI-driven devices that perform intricate diagnostics outside central laboratories. These systems allow onsite field diagnostics, making them available in regions without infrastructure or skilled personnel for conventional testing (Chakraborty, 2024).

Table 1. AI-Powered POCT‘s Impact in Remote and Resource-Limited Areas

Challenge	Traditional Diagnostic Approach	AI-Powered POCT Solution	Impact on Patient Care	References
Limited Infrastructure	Requires centralized labs, expensive equipment, and skilled personnel	Portable, low-cost, AI-driven devices that function without lab infrastructure	Expand access to diagnostics in rural and underserved areas.	(Sharma et al., 2017)
Lack of Skilled Healthcare Workers	Skilled technicians are required for accurate result interpretation	AI guides users and automates the diagnostic process	Enables non-specialists to perform and interpret complex tests, improving healthcare delivery.	(Adler-Milstein et al., 2022)
Delayed Diagnosis	Sample transport to labs delays diagnosis and treatment	AI provides real-time results, eliminating the need for sample transport	Enables immediate diagnosis and treatment, improving outcomes, especially in time-sensitive conditions.	(Quig et al., 2019)

POCT systems that utilize AI effectively address major bottlenecks in healthcare delivery, especially in areas of low resource availability, as outlined in Table 1. By eliminating the need for

centralized labs, these portable AI machines open up possibilities for diagnostic services to reach distant locations with little infrastructure. Also, while AI can guide or automate diagnostic tasks, helping non-specialists conduct tests and interpret results, it helps to bridge the gap created by the shortage of skilled human resources. Real-time processing means there are no delays in transporting samples to distant laboratories, leading to the rapid diagnosis and treatment of diseases that, on account of their nature, require time-sensitive intervention. Such technology shows great promise for improving health outcomes in these neglected areas. Hopefully, it will be formally entered into global health systems to offer better access to quality healthcare (Plebani et al., 2025).

AI in Infectious Disease Diagnostics

The rise of new diseases seriously threatens modern medicine and global healthcare systems. When integrated into POCT systems, AI revolutionizes diagnosing and managing infectious diseases and outbreaks. Some of the key benefits of AI include its ability to process large volumes of data and provide real-time diagnostics capabilities that have been especially critical during the pandemic. This section will explore the potential of AI-powered POCT in the COVID-19 response, its role in tracking disease transmission and mutations, and its application in identifying other pathogens such as Ebola, Zika, and other emerging infectious diseases (Catania, 2022).

1) AI in Pandemic Response and Management: The COVID-19 pandemic saw AI technologies on the ground. China was one of the first countries to employ AI-assisted point-of-care testing (POCT) systems to measure SARS-CoV-2 transmission rates. AI was used in combination with molecular diagnostics like RT-PCR and antigen tests for the rapid detection of viral RNA and proteins, from seconds, compared with hours or days in traditional laboratory testing methods.AI also performed functions such as monitoring infection trends, finding hotspots, and predicting epidemic surges so that public health authorities could respond more effectively by planning lockdowns, social distancing measures, and resource allocation upon real-time data. Beyond diagnostics, AI was also utilized for the genomic sequencing analysis of emerging variants such as Delta and Omicron. With the help of artificial neural networks, AI did mutation detection that was crucial in informing government intervention, vaccine manufacturers, and healthcare professionals on therapeutic strategies, thus contributing to an overall improvement in pandemic response(Hick et al., 2020). The examples above demonstrate how AI has greatly accelerated diagnostics and informed timely decision-making during the pandemic.

2) Biases and Ethical Considerations of AI Diagnostics: Artificial Intelligence systems may yield varying degrees of performance because of the data used during training, leading to biased diagnostic results, especially against marginalized groups. A classic example is AI algorithms employed for skin cancer detection, which indicates that its diagnostic did not work well for darker skin, as most of the data was from lighter-skinned individuals. Again, models developed within the U.S., which were mainly based on Caucasian populations, turned out to provide inaccurate results for patients from other ethnic backgrounds and caused unequal health outcomes (Zhu et al., 2019). Such biases must be addressed using diverse data sets that indicate diverse demographics from race to age and gender. Additionally, bias assessment and validation of the AI models must be undertaken before implementation to guarantee fair and reliable results across all population

categories. Figure 3 outlines the workflow of AI health monitoring systems, emphasizing ethical data usage.

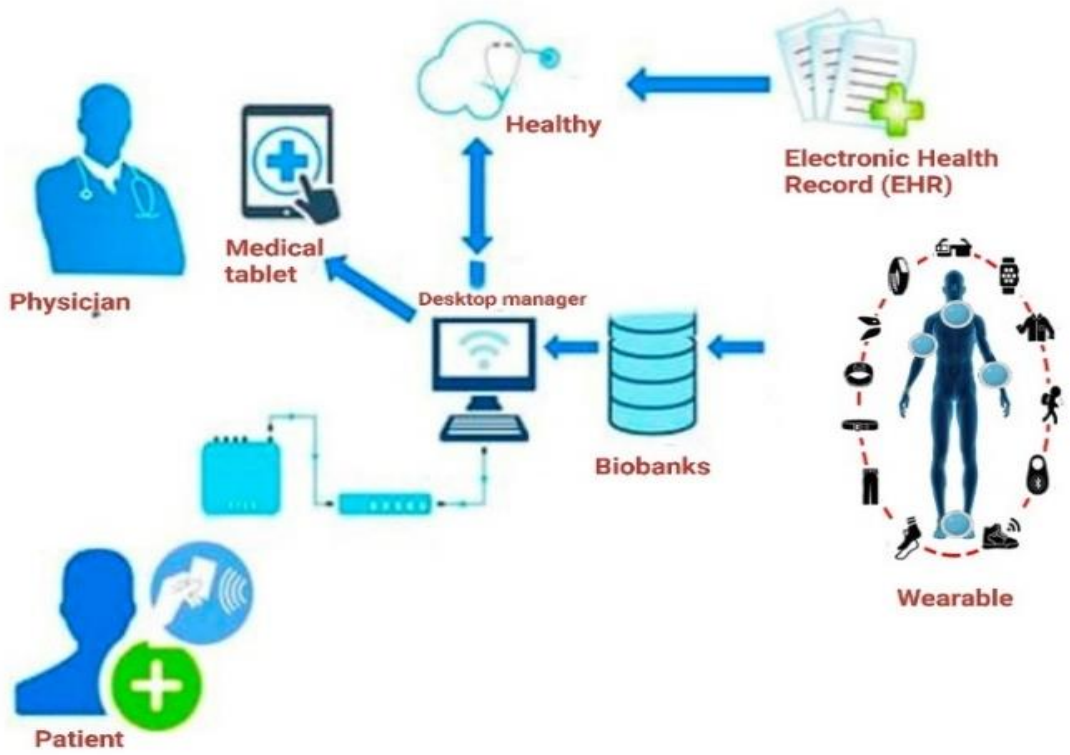


Figure 3. AI-Integrated Wearable Health Monitoring

Future Prospects: The Next Generation of AI-Powered POCT

AI has a promising future with point-of-care testing (POCT) to remold diagnostics and health service delivery in the next decade. The current trends in AI are expected to be integrated into aspects such as deep learning, autonomous diagnostic systems, and possibly genomic data for revolutionary advances in diagnostics. It will most likely be associated with improved accuracy and speed of diagnosis. It will help demystify access to and delivery of quality health care globally, especially in remote and underserved regions. This section explores the anticipated leap forward for AI and POCT in the changeable mode.

1) Progress in Artificial Intelligence for POCT: POCT will become better and more accurate in diagnosis with further advancement of AI, primarily through deep learning and neural network models. With convolutional neural networks (CNNs) and recurrent neural networks (RNNs), deep learning architectures have already shown remarkable results in image interpretation, disease prediction, and anomaly detection, among others. For instance, CNNs have been shown to produce human-level accuracy in detecting malignant lesions and diabetic retinopathy from medical images (Patrício et al., 2023). In the years to come, such technologies will have broader capabilities, wherein they will be incorporated into POCT systems for real-time analysis of genomic sequences, proteomic datasets, and other complex molecular data. Advancements in generating artificial

intelligence systems and generative adversarial networks (GANs) will allow POCT systems to simulate patently unique patient states and estimate disease progression limits more accurately than previously possible (Feldman, 2024). Thus, these systems will diffuse novel personalized medicine techniques and enhance prompt intervention measures, resulting in better patient outcomes.

2) Hardware-Software Integration: AI-sophisticated hardware integration will redefine the whole scenario of POCT systems. Compact biosensors, lab-on-a-chip devices, and handheld diagnostic devices are expected to be integrated with AI algorithms to facilitate fast tests with high sensitivities in clinical and field settings (Ardila, 2025). These small systems will eventually return results in real time, enabling healthcare professionals to make quick decisions confidently. In addition, with better hardware-software synchronization, mobile diagnostics will allow users to perform tests in a laboratory setting in their homes or even in a disaster zone, currently, with AI-enabled POCT capabilities.

Conclusion

The role of artificial intelligence (AI) in molecular diagnostics is increasing in importance because of its substantial influence in terms of speed and accuracy, with engine-fueled algorithms, studying enormous amounts of data streams in little time, results in more accurate diagnoses of complex illnesses and diseases. Drug discovery and medicinal care, including cancer, meningitis, cardiovascular diseases, and many other communicable maladies, will be heightened, causing overall improvement in therapeutic medicine, cost reduction, and harm reduction brought about by improved diagnostics. Beyond innovation in personal medicine, AI encompasses the transformation of diagnostics, making learning more accessible to people in the second or third regions. The dissociation of therapeutic services appears behind technological facets: joint learning of AI enables the patient agency to interact in diagnostic processes, and interconnectivity via wider usage of telemedicine facilitates the use of wearables and health apps to monitor and diagnose diseases remotely. Healthcare costs are plummeting pathetically, and health diagnostics are gaining traction toward universal accessibility via the power of AI. The research of AI in the healthcare sector seems to revolve around making healthcare delivery more efficient, universal, and personalized.

References

Adler-Milstein, J., Aggarwal, N., Ahmed, M., Castner, J., Evans, B. J., Gonzalez, A. A., James, C. A., Lin, S., Mandl, K. D., & Matheny, M. E. (2022). Meeting the moment: addressing barriers and facilitating clinical adoption of artificial intelligence in medical diagnosis. *NAM perspectives*, *2022*.

Adnan, M., Xiao, B., Ali, M. U., Bibi, S., Yu, H., Xiao, P., Zhao, P., Haiyan, W., & An, X. (2024). Human inventions and its environmental challenges, especially artificial intelligence: New challenges require new thinking. *Environmental Challenges*, 100976.

Aminizadeh, S., Heidari, A., Dehghan, M., Toumaj, S., Rezaei, M., Navimipour, N. J., Stroppa, F., & Unal, M. (2024). Opportunities and challenges of artificial intelligence and distributed systems to improve the quality of healthcare service. *Artificial Intelligence in Medicine*, *149*, 102779.

Ardila, C. M. (2025). Advancing healthcare through laboratory on a chip technology: Transforming microorganism identification and diagnostics. *World Journal of Clinical Cases*, *13*(3).

Attwood, M. M., Fabbro, D., Sokolov, A. V., Knapp, S., & Schiöth, H. B. (2021). Trends in kinase drug discovery: targets, indications and inhibitor design. *Nature Reviews Drug Discovery*, *20*(11), 839-861.

Catania, L. J. (2022). Immunology: The science of pandemics, infectious disease and COVID-19. *The Paradox of the Immune System*, 181.

Chakraborty, S. (2024). Democratizing nucleic acid-based molecular diagnostic tests for infectious diseases at resource-limited settings–from point of care to extreme point of care. *Sensors & Diagnostics*, *3*(4), 536-561.

Chan, C. P. Y., Mak, W. C., Cheung, K. Y., Sin, K. K., Yu, C. M., Rainer, T. H., & Renneberg, R. (2013). Evidence-based point-of-care diagnostics: current status and emerging technologies. *Annual review of analytical chemistry*, *6*(1), 191-211.

Chanchal, D. K., Chaudhary, J. S., Kumar, P., Agnihotri, N., & Porwal, P. (2024). CRISPR-Based therapies: revolutionizing Drug Development and Precision Medicine. *Current gene therapy*, *24*(3), 193-207.

Chandrashekar, K., Niranjan, V., Vishal, A., & Setlur, A. S. (2024). Integration of artificial intelligence, machine learning and deep learning techniques in genomics: review on computational perspectives for NGS analysis of DNA and RNA seq data. *Current Bioinformatics*, *19*(9), 825-844.

Chen, X., Wang, Z., Hua, Q., Shang, W.-L., Luo, Q., & Yu, K. (2022). AI-empowered speed extraction via port-like videos for vehicular trajectory analysis. *IEEE Transactions on Intelligent Transportation Systems*, *24*(4), 4541-4552.

Feldman, R. (2024). Artificial Intelligence and Cracks in the Foundation of Intellectual Property. *Available at SSRN 4736929*.

Gupta, R. G., Li, F., Roszik, J., & Lizée, G. (2021). Exploiting tumor neoantigens to target cancer evolution: current challenges and promising therapeutic approaches. *Cancer discovery*, *11*(5), 1024-1039.

Haleem, A., Javaid, M., Singh, R. P., & Suman, R. (2022). Medical 4.0 technologies for healthcare: Features, capabilities, and applications. *Internet of Things and Cyber-Physical Systems*, *2*, 12-30.

Henderson, L. M. (2019). *Impact of Warfarin Pharmacogene Variation on Drug Metabolism and Pharmacological Response in Alaska Native and American Indian Populations*

Hick, J. L., Hanfling, D., Wynia, M. K., & Pavia, A. T. (2020). Duty to plan: health care, crisis standards of care, and novel coronavirus SARS-CoV-2. *NAM perspectives*, *2020*.

Hunt, C., Montgomery, S., Berkenpas, J. W., Sigafoos, N., Oakley, J. C., Espinosa, J., Justice, N., Kishaba, K., Hippe, K., & Si, D. (2022). Recent progress of machine learning in gene therapy. *Current gene therapy*, *22*(2), 132-143.

Johansson, Å., Andreassen, O. A., Brunak, S., Franks, P. W., Hedman, H., Loos, R. J., Meder, B., Melén, E., Wheelock, C. E., & Jacobsson, B. (2023). Precision medicine in complex diseases—Molecular subgrouping for improved prediction and treatment stratification. *Journal of internal medicine*, *294*(4), 378-396.

Land, K. J., Boeras, D. I., Chen, X.-S., Ramsay, A. R., & Peeling, R. W. (2019). REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. *Nature microbiology*, *4*(1), 46-54.

Patrício, C., Neves, J. C., & Teixeira, L. F. (2023). Explainable deep learning methods in medical image classification: A survey. *ACM Computing Surveys*, *56*(4), 1-41.

Plebani, M., Nichols, J. H., Luppa, P. B., Greene, D., Sciacovelli, L., Shaw, J., Khan, A. I., Carraro, P., Freckmann, G., & Dimech, W. (2025). Point-of-care testing: state-of-the art and perspectives. *Clinical Chemistry and Laboratory Medicine (CCLM)*, *63*(1), 35-51.

Quig, K., Wheatley, E. G., & O'Hara, M. (2019). Perspectives on blood-based point-of-care diagnostics. *Open Access Emergency Medicine*, 291-296.

Sahoo, O. S., Aidasani, H., Nayek, A., Tripathi, S., Talukdar, J., Gul, A., Kumar, D., Dhar, R., & Karmakar, S. (2024). Role of next-generation sequencing in revolutionizing healthcare for cancer management. *MedComm–Future Medicine*, *3*(4), e70001.

Sharafaddini, A. M., Esfahani, K. K., & Mansouri, N. (2024). Deep learning approaches to detect breast cancer: a comprehensive review. *Multimedia Tools and Applications*, 1-112.

Sharma, S., Crawley, A., & O'Kennedy, R. (2017). Strategies for overcoming challenges for decentralised diagnostics in resource-limited and catastrophe settings. *Expert review of molecular diagnostics*, *17*(2), 109-118.

Vinchurkar, R., Kole, S., Desai, R., Wagh, P., Gawade, A., & Kuchekar, A. (2024). Precision Medicine Paradigms in Allergic Rhinitis: Navigating Immunotherapy and Digital Healthcare. *Expert review of precision medicine and drug development*, *9*(1), 29-41.

Zare Harofte, S., Soltani, M., Siavashy, S., & Raahemifar, K. (2022). Recent advances of utilizing artificial intelligence in lab on a chip for diagnosis and treatment. *Small*, *18*(42), 2203169.

Zhu, Y., Sidell, M. A., Arterburn, D., Daley, M. F., Desai, J., Fitzpatrick, S. L., Horberg, M. A., Koebnick, C., McCormick, E., & Oshiro, C. (2019). Racial/ethnic disparities in the prevalence of diabetes and prediabetes by BMI: Patient Outcomes Research To Advance Learning (PORTAL) multisite cohort of adults in the US. *Diabetes care*, *42*(12), 2211-2219.

About the Authors

Maria Nazir is an MPhil Scholar of Microbiology at the Cholistan University of Veterinary and Animal Sciences (CUVAS) in Bahawalpur, Pakistan. She is involved in a National Research Program for Universities (NRPU) Project, with research interests in Molecular Characterization. She has submitted abstracts to various conferences and authored and coauthored of several book chapters. Her research focuses on the isolation and molecular characterization of *Corynebacterium pseudotuberculosis* and its applications in promoting animal and human health. Her research criteria are based on genetics, molecular biology, and bioinformatics.

Email: nazirmaria545@gmail.com ORCID 0009-0004-6714-6212

Samra Maqsood received her M.Phil. in Molecular Biology from the National Center of Excellence in Molecular Biology (CEMB), University of Punjab, Lahore, Pakistan. She is passionate about teaching and research in biomedical and forensic sciences. Her research interests include forensic serology, DNA typing, virology, stem cell therapies, and molecular biology techniques. She has worked on several research projects, including genetic polymorphism studies, DNA isolation, human genetics, and epidemiological studies on COVID-19.

Email: samrama.qsoodmaqsood@gmail.com ORCID: 0009-0004-5674-1091

Syed Sohail Ahmad completed an MPhil in Medical Microbiology from Hazara University, Mansehra. I have more than three years of teaching and research experience. Now, I am working as a Lecturer at the Rehman College of Allied Health Sciences, Rehman Medical Institute, Peshawar. My research interests are Biofilm and its eradication, Novel drugs and vaccine development for mycobacterium infections, and cancer. I worked on different anti-biofilm compounds and cancer cell lines. I have experience in in vivo and in vitro work.

Emails: syedsohailedu@gmail.com ORCID: 0009-0001-9652-7565

Miss. Nimra Shahzadi did BS and MS in Microbiology and Molecular Genetics and Molecular Biology, respectively. My research interests are microbiology, molecular biology, nanotechnology, immunology, cancer, and proteomics. I have experience working on nanoparticles and proteins.

Email: shnimra.talib@gmail.com ORCID: 0009-0007-4628-2292

Ghanwa Maryam received her Bechalor degree in 2023 from Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan. Her research interests include Molecular, biology, Fisheries and conservation and biodiversity of animals (Vertebrates and Invertebrates).

Email: ghanwamaryam@gmail.com ORCID: 0009-0000-5968-3246

Dr. Muhammad SAFDAR earned his PhD in molecular biology and genetics from Gaziantep University, Turkey. He is a lecturer in the Breeding and Genetics department at Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, Pakistan. His research interests are molecular genetics and genomics, nutrigenomics, nano-genomics, bioinformatics, biotechnology, and their applications. He has published more than 70 research articles in national and international journals. He has also written many book chapters as well as an edited book. He is an associate editor for international journals.

E-mail: drmuhammadsafdar8@gmail.com, ORCID: 0000 0002 3720 2090

Similarity Index
The similarity index obtained from the plagiarism software for this book chapter is 9%.

To Cite This Chapter:
Nazir, M. (2025). AI-Powered Molecular Diagnostics: Revolutionizing Point-of-Care Testing. 2025 ISRES Publishing. (pp.140-153) ISRES Publishing.

Nanotechnology in Healthcare innovation in Drug delivery, Diagnostics and Therapeutics

Khawar ALI
Ghulam MURTAZA
Muhammad Masood AHMED
Mehmet OZASLAN

Introduction

Nanotechnology has rapidly evolved as a pivotal enabler of innovation across the biomedical sciences, with transformative implications for drug delivery, diagnostics, and therapeutics. By leveraging the unique physicochemical properties of materials at the nanoscale (1–100 nm), such as increased surface area-to-volume ratio, quantum effects, and tunable surface chemistry, researchers are overcoming longstanding limitations in conventional medical approaches. The interdisciplinary convergence of materials science, pharmacology, and molecular biology has catalyzed the development of nano-systems that offer enhanced precision, bioavailability, and patient-specific targeting. In drug delivery, nanocarrier platforms ranging from liposomes and dendrimers to polymeric nanoparticles and solid lipid nanoparticles have demonstrated the capacity to improve pharmacokinetics, enable controlled release, and facilitate intracellular drug transport. Notably, the clinical success of Pegylated Liposomal Doxorubicin (Doxil®) marked a significant milestone, offering reduced cardiotoxicity while maintaining therapeutic efficacy in oncology settings (Bisht et al., 2025). Beyond passive targeting through the Enhanced Permeability and Retention (EPR) effect, functionalized Nanocarriers employing ligands such as antibodies or peptides have enabled active targeting to specific cellular receptors, improving therapeutic index and reducing off-target effects. Advancements in nanoscale diagnostics have significantly enhanced sensitivity, specificity, and real-time detection capabilities. Nanoparticles are increasingly used as contrast agents in magnetic resonance imaging (MRI), computed tomography (CT) and positron emission tomography (PET), allowing for earlier detection of pathophysiological changes at the cellular and molecular levels. Moreover, the integration of nanomaterials into biosensors has enabled ultra-sensitive point-of-care diagnostic platforms, capable of detecting low-abundance biomarkers for diseases such as cancer, neurodegenerative disorders, and viral infections. Therapeutic applications of nanotechnology extend beyond drug delivery to include novel intervention strategies such as photothermal therapy (PTT) and photodynamic therapy (PDT). Nanoparticles convert external stimuli typically light into cytotoxic effects selectively within tumor microenvironments. The emergence of theragnostic platforms, which integrate diagnostic and therapeutic functionalities into a single nanostructure, exemplifies the shift toward personalized and precision medicine by enabling real-time monitoring of therapeutic responses and dynamic modulation of treatment regimens. Despite these advancements, several translational barriers persist. Key challenges include potential nanotoxicity, immunogenicity, bioaccumulation, and the lack of harmonized regulatory frameworks for clinical approval (Souto et al., 2024). Addressing these hurdles through rigorous preclinical evaluation, scalable manufacturing, and interdisciplinary collaboration will be essential to fully realize the clinical potential of nanotechnology in healthcare.

Nanorobotic Drug Delivery for Cancer Treatment

Cancer remains a formidable global health burden, accounting for nearly 10 million deaths in 2020 (Ibraheem et al., 2025). Despite advances in oncological therapeutics, conventional treatments such as chemotherapy and radiotherapy are limited by systemic toxicity, low tumor specificity, and the development of multidrug resistance. The advent of nanotechnology, particularly nanorobotic systems, has introduced a paradigm shift in cancer therapeutics, enabling targeted, intelligent, and minimally invasive drug delivery strategies. Nanorobots nanoscale devices engineered for autonomous or guided operations within the human body offer unprecedented precision in delivering therapeutic agents directly to tumor microenvironments while minimizing off-target. Figure 1, shows the nanorobotic platforms enable highly targeted delivery of anticancer agents through precise navigation, stimulus-responsive release mechanisms, and selective tumor accumulation, thereby improving treatment outcomes and reducing off-target effects (Kong et al., 2023).

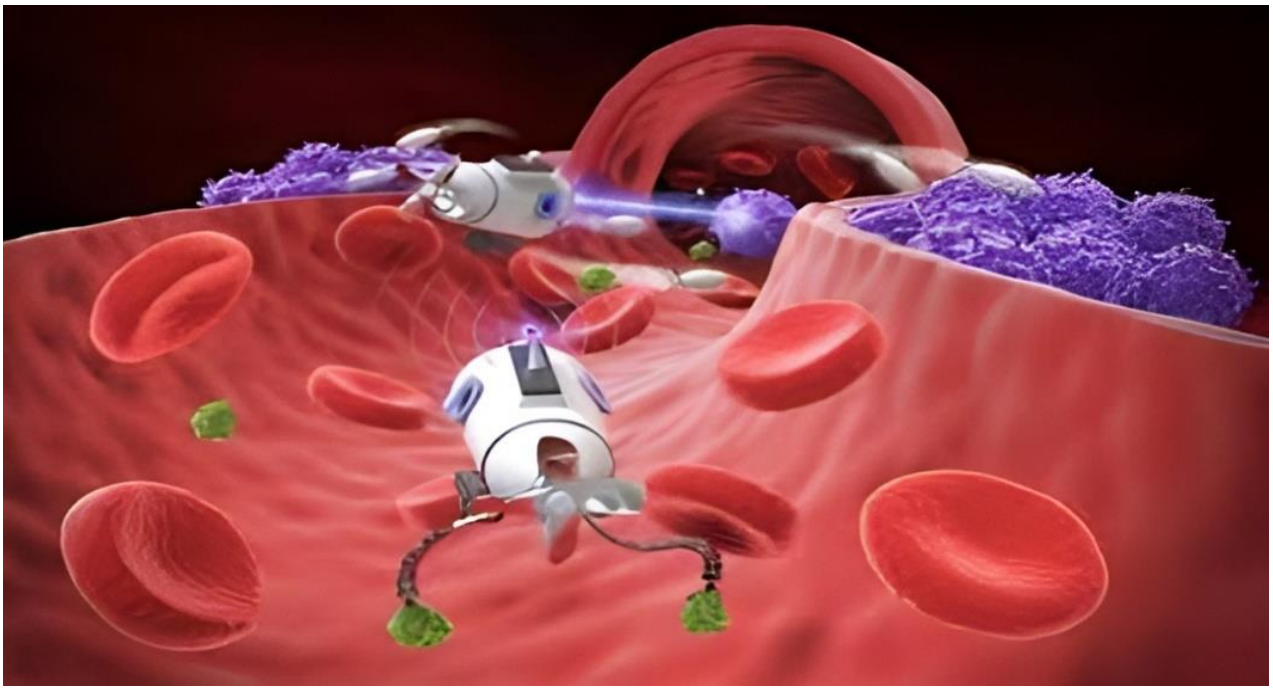


Figure 1. Nanorobotic Approaches for Precision Drug Delivery in Cancer Treatment (Kong et al., 2023)

Design and Mechanism of Nanorobots in Oncology

Nanorobots are typically constructed from a variety of materials, including DNA origami, polymers, lipids, or metallic nanoparticles, depending on the intended functionality and biocompatibility requirements. Their size (generally <1000 nm), shape, and surface chemistry can be finely tuned to navigate biological barriers and evade immune detection. A critical feature of nanorobotic platforms is their ability to respond to specific internal or external stimuli such as pH, temperature, magnetic fields, or enzymatic activity allowing on-demand drug release within the tumor microenvironment. One promising design involves DNA-based nanorobots that are programmed to recognize molecular markers on tumor cells, such as nucleolin or HER2, and

trigger the release of chemotherapeutic agents upon binding. Biohybrid nanorobots powered by flagellated magnetotactic bacteria are guided by magnetic fields to hypoxic regions of solid tumors, where traditional drug delivery fails. These biohybrid systems not only enhance tissue penetration but also allow real-time tracking through MRI contrast agents integrated into the nanorobot chassis.

Therapeutic Advantages over Conventional Modalities

The precision and responsiveness of nanorobotic systems confer several therapeutic advantages. Firstly, site-specific delivery significantly increases the local concentration of therapeutic agents within the tumor while reducing systemic exposure and toxicity. This is particularly critical for drugs with narrow therapeutic indices, such as doxorubicin or paclitaxel. Secondly, nanorobots can be engineered to bypass drug efflux mechanisms, a key contributor to chemoresistance in solid tumors (Mao et al., 2024). Moreover, the ability of nanorobots to integrate diagnostic and therapeutic functions termed theranostics represents a major advancement in personalized medicine. For example, certain nanorobots incorporate quantum dots or MRI-active elements, enabling clinicians to visualize tumor accumulation, monitor the convergence of diagnostics, controlled drug release, and active navigation enhances both treatment efficacy and clinical decision-making.

Despite the promising preclinical outcomes, several translational challenges must be addressed before clinical deployment. Biocompatibility remains a primary concern, particularly regarding immunogenicity, long-term toxicity, and clearance pathways. While PEGylation and biomimetic coatings have improved circulation time and immune evasion, the heterogeneity of the tumor microenvironment complicates targeting efficacy. Furthermore, mass production of nanorobots with reproducible performance and regulatory compliance poses significant engineering and economic hurdles. From a regulatory perspective, nanorobotic systems do not fit neatly into existing frameworks for either pharmaceuticals or medical devices. The U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) have begun issuing guidance on nanomedicines, but nanorobots, particularly those with autonomous behavior, require novel evaluation paradigms. Ethical concerns also arise regarding autonomous control and long-term biodistribution, necessitating transparent clinical trial protocols and robust post-marketing surveillance. The integration of artificial intelligence, bioinformatics, and synthetic biology is anticipated to further enhance the capabilities of nanorobotic platforms. Adaptive nanorobots capable of learning from environmental feedback or reprogramming themselves in vivo are on the horizon, potentially enabling closed-loop therapeutic interventions. Additionally, developments in soft robotics and biodegradable nanomaterials may alleviate safety concerns, accelerating regulatory approval and clinical adoption. Nanorobotic drug delivery systems hold transformative potential in oncology by addressing the long-standing challenges of specificity, efficacy, and patient safety. While clinical translation is still emerging, the convergence of multidisciplinary research in materials science, robotics, and molecular medicine is rapidly advancing this field toward tangible clinical impact.

Nanogels for Multifunctional Drug Delivery

Nanogels nanoscale, crosslinked hydrogel particles have emerged as promising platforms for advanced drug delivery due to their unique physicochemical properties. High water content, tunable size, and responsiveness to various stimuli make them suitable for targeted therapy, imaging, and sustained drug release, particularly in challenging therapeutic areas such as cancer, ophthalmology, and neurological disorders.

Structural and Functional Attributes of Nanogels

Nanogels are three-dimensional, hydrophilic polymer networks capable of swelling in aqueous environments without dissolving. This allows for the encapsulation of diverse therapeutic agents, including small molecules, proteins, and nucleic acids. Their nanoscale size and modifiable surface properties enable them to respond to stimuli such as pH, temperature, and redox potential, facilitating site-specific and controlled drug release.

Applications in Cancer Therapy

In oncology, nanogels have shown potential in enhancing drug solubility, efficacy, and safety. For instance, curcumin-loaded nanogels have demonstrated a significant increase in water solubility, improving its bioavailability and therapeutic impact. Moreover, nanogels facilitate combination therapies; co-delivery systems comprising paclitaxel and interleukin-2 have been developed to improve therapeutic synergy in triple-negative breast cancer. Additionally, hyaluronate-based nanogels carrying doxorubicin and cisplatin have been shown to effectively reverse tumor cell resistance mechanisms.

Theragnostic Capabilities

Beyond their therapeutic roles, nanogels are now recognized for their theragnostic applications by combining therapeutic delivery and diagnostic imaging functionalities. These systems often incorporate contrast agents or dyes, enabling real-time monitoring of drug biodistribution and therapeutic response. For instance, nanogels embedded with chlorine6 have facilitated simultaneous photodynamic therapy and fluorescence imaging in tumors.

Neurological Applications

Crossing the blood-brain barrier (BBB) remains one of the primary obstacles in treating central nervous system (CNS) disorders. Nanogels have been engineered with properties that allow them to bypass or penetrate the BBB. Methotrexate-loaded nanogels, for example, significantly increased brain drug concentration by approximately 10 to 15-fold compared to free drug administration. Moreover, nanogels have been employed in delivering microRNAs and gene therapies for glioblastoma, showing great promise in gene-based interventions.

Ophthalmological Applications

In ophthalmology, nanogels offer a solution to poor drug retention and limited tissue permeability. Their mucoadhesive properties enable extended residence time on the ocular surface, improving drug absorption. Recent studies involving carbonized lysine-derived nano-gels demonstrated effective treatment of dry eye disease at significantly lower doses than conventional drops, reducing side effects while maintaining efficacy. Nano-gels represent a transformative platform in multifunctional drug delivery. Their adaptability to deliver diverse payloads, responsiveness to physiological stimuli, and capacity for diagnostic imaging underscore their potential in personalized medicine. While their utility in cancer, ophthalmology, and neurological disorders are already evident, further clinical trials and translational research will solidify their role in modern therapeutics.

siRNA and miRNA-Loaded Nanocarriers for Breast Cancer

Breast cancer remains one of the most commonly diagnosed malignancies among women worldwide. Despite advancements in early detection and systemic therapies, certain subtypes such as triple-negative breast cancer (TNBC) continue to pose significant treatment challenges due to the lack of estrogen, progesterone, and HER2 receptors, which limits the effectiveness of conventional targeted therapies. Recent progress in nanotechnology and molecular biology has paved the way for novel treatment strategies that leverage the potential of RNA interference (RNAi), particularly small interfering RNA (siRNA) and microRNA (miRNA), for gene silencing and regulation of cancer-related pathways. siRNAs are synthetic double-stranded RNA molecules that induce the degradation of complementary messenger RNA (mRNA), effectively silencing specific genes implicated in tumor growth, metastasis, and chemoresistance. miRNAs, on the other hand, are endogenous non-coding RNAs that regulate gene expression post-transcriptionally by binding to the 3' untranslated regions (UTRs) of target mRNAs. Aberrant expression of miRNAs has been closely associated with breast cancer progression and prognosis, and restoring normal miRNA function through delivery systems has emerged as a promising therapeutic approach. However, delivering siRNA and miRNA to tumor cells in vivo presents significant challenges due to their instability in the bloodstream, susceptibility to enzymatic degradation, and poor cellular uptake. To address these limitations, researchers have developed nanocarriers engineered nanoparticles that protect RNA molecules from degradation and facilitate targeted delivery to tumor tissues. Among the various platforms explored, lipid-based nanoparticles, polymeric nanoparticles, and inorganic nanostructures have demonstrated potential in preclinical studies for effective RNA delivery (Rosenblum et al., 2020). In the context of TNBC, hybrid nanoparticles combining siRNA and therapeutic phytochemicals such as quercetin have gained particular interest. Quercetin, a flavonoid found in fruits and vegetables, exhibits antioxidant, anti-inflammatory, and anticancer properties. It has been shown to modulate key signaling pathways such as PI3K/AKT and NF-κB, both of which are often dysregulated in TNBC. By co-encapsulating quercetin with siRNA in a single nanocarrier, researchers aim to achieve synergistic effects that enhance apoptosis, inhibit proliferation, and reduce metastatic potential. For instance, a study demonstrated that a lipid–polymer hybrid nanoparticle loaded with quercetin and siRNA targeting the Bcl-2 gene a key anti-apoptotic protein significantly induced apoptosis in TNBC cells

and reduced tumor growth in xenograft models. The hybrid design leverages the structural stability of polymeric cores and the biocompatibility of lipid shells, improving circulation time and tumor penetration. Furthermore, surface modifications such as polyethylene glycol (PEG) coating and targeting ligands like folic acid or antibodies enhance selectivity toward cancer cells while minimizing off-target effects. miRNA-loaded nanocarriers are also being explored to restore tumor-suppressive miRNAs that are typically downregulated in TNBC. For example, miR-34a and let-7 have shown tumor-suppressive roles by targeting oncogenes involved in proliferation and epithelial–mesenchymal transition (EMT). Delivery of these miRNAs using biodegradable polymeric nanoparticles or exosomes has shown promising results in preclinical models. The integration of siRNA or miRNA with natural compounds like quercetin in nanocarriers offers a multifaceted strategy for targeting TNBC. Figure 2, illustrates nanocarrier-based platforms enable precise delivery of siRNA and miRNA to breast cancer cells, facilitating targeted gene silencing, inhibition of tumor growth, and overcoming of therapy resistance mechanisms (Ashique et al., 2022). These systems not only silence oncogenes but also sensitize tumor cells to therapy and overcome multidrug resistance. As research advances, the optimization of nanoparticle size, surface charge, release kinetics, and targeting specificity will be critical for clinical translation. Clinical trials assessing the safety and efficacy of RNA-loaded nanocarriers are already underway, offering hope for more effective and personalized treatments for aggressive breast cancer subtypes.

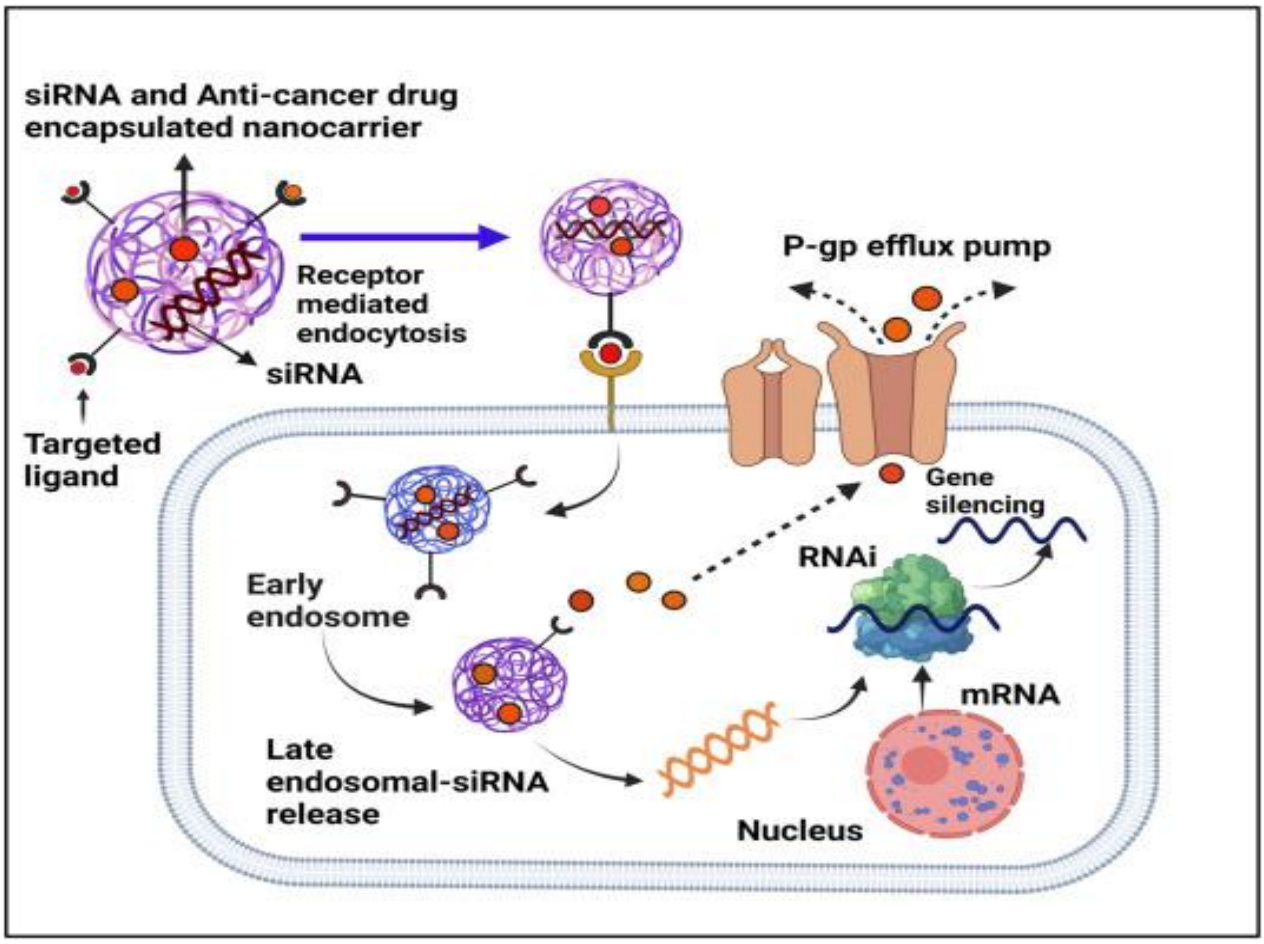


Figure 2. Nanocarrier-Based Delivery of siRNA and miRNA for Breast Cancer Treatment (Ashique et al., 2022).

Ultrasound-Triggered Nanoparticles for Thrombolysis

Thrombosis, the formation of a blood clot within a blood vessel, remains a major cause of morbidity and mortality worldwide, particularly in conditions like ischemic stroke, myocardial infarction, and pulmonary embolism. Conventional thrombolytic therapy typically involves systemic administration of agents such as tissue plasminogen activator (tPA), which catalyzes the conversion of plasminogen to plasmin, promoting fibrin degradation. However, this approach is limited by significant off-target effects, short drug half-life, and high risk of hemorrhagic complications. To address these shortcomings, researchers are increasingly investigating ultrasound-triggered nanoparticles (UTNs) as a targeted and controlled drug delivery system for site-specific thrombolysis.

Mechanism of Ultrasound Activation and Nanoparticle Design

UTNs are engineered to remain stable during systemic circulation and to release their therapeutic payload only upon exposure to externally applied ultrasound. Ultrasound serves as a non-invasive, spatiotemporally precise trigger that enhances drug delivery at the thrombus site through mechanisms such as cavitation, acoustic streaming, thermal effects, and mechanical vibration. Physical forces can disrupt the thrombus matrix while simultaneously releasing the encapsulated thrombolytic agents, thereby enhancing clot penetration and dissolution. A variety of nanoparticle platforms have been developed for ultrasound-mediated thrombolysis, including liposomes, polymeric nanoparticles, microbubbles, and perfluorocarbon nanodroplets. Microbubbles, often composed of phospholipid or protein shells encapsulating inert gases, oscillate under ultrasound exposure. Their collapse known as inertial cavitation can transiently permeabilize surrounding biological barriers and mechanically dislodge fibrin, while releasing surface-conjugated or encapsulated thrombolytic drugs like tPA. Additionally, these microbubbles can be functionalized with fibrin-targeting ligands to enhance their localization at the clot site.

Emerging Platforms and Innovations

Among the most promising UTNs are perfluorocarbon nanodroplets, which remain in a superheated liquid state until activated by ultrasound via acoustic droplet vaporization (ADV). Upon sonication, these nanodroplets transition into microbubbles, exerting localized mechanical pressure that disrupts clot integrity while releasing therapeutic agents. This vaporization process enables deeper penetration into thrombi compared to microbubbles alone and enhances treatment efficacy in both arterial and venous thrombotic models. Researchers are also exploring ultrasound-responsive polymeric nanoparticles, particularly those composed of biocompatible polymers such as poly (lactic-co-glycolic acid) (PLGA). These nanoparticles can be loaded with thrombolytic drugs and designed to degrade or release their cargo in response to ultrasound-induced heating or pressure. Some systems incorporate phase-change materials or thermo-sensitive polymers that melt or rupture upon ultrasound exposure, enabling precise, on demand drug release. Furthermore, theragnostic nanoparticles are being developed to combine therapy and diagnostics. For example, nanoparticles incorporating gold nanorods or iron oxide cores offer ultrasound-triggered drug delivery alongside imaging capabilities using photoacoustic or magnetic resonance imaging. This

dual functionality enables real-time visualization of nanoparticle accumulation and thrombus resolution, allowing for more personalized and adaptive treatment protocols.

Preclinical Studies and Therapeutic Impact

The therapeutic efficacy of UTNs has been demonstrated in various preclinical models. Perfluorocarbon nanodroplets encapsulating tPA and demonstrated that ultrasound activation enhanced thrombolysis by over threefold compared to free tPA in a rat embolism model. Similarly, microbubble-based carriers functionalized with fibrin-specific peptides, achieving high targeting efficiency and effective clot lysis with reduced systemic exposure. Importantly, UTNs offer the potential to reduce the required dosage of thrombolytic agents, thereby minimizing the risk of bleeding complications. The ability to localize drug release to the thrombus site allows for lower systemic drug concentrations, improving the safety profile an essential consideration for translating these technologies into clinical practice.

Despite their promise, several challenges must be addressed before UTNs can be widely adopted in clinical settings. These include optimizing nanoparticle circulation time, ensuring biocompatibility, scaling up manufacturing under Good Manufacturing Practice (GMP) conditions, and standardizing ultrasound parameters for effective activation across diverse anatomical regions. Moreover, the interaction of UTNs with the immune system, blood components, and endothelial surfaces requires further study to prevent unintended side effects. Future developments may involve multimodal nanoparticles capable of responding to multiple stimuli (e.g., pH, enzymes, and ultrasound), patient-specific ultrasound modulation, and AI-guided imaging integration for real-time feedback and dose adjustment. As research continues to progress, UTNs are likely to become a transformative tool in thrombolytic therapy, offering enhanced precision, safety, and efficacy compared to current standards.

Biodegradable Nanocarriers for Regenerative Medicine

The advent of regenerative medicine has redefined therapeutic paradigms, offering innovative strategies to repair, replace, or regenerate damaged tissues and organs. Central to this field is the development of advanced delivery systems that can precisely transport bioactive agents to target sites while minimizing off-target effects. Among these, biodegradable nanocarriers have emerged as a versatile and promising platform, owing to their controlled degradation, tunable release profiles, and superior biocompatibility. These nanocarriers enable spatial and temporal control over the delivery of a broad range of therapeutics, including growth factors, nucleic acids, and stem cell modulators, making them integral to the next generation of regenerative therapies.

Composition and Design Strategies

Biodegradable nanocarriers are primarily engineered from natural or synthetic polymers that undergo enzymatic or hydrolytic degradation into non-toxic byproducts. Common synthetic polymers include poly (lactic acid) (PLA), poly (lactic-co-glycolic acid) (PLGA), and polycaprolactone (PCL), which offer controllable degradation kinetics and mechanical stability.

Natural biopolymers such as chitosan, alginate, and gelatin provide intrinsic biological activity, such as cell adhesion and hemostasis, and exhibit minimal immunogenicity. Advanced design strategies incorporate surface modification and core–shell architectures to enhance functionality. Functionalization with ligands such as peptides, antibodies, or aptamers facilitates targeted delivery, while responsive polymers enable stimuli-triggered release in response to pH, redox conditions, or specific enzymes (Majumder et al., 2021). Moreover, multifunctional nanocarriers, combining imaging agents with therapeutic payloads, are being developed for theragnostic applications in tissue repair.

Applications in Regenerative Therapeutics

Growth Factor Delivery

Controlled and localized delivery of growth factors is critical for stimulating tissue regeneration without systemic side effects. Biodegradable nanocarriers have demonstrated considerable efficacy in encapsulating and sustaining the release of fragile proteins such as vascular endothelial growth factor (VEGF) and bone morphogenetic proteins (BMPs). For instance, PLGA nanoparticles encapsulating BMP-2 have shown enhanced Oste inductive capacity in critical-sized bone defects, improving tissue integration and minimizing ectopic calcification (Qi et al., 2024).

Nucleic Acid-Based Therapies

Nanocarriers also provide a robust platform for gene therapy, delivering nucleic acids such as siRNA, miRNA, or plasmid DNA to modulate gene expression at the site of injury. Chitosan-based nanoparticles, for example, have effectively delivered siRNA targeting inflammatory cytokines in models of myocardial infarction, enhancing cardiac tissue repair. The ability of these carriers to protect nucleic acids from nuclease degradation and facilitate endosomal escape is crucial for therapeutic efficacy.

Stem Cell Engineering

Biodegradable nanocarriers play a pivotal role in stem cell-based regenerative approaches by enhancing stem cell survival, homing, and differentiation. For example, lipid-polymer hybrid nanoparticles delivering retinoic acid have successfully directed the neural differentiation of human embryonic stem cells within three-dimensional culture systems. Furthermore, nanocarrier-mediated delivery of chemokines or genetic material can enhance stem cell recruitment and engraftment at injury sites, which is critical for improving outcomes in ischemic and neurodegenerative conditions.

Targeted and Stimuli-Responsive Delivery

Targeted delivery remains a cornerstone for the successful translation of nanomedicine in regenerative applications. Ligand-directed nanoparticles can selectively bind to cell surface markers expressed in damaged or inflamed tissues, such as integrins or CD44, enhancing

therapeutic precision while minimizing systemic exposure (Liu et al., 2024). Concurrently, stimuli-responsive nanocarriers, designed to respond to pathophysiological cues (e.g., acidic pH in ischemic tissues or enzyme-rich extracellular matrices), offer on-demand release of therapeutics. Enzyme-sensitive polymers that degrade in response to matrix metalloproteinases have demonstrated site-specific drug release in models of chronic wounds and inflammatory diseases.

Despite significant advancements, several barriers impede the clinical translation of biodegradable nanocarriers. These include batch-to-batch variability, challenges in scaling up production, limited understanding of in vivo degradation kinetics, and potential off-target immunogenic responses. Additionally, regulatory frameworks for complex nanomaterials remain underdeveloped, necessitating rigorous safety and efficacy evaluations (Souto et al., 2024).
Future efforts are likely to focus on intelligent nanocarriers that integrate biosensors and feedback-controlled release mechanisms. Moreover, synergistic combinations of nanocarriers with 3D bioprinted scaffolds, microfluidic systems, and CRISPR-Cas9 technologies hold the potential to revolutionize personalized regenerative therapies. The incorporation of machine learning algorithms to predict optimal nanocarrier compositions and release profiles could further enhance translational success. Biodegradable nanocarriers represent a critical frontier in regenerative medicine, offering tailored, safe, and effective delivery systems for a wide array of therapeutic agents. Their ability to interface with complex biological systems, modulate cellular responses, and degrade into innocuous products underscores their utility in clinical tissue regeneration. Continued interdisciplinary research and refinement of biomaterials, coupled with robust clinical validation, will be pivotal in realizing their full potential in restoring tissue function and improving patient outcomes.

Diagnostic Advancements

Nanoparticle-Enhanced Nuclear and Optical Imaging

The convergence of nanotechnology and molecular imaging has opened transformative avenues in diagnostics, enabling the visualization of cellular and molecular processes in real-time with unparalleled sensitivity and precision. Among the most widely used modalities, nuclear imaging including positron emission tomography (PET) and single-photon emission computed tomography (SPECT) and optical imaging, particularly fluorescence and photoacoustic imaging, are vital tools for disease detection, therapy monitoring, and surgical guidance. Despite their clinical value, both modalities face intrinsic limitations such as low spatial resolution (in nuclear imaging) and shallow tissue penetration (in optical imaging). The integration of engineered nanoparticles into these imaging systems addresses these limitations, enhancing signal fidelity, target specificity, and multimodal functionality.

Nanoparticles in Nuclear Imaging

Nuclear imaging relies on the detection of radiolabeled tracers to visualize physiological and pathological processes. However, conventional tracers are often limited by short circulation times, poor target specificity, and rapid systemic clearance. Nanoparticle-based radiotracers

overcome these challenges by serving as versatile platforms for the co-delivery of radionuclides and targeting moieties, significantly improving imaging quality. For example, PEGylated gold nanoparticles (AuNPs) radiolabeled with copper-64 (^64Cu) have demonstrated superior tumor uptake and extended circulation times in murine models, enabling prolonged PET imaging and high tumor-to-background contrast. The high atomic number of gold also contributes to enhanced contrast in computed tomography (CT), facilitating dual-modality PET/CT imaging. Similarly, iron oxide nanoparticles conjugated with chelators like DOTA allow for stable radiolabeling with ^68Ga, providing PET contrast while simultaneously serving as T2-weighted MRI agents (Karageorgou et al., 2023). Another notable approach involves mesoporous silica nanoparticles functionalized with tumor-targeting ligands and loaded with ^99mTc for SPECT imaging. These particles offer high payload capacity, surface modifiability, and tunable release kinetics, enabling both targeted delivery and controlled signal output. Such hybrid nanoplatforms enable real-time tracking of nanoparticle biodistribution while minimizing off-target radiation exposure.

Nanoparticles in Optical Imaging

Optical imaging provides exceptional sensitivity and spatial resolution in preclinical and intraoperative settings but suffers from limited tissue penetration and photo-bleaching of conventional dyes. Nanoparticles address these barriers by enhancing fluoro-phore stability, brightness, and spectral tunability. Quantum dots (QDs) are semiconductor nano-crystals with tunable emission spectra and high photo-stability, making them ideal for long-term fluorescence imaging. Functionalized with tumor-targeting ligands such as folate or RGD peptides, QDs enable specific labeling of cancer cells and lymph nodes, facilitating intraoperative tumor margin delineation. However, concerns about heavy metal toxicity have shifted focus to alternatives like silicon-based QDs and carbon dots, which offer improved biocompatibility. Up-conversion nanoparticles (UCNPs) represent another class of optical agents that absorb near-infrared (NIR) light and emit visible or UV light via anti-Stokes processes. This NIR excitation minimizes tissue auto-fluorescence and permits deeper penetration, making UCNPs ideal for imaging of deep-seated tissues (Yang et al., 2023). When surface-modified with peptides or antibodies, UCNPs enable highly selective tumor imaging with minimal background interference. Moreover, polymeric nanoparticles such as PLGA or PEGylated micelles can encapsulate NIR fluorophores like indocyanine green (ICG) for enhanced photoacoustic imaging. This hybrid technique combines the molecular contrast of optical imaging with the spatial resolution of ultrasound. ICG-loaded nanoparticles were developed that provided high-resolution imaging of tumor vasculature and clear surgical margins during tumor resection.

Multimodal Nanoparticles

A key advancement in the field is the development of multimodal nanoparticles that integrate nuclear and optical imaging within a single nanostructure. These platforms offer complementary advantages: nuclear imaging provides whole-body sensitivity, while optical imaging offers high-resolution, real-time visualization during procedures. For instance, a dual-labeled nanoprobe incorporating both ^64Cu for PET and a NIR dye was created for intraoperative fluorescence imaging. This allowed preoperative tumor localization followed by real-time surgical

guidance, improving resection accuracy and minimizing residual disease. Such theragnostic nanoparticles are at the forefront of personalized medicine, enabling diagnosis, therapy delivery, and monitoring in a unified platform.

Despite significant progress, the clinical translation of nanoparticle-enhanced imaging agents faces multiple hurdles. Key concerns include nanoparticle toxicity, immune recognition, biodistribution unpredictability, and regulatory complexities. Ensuring batch-to-batch consistency, biodegradability, and safe clearance is crucial for human application. To address these concerns, researchers are exploring biodegradable nanomaterials, activatable probes triggered by local stimuli (e.g., pH, enzymes), and "stealth" coatings to reduce immune uptake. Artificial intelligence (AI)-assisted imaging analysis and smart contrast agents that adapt in response to physiological changes are also under development. As imaging becomes more integrated with therapy, nanoparticles that combine imaging with drug delivery or photothermal therapy will become central to image-guided the ranostics. The synergy between nanotechnology and molecular imaging continues to push the boundaries of non-invasive diagnostics, promising earlier detection, better treatment planning, and improved patient outcomes.

Label-Free Leukemia Diagnosis via SERS and AI

Acute leukemia, characterized by the rapid proliferation of immature hematopoietic cells, remains a critical challenge in hematologic oncology due to its aggressive course and the urgent need for early diagnosis. Conventional diagnostic approaches including flow cytometry, cyto-genetics, and molecular testing often rely on invasive biopsies, require specific biomarkers, and entail time-intensive processing. In response to these limitations, an innovative label-free diagnostic paradigm has emerged, combining Surface-Enhanced Raman Scattering (SERS) with Artificial Intelligence (AI) for the non-invasive analysis of exosomes nanoscale extracellular vesicles secreted by cells that harbor molecular signatures of their origin. This integrated approach holds immense potential for early, accurate, and minimally invasive detection of acute leukemia.

Surface-Enhanced Raman Scattering (SERS)

SERS is a powerful analytical technique that amplifies the inherently weak Raman scattering signals of molecules when they are adsorbed onto plasmonic metal nanostructures, typically silver or gold. The localized surface plasmon resonance (LSPR) of these nanoparticles enhances the electromagnetic field around the molecules, yielding signal enhancements of up to 10^8-fold. This ultra-sensitivity enables the detection of minute quantities of biomolecules, making SERS highly suited for early-stage cancer diagnostics. When applied to exosome analysis, SERS enables the direct acquisition of vibrational fingerprints from their biochemical constituents‘ proteins, lipids, RNA, and metabolites without the need for fluorescent or radioactive labels. Studies have demonstrated that SERS spectra of exosomes derived from patients with acute myeloid leukemia (AML) differ markedly from those of healthy controls, particularly in spectral regions associated with nucleic acids and protein conformations. These biochemical alterations reflect the leukemic transformation at the molecular level and form the basis for diagnostic discrimination. Figure 3, demonstrates the surface-enhanced Raman spectroscopy (SERS) provides

a highly sensitive and specific molecular profiling approach, leveraging plasmonic nanostructures to amplify Raman signals for rapid and multiplexed diagnostic applications (Xiong et al., 2023).

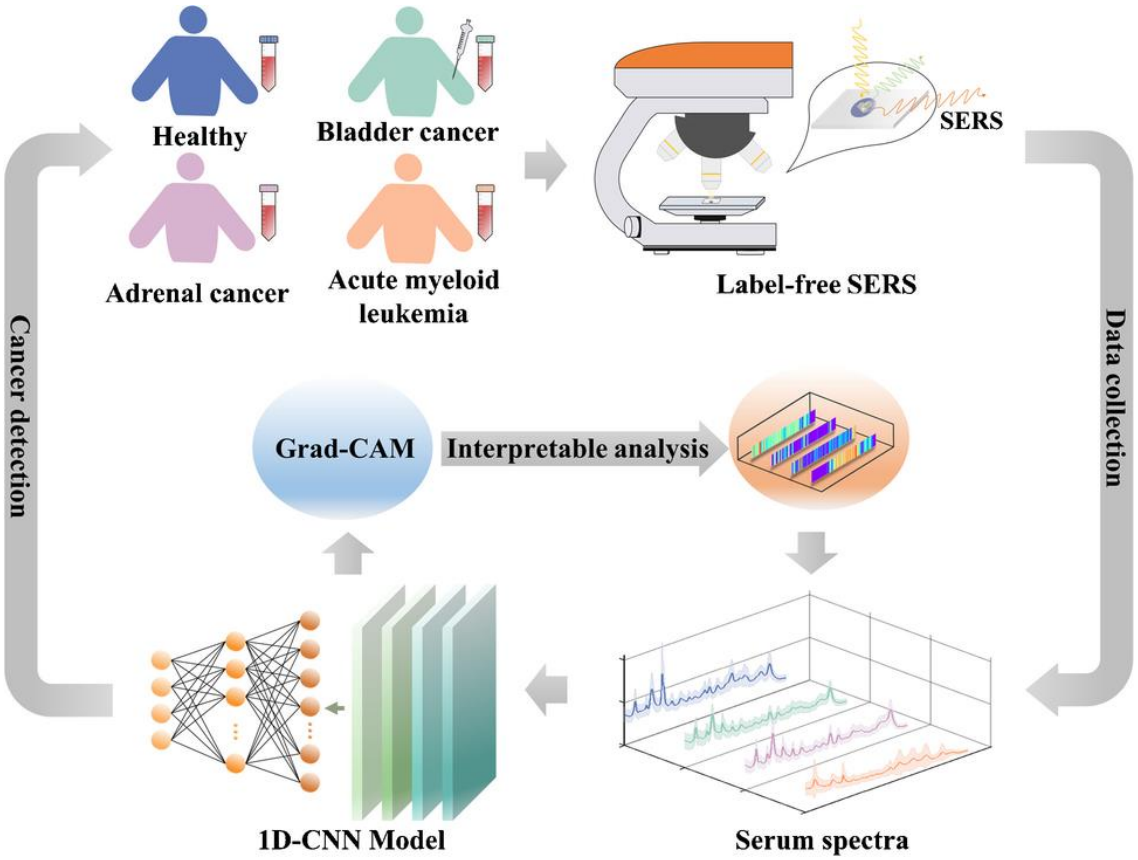


Figure 3. SERS in Molecular Diagnostics: A Platform for Ultra-Sensitive Profiling (Xiong et al., 2023)

Artificial Intelligence: Unlocking Hidden Patterns in SERS Spectra

Surface-Enhanced Raman Spectroscopy (SERS) is a highly sensitive analytical technique that enhances Raman scattering via metallic nanostructures, enabling the detection of low-concentration analytes. However, the interpretation of SERS data is often impeded by spectral complexity, noise, and overlapping vibrational modes challenges that are increasingly being addressed through the integration of Artificial Intelligence (AI). AI, particularly machine learning (ML) and deep learning (DL), excels at discerning patterns in high-dimensional and nonlinear data, making it well-suited for spectral analysis. Traditional chemometric tools like Principal Component Analysis (PCA) and Support Vector Machines (SVMs) have been widely used to classify SERS spectra. More recently, deep learning models especially Convolutional Neural Networks (CNNs) have demonstrated superior performance in analyzing complex spectral datasets. For instance, CNNs trained on SERS spectra of blood samples have been shown to distinguish between healthy individuals and cancer patients with over 90% accuracy (Lin et al., 2025), significantly improving early diagnostic capabilities. In clinical applications, AI-powered SERS has enabled the identification of disease biomarkers in biological fluids such as saliva, urine, and serum with high specificity and minimal sample preparation. This approach is particularly

promising for early cancer detection, including breast and prostate cancers, where early diagnosis is critical for improved outcomes (Wasilewski et al., 2025). By training on large spectral datasets, AI algorithms can learn subtle spectral variations associated with disease states that might elude human interpretation. Beyond medicine, AI-enhanced SERS is being used in environmental monitoring to detect pollutants such as pesticides, heavy metals, and organic dyes. Machine learning algorithms like Random Forests and k-Nearest Neighbors (k-NN) can classify spectral signatures even in the presence of significant noise and interference. For example, the successful detection of trace pollutants in water samples using SERS combined with supervised learning models, highlighting its potential for field-deployable environmental diagnostics. Despite these advances, several challenges persist. A major limitation is the variability in SERS data arising from differences in substrate fabrication, laser excitation, and sample preparation. This heterogeneity can compromise model generalizability. Transfer learning and domain adaptation have been proposed as solutions, allowing pre-trained models to adapt to new but related datasets. Another pressing issue is the interpretability of AI models, especially deep learning architectures that function as "black boxes." In sensitive fields such as medicine and regulatory science, the inability to explain a model's decision-making process poses ethical and practical concerns. To address this, researchers are developing explainable AI (XAI) frameworks that can highlight the most influential spectral features contributing to a classification outcome, thereby enhancing trust and facilitating scientific understanding. Looking forward, the convergence of AI and SERS holds significant promise. Automation, data standardization, and the integration of AI with portable SERS platforms could revolutionize real-time, point-of-care diagnostics and on-site environmental analysis. To fully exploit this synergy, future research must focus on creating large, well-annotated spectral databases, improving the robustness of AI models across experimental conditions, and ensuring transparency in algorithmic decision-making. Artificial intelligence is transforming SERS from a complex laboratory tool into a practical, scalable platform for real-world chemical and biological sensing. By unlocking hidden patterns in spectra, AI is not only enhancing sensitivity and specificity but also expanding the frontiers of what SERS can achieve in clinical, environmental, and industrial applications.

In recent investigations, supervised ML models such as support vector machines (SVM) and convolutional neural networks (CNN) have been trained to classify SERS spectra of exosomes with remarkable accuracy. Gold nanoparticle-coated substrates were employed to acquire SERS spectra from plasma-derived exosomes of AML patients and healthy donors. An SVM model trained on these spectra achieved diagnostic accuracy exceeding 90%, demonstrating the method‘s potential as a label-free and highly sensitive diagnostic tool. Such AI-enhanced SERS platforms can not only differentiate between healthy and diseased states but also stratify leukemia subtypes and potentially track disease progression. Additionally, AI enables real-time analysis and can be integrated into automated diagnostic platforms, paving the way for point-of-care (POC) applications. Feature extraction methods like principal component analysis (PCA) and t-distributed stochastic neighbor embedding (t-SNE) aid in dimensionality reduction and visualization, improving model interpretability and robustness.

Advantages Over Conventional Diagnostic Modalities

The integration of SERS with AI for leukemia diagnostics presents several transformative advantages:

Label-Free Operation: The approach eliminates the need for antibody labeling or enrichment steps, reducing time, cost, and variability.

Minimally Invasive Sampling: Exosomes can be isolated from blood or other body fluids, avoiding bone marrow biopsies and making the method suitable for longitudinal monitoring.

High Sensitivity and Specificity: The enhanced Raman signal coupled with AI-driven classification allows for the detection of subtle biochemical differences with high diagnostic accuracy.

Rapid and Scalable: The analysis can be completed within hours and lends itself to high-throughput formats, ideal for clinical screening.

Furthermore, since exosomes dynamically reflect the physiological state of their cells of origin, this method may allow for early disease detection, relapse monitoring, and evaluation of therapeutic response in real-time hallmarks of precision oncology. Despite the promising progress, several challenges remain. Standardization is a critical hurdle: differences in exosome isolation techniques, SERS substrate fabrication, and spectral acquisition protocols can affect reproducibility and data comparability across laboratories. Establishing universal standards and quality controls is essential for clinical translation. Large-scale, multicenter studies are needed to validate AI models across diverse populations and leukemia subtypes. Avoiding overfitting and ensuring generalizability are key to regulatory approval and widespread adoption. Moreover, interdisciplinary collaboration between oncologists, nanotechnologists, and data scientists will be vital to refine algorithms and adapt them to real-world clinical settings. Future efforts may focus on integrating SERS-AI platforms into lab-on-a-chip or handheld diagnostic devices, enabling decentralized testing in resource-limited environments. Development of open-access spectral databases and AI training repositories will also accelerate innovation and transparency. The convergence of SERS and AI represents a cutting-edge strategy for label-free diagnosis of acute leukemia through exosome profiling. This technique combines the molecular sensitivity of SERS with the analytical power of AI to achieve rapid, accurate, and non-invasive diagnostics. With further development and validation, this technology has the potential to redefine hematologic cancer diagnostics and usher in a new era of exosome-based precision medicine.

Nanorobots for Early Disease Detection

The convergence of nanotechnology and biomedical engineering has given rise to a groundbreaking innovation in healthcare: nanorobots. These nanoscale devices typically ranging from 1 to 100 nanometers in size are engineered to operate within the human body, where they can detect disease biomarkers, analyze physiological conditions, and communicate diagnostic data. Their ability to operate at the cellular and molecular level enables them to identify early

pathological changes that are undetectable by conventional diagnostic methods, offering transformative potential for early disease detection.

Design and Mechanisms of Action

Nanorobots are constructed using a range of biocompatible materials, including DNA origami, polymers, metals (e.g., gold, iron oxide), or carbon-based nanostructures such as carbon nanotubes. They are often equipped with nanoscale sensors, actuators, and communication modules. These devices can be functionalized with antibodies, aptamers, or molecular probes to recognize disease-specific targets like proteins, nucleic acids, or metabolites. Some advanced designs are powered by external fields (e.g., magnetic or ultrasonic), while others rely on internal biochemical gradients for autonomous navigation. A particularly innovative development is the DNA nanobot a self-assembling, programmable DNA structure that responds to molecular cues to release payloads or signal detection events.

Applications in Early Cancer Detection

Cancer is a major focus of nanorobot-based diagnostics due to the disease's reliance on early detection for favorable outcomes. DNA nanorobots have been engineered to recognize tumor-specific biomarkers such as overexpressed nucleolin or HER2 receptors. Once bound, the nanorobot can release a payload (e.g., thrombin or cytotoxins) or emit a signal detectable via imaging or biosensing platforms (Kong et al., 2023). These systems achieve high sensitivity and specificity, often outperforming traditional biomarker assays. Magnetic nanorobots functionalized with targeting ligands have also demonstrated the ability to locate circulating tumor cells (CTCs) in blood, a critical step in metastasis detection. By isolating and analyzing these rare cells, clinicians can detect cancer progression before symptoms appear.

Infectious Diseases and Rapid Diagnostics

Nanorobots offer significant advantages in the detection of infectious agents. For instance, micro- and nanorobots functionalized with pathogen-specific aptamers can identify bacterial or viral antigens in bodily fluids within minutes. These devices not only reduce diagnostic time but also enhance detection sensitivity by orders of magnitude compared to ELISA or PCR-based techniques. Moreover, magnetic or enzyme-powered nanorobots can actively seek out pathogens in complex fluids such as blood or saliva, concentrating them for downstream molecular analysis. This technology has shown promise in the early diagnosis of sepsis and viral infections, where speed and accuracy are critical.

Cardiovascular and Neurological Applications

Early detection of cardiovascular diseases (CVDs) relies heavily on identifying biomarkers like cardiac troponins. Gold nanoparticle-modified nanobiosensors have been developed to bind cardiac troponin I with high affinity, allowing real-time monitoring of myocardial injury. These nanorobots can be integrated into wearable diagnostic platforms for continuous monitoring in at-risk individuals. In the context of neurodegenerative diseases, such as Alzheimer‘s and

Parkinson‘s, nanorobots have been functionalized to detect amyloid-beta peptides and alpha-synuclein aggregates key pathological hallmarks. They can also be engineered to cross the blood-brain barrier (BBB) using targeting ligands like transferrin or lactoferrin, enabling early diagnostic imaging and biomarker sampling directly from the central nervous system. Despite their promise, several technical and translational challenges hinder the widespread adoption of nanorobots in clinical diagnostics. These include:

Manufacturing complexity and reproducibility at nanoscale

1. Precise control of movement and targeting in vivo.

2. Avoidance of immune system clearance and ensuring long-term biocompatibility.

3. Regulatory and ethical concerns regarding autonomous nanosystems in the human body.

Ongoing studies aims to address these issues through advanced fabrication techniques, improved targeting specificity, and development of ~~–s~~mart" biodegradable materials. Furthermore, clinical translation will require rigorous safety evaluations and standardized regulatory frameworks. Nanorobots represent a paradigm shift in early disease detection, combining real-time biosensing, molecular recognition, and active targeting in a single platform. Their ability to detect disease at the molecular level before the onset of symptoms holds promise for significantly improving outcomes across cancer, infectious diseases, cardiovascular disorders, and neurodegeneration. As the technology matures, nanorobots are poised to become indispensable tools in the future of precision diagnostics.

Point-of-Care Diagnostics Using Nanodevices

A Technological Revolution in Rapid Healthcare, Point-of-care (POC) diagnostics represent a paradigm shift in healthcare delivery by enabling rapid, decentralized testing at or near the site of patient care. Traditional diagnostic procedures often involve centralized laboratories, time-consuming sample processing, and delayed results. In contrast, POC technologies provide immediate diagnostic feedback, facilitating timely clinical decisions and improved patient outcomes. Recent advances in nanotechnology have significantly enhanced the capabilities of POC systems. Nanodevices defined as tools and systems operating at the nanoscale offer exceptional sensitivity, specificity, and multifunctionality. Their integration into POC platforms has catalyzed the development of compact, cost-effective, and highly responsive diagnostic tools (Moulahoum et al., 2023).

Advantages of Nanodevice-Enabled POC Systems

Nanodevices confer multiple benefits over conventional diagnostic tools, especially in the context of resource-limited settings and time-sensitive conditions. High Sensitivity and Specificity: Nanomaterials exhibit unique optical, magnetic, and electrical properties that amplify signal detection, enabling identification of biomarkers at ultralow concentrations. Miniaturization and Portability: Devices incorporating nanoscale components are compact and lightweight, allowing

for handheld or wearable formats ideal for field use. Rapid Turnaround Time: Nanodevices can drastically reduce assay duration, with some delivering results in under 15 minutes.

Low Sample Volume: These devices often require only microliter-scale biological fluids, facilitating painless and minimally invasive testing.

1. Nanomaterials Utilized in POC Diagnostics

2. Gold Nanoparticles (AuNPs)

Gold nanoparticles are frequently employed in colorimetric assays due to their surface plasmon resonance (SPR) properties. Upon aggregation, AuNPs exhibit a visible color shift, making them ideal for rapid, visual detection methods such as lateral flow assays.

3. Quantum Dots (QDs)

Quantum dots are semiconductor nanocrystals with tunable fluorescence and high photostability. These characteristics make them suitable for multiplexed detection in optical biosensing platforms, particularly in the early detection of diseases like cancer.

4. Magnetic Nanoparticles

Magnetic nanoparticles (MNPs) are used for target enrichment, separation, and signal enhancement in biosensors. Their functionalization with antibodies or aptamers allows for selective capture of pathogens or biomarkers from complex biological matrices.

5. Carbon Nanotubes and Nanowires

These materials serve as transducers in electrochemical and field-effect transistor (FET)-based sensors. Their high surface area and electrical conductivity make them ideal for detecting low-concentration analytes in real-time.

Detection Modalities in Nanodevice-Based POC Diagnostics

Colorimetric assays using AuNPs are the backbone of widely used lateral flow devices. Their visual simplicity and low cost make them ideal for home or rural applications, such as pregnancy tests or COVID-19 antigen kits.

Fluorescence-Based Detection

Fluorescent probes, including QDs and dye-doped silica nanoparticles, provide highly sensitive detection and are compatible with smartphone-based readers. These systems are particularly suited for diseases requiring multiplex analysis.

Electrochemical Detection

Electrochemical biosensors convert biochemical interactions into electrical signals. Modified electrodes with nanomaterials enhance conductivity and target capture efficiency, making them indispensable for applications like blood glucose monitoring and cardiac biomarker detection.

Clinical Applications

Nanodevice-based diagnostics have played a critical role in detecting infectious pathogens such as HIV, tuberculosis, malaria, and more recently, SARS-CoV-2. Rapid antigen tests for COVID-19, which utilize nanoparticle-enhanced immunoassays, are a prominent example.

Oncology

Nanodevices enable early cancer detection by targeting circulating tumor cells and specific biomarkers like PSA, CEA, or HER2. Their high sensitivity allows detection even at early disease stages, potentially improving prognosis.

Cardiovascular Diseases

Cardiac biomarker detection, such as troponin I and B-type natriuretic peptide (BNP), has been enhanced through POC devices using carbon nanomaterials. These allow real-time monitoring of acute cardiac events at the bedside.

Commercial and Emerging Platforms

Several nanotechnology-enabled POC systems have reached the market or are under advanced development:

1. Abbott BinaxNOW™: Utilizes nanoparticle technology for SARS-CoV-2 antigen detection in under 15 minutes.
2. Cepheid GeneXpert®: A microfluidic cartridge-based system with embedded nanostructures for automated molecular diagnostics.
3. i-STAT System by Abbott: Employs biosensor cartridges enhanced with nanomaterials for blood analysis in emergency care settings.

Despite their immense promise, nanodevice-based POC diagnostics face significant barriers:

Regulatory Compliance: The novelty of nanomaterials raises concerns about safety, reproducibility, and long-term biocompatibility, necessitating rigorous validation.

Scalability and Manufacturing: Producing uniform nanostructures at commercial scale remains challenging, often limiting cost-effectiveness.

Integration with Digital Health: The next generation of POC devices must integrate seamlessly with mobile platforms, cloud databases, and AI-based decision-support systems for telemedicine and remote care. Future development is expected to focus on multi-analyte detection, wireless connectivity, and real-time health monitoring ushering in a new era of personalized, decentralized diagnostics. The convergence of nanotechnology and point-of-care diagnostics marks a transformative leap in medical testing, offering accessible, accurate, and immediate results across a wide range of health conditions. Nanodevices not only enhance traditional diagnostic methods but also pave the way for innovative tools that can operate in resource-limited, remote, and emergency settings. With continued interdisciplinary collaboration and regulatory advancements, nanodevice-enabled POC diagnostics hold great potential for reshaping global health outcomes.

Nano-informatics for Personalized Diagnostics

Nano-informatics is a cutting-edge interdisciplinary field that merges nanotechnology, bioinformatics, artificial intelligence (AI), and systems biology to manage, model, and interpret the vast datasets generated from nanoscale materials and devices. In personalized diagnostics, nano-informatics plays a transformative role by enabling the development of intelligent, patient-specific diagnostic platforms that facilitate early detection, continuous monitoring, and tailored therapeutic decision-making.

Bridging Nanotechnology and Informatics

The use of nanomaterials such as gold nanoparticles, quantum dots, and carbon-based nanostructures in diagnostic devices has grown significantly due to their unique physicochemical properties. However, their biological interactions are complex and highly dependent on factors such as size, shape, surface chemistry, and the patient's physiological environment. Nano-informatics provides computational tools and predictive models to decode these interactions and optimize nanoparticle design for specific diagnostic applications. For instance, AI-driven algorithms can predict the composition and behavior of the protein corona that forms around nanoparticles upon exposure to biological fluids. This corona significantly influences nanoparticle biodistribution, cellular uptake, and immune recognition. Accurately modeling this process enables the customization of nano-diagnostic agents based on individual biomolecular profiles, enhancing both sensitivity and specificity.

Personalized Diagnostics through Nano-informatics

Personalized diagnostics aims to detect disease markers unique to each individual, often at the molecular or genetic level. Nano-informatics accelerates the development of nano-biosensors that can identify ultra-low concentrations of disease biomarkers such as circulating tumor DNA (ctDNA), microRNAs, or exosomal proteins with high precision. These sensors are increasingly being designed using machine learning models that optimize surface functionalization, signal amplification, and molecular recognition capabilities. In oncology, nano-informatics enables the stratification of patients by modeling how nanoparticles interact with different tumor microenvironments. This supports the selection of nano-diagnostics suited to specific cancer subtypes, such as HER2-positive or triple-negative breast cancer. Moreover, real-time data from

wearable nanosensors can be processed via cloud-integrated platforms to offer continuous, dynamic monitoring of health parameters, which is especially valuable in managing chronic conditions or monitoring cancer recurrence (Imoize et al., 2023). Despite its enormous potential, nano-informatics faces several challenges. Standardization of data formats, interoperability among databases, and reproducibility of predictive models remain major hurdles. Additionally, the clinical translation of nano-informatics tools requires rigorous validation, regulatory approval, and close collaboration between researchers, clinicians, and industry stakeholders. Nevertheless, efforts such as the National Cancer Institute‘s Cancer Nanotechnology Laboratory (CaNanoLab) portal and the NanoHub platform are addressing these issues by offering open-access repositories for nanomaterial characterization and modeling. These initiatives support data sharing and foster collaboration across disciplines (NCI, 2024). Looking ahead, the integration of nano-informatics with multi-omics data (genomics, proteomics, metabolomics), digital health platforms, and next-generation AI tools will enable the creation of intelligent diagnostic systems. These systems will not only detect disease at earlier stages but will also adapt over time to reflect the patient‘s evolving health status. In doing so, nano-informatics is poised to become a cornerstone of precision medicine offering diagnostics that are not only accurate, but truly personalized.

Therapeutic Breakthroughs

Nanoparticle-Based Immunotherapy for Cancer

Cancer immunotherapy has transformed the landscape of oncology by leveraging the immune system to selectively identify and eradicate malignant cells. However, clinical responses remain heterogeneous due to several challenges, including poor pharmacokinetics, immune-related adverse events, and the immunosuppressive tumor microenvironment (TME). Nanoparticle-based delivery platforms offer promising solutions by enhancing the precision, potency, and safety of immunotherapeutic agents.

Nanoparticle Platforms for Immune Modulation

Nanoparticles (NPs) ranging from 1 to 100 nanometers are versatile carriers capable of delivering a wide spectrum of immune-modulators, including immune checkpoint inhibitors, cytokines, adjuvants, and nucleic acids. Their high surface-area-to-volume ratio allows for efficient drug loading, while surface modification strategies enable active targeting of immune cells or tumor tissues. Furthermore, NPs protect encapsulated agents from enzymatic degradation and can be engineered for controlled or stimuli-responsive release. Lipid-based nanoparticles (LNPs) are among the most clinically advanced platforms, exemplified by their role in mRNA vaccine delivery. In cancer immunotherapy, LNPs can deliver mRNA encoding tumor-associated antigens (TAAs), neoantigens, or immunostimulatory cytokines to dendritic cells (DCs), promoting antigen presentation and cytotoxic T lymphocyte (CTL) activation (Han et al., 2023). Polymeric nanoparticles, particularly those composed of FDA-approved PLGA, have also demonstrated efficacy in co-delivering TAAs with adjuvants such as toll-like receptor (TLR) agonists, enhancing both humoral and cellular immune responses. Additionally, inorganic nanoparticles, such as gold or silica-based systems, offer tunable physicochemical properties for applications in photothermal

therapy and immune activation. For instance, gold nanoparticles conjugated with tumor antigens can act as both immune enhancers and localized heat inducers, facilitating immunogenic cell death and subsequent immune priming (Huang et al., 2023).

Reprogramming the Tumor Microenvironment

The immunosuppressive TME remains a significant obstacle to effective immunotherapy. Key suppressive cell populations tumor-associated macrophages (TAMs), regulatory T cells (Tregs), and myeloid-derived suppressor cells (MDSCs) limit immune activation and foster tumor progression. Nanoparticles can be functionalized to deliver agents such as CSF1R or PI3Kγ inhibitors that selectively deplete or reprogram TAMs, converting the TME into a more immunostimulatory environment. Moreover, immune checkpoint inhibitors, including antibodies targeting PD-1/PD-L1 and CTLA-4, have shown remarkable efficacy but are often associated with systemic toxicity. Nanocarriers enable localized delivery of these agents or small interfering RNAs (siRNAs) targeting checkpoint molecules, thereby increasing tumor specificity and minimizing off-target immune activation (Choi et al., 2024).

Personalized Nanovaccines and Combination Strategies

Personalized cancer vaccines are gaining traction, particularly in the context of neoantigen-targeted immunotherapy. Nanoparticles encapsulating patient-specific mRNA neoantigens can be rapidly manufactured and administered to elicit robust T cell responses. A recent clinical study demonstrated that mRNA-loaded LNPs induced durable polyclonal immune responses in melanoma patients, with promising therapeutic outcomes. NPs also enable rational combination therapies by co-delivering immune agonists with chemotherapeutics, photodynamic agents, or gene-editing tools like CRISPR-Cas9. These combinations can induce immunogenic cell death and simultaneously activate multiple arms of the immune system, effectively converting "cold" tumors characterized by low T cell infiltration into "hot" tumors that respond more favorably to immunotherapy. Despite substantial progress, several challenges impede the widespread clinical translation of nanoparticle-based immunotherapies. These include complex manufacturing processes, variability in pharmacokinetics, and limited understanding of long-term biodistribution and safety. Furthermore, the inter- and intra-tumoral heterogeneity necessitates the development of adaptive and personalized NP systems. Future innovations will likely focus on the integration of theragnostic nanoparticles combining diagnostic imaging with therapeutic function as well as the use of biomimetic carriers such as exosomes for enhanced biocompatibility and immune evasion. Additionally, advances in artificial intelligence and machine learning may facilitate the design of predictive nanoparticle formulations optimized for individual patient profiles. Nanoparticle-based immunotherapy represents a rapidly evolving frontier with the potential to reshape the future of cancer treatment. By addressing current limitations and leveraging multidisciplinary innovation, this approach could enable more precise, durable, and accessible immunotherapeutic solutions.

Nanoparticle-Assisted Gene Therapy

Gene therapy holds transformative potential for treating genetic and acquired diseases by introducing, modifying, or silencing specific genes within a patient's cells. However, the clinical

application of gene therapy has long been limited by challenges in delivering nucleic acids effectively and safely. Naked DNA or RNA is rapidly degraded in biological fluids, exhibits poor cellular uptake, and often fails to reach its target within the body. Nanoparticle-assisted gene delivery offers a powerful strategy to overcome these barriers, enabling protection, targeted transport, and controlled release of therapeutic genetic material.

Nanoparticles as Vectors for Gene Delivery

Nanoparticles (NPs) are sub-micrometer structures that can be engineered to encapsulate or bind nucleic acids such as plasmid DNA, small interfering RNA (siRNA), microRNA (miRNA), and messenger RNA (mRNA). Their high surface area, customizable surface chemistry, and ability to cross biological membranes make them ideal candidates for gene delivery. Among the most advanced systems are lipid nanoparticles (LNPs), which are particularly well-suited for mRNA and CRISPR-Cas9 delivery. LNPs form stable complexes with nucleic acids, shielding them from nuclease degradation while promoting efficient endocytosis and endosomal escape. Their utility was demonstrated at scale during the COVID-19 pandemic, where they served as the backbone for mRNA vaccine delivery. Polymeric nanoparticles, such as those based on poly (lactic-co-glycolic acid) (PLGA), polyethyleneimine (PEI), or chitosan, are widely used for DNA and siRNA delivery. These systems offer excellent biocompatibility and tunable release profiles. However, cationic polymers like PEI can exhibit dose-dependent cytotoxicity, necessitating optimization to balance efficacy and safety. Inorganic nanoparticles, including gold nanoparticles (AuNPs), magnetic nanoparticles, and mesoporous silica, provide additional functionalities such as imaging, magnetic targeting, and external stimulus-responsiveness. For instance, AuNPs can be functionalized with siRNA and triggered by light or heat to release their cargo in a controlled manner.

Mechanisms of Delivery and Cellular Uptake

The delivery of genetic material via nanoparticles involves a series of critical steps: systemic circulation, extravasation into target tissue, cellular internalization, and intracellular release. Once administered, nanoparticles must evade immune clearance and enzymatic degradation. Surface modification with hydrophilic polymers like polyethylene glycol (PEG) prolongs circulation and reduces recognition by the mononuclear phagocyte system. Following accumulation in the target tissue often through passive mechanisms like the enhanced permeability and retention (EPR) effect or active ligand-mediated targeting nanoparticles are internalized by cells via endocytosis. For gene therapy to be effective, endosomal escape is essential. Many nanoparticle formulations are designed to exploit the acidic environment of endosomes, triggering structural changes that disrupt the membrane and release the payload into the cytosol (Degors et al., 2019). For DNA or CRISPR-based therapies, transport to the nucleus may also be required for full activity.

Clinical Applications and Emerging Therapies

Nanoparticle-assisted gene delivery has shown promise across a wide range of therapeutic areas. In oncology, nanoparticles are used to deliver siRNAs or miRNAs to modulate gene

expression in tumors. For example, siRNA-loaded LNPs targeting mutant KRAS have shown therapeutic efficacy in models of pancreatic and lung cancers. Combinatorial nanocarrier systems delivering both siRNA and chemotherapeutic agents are also being explored for synergistic effects. In genetic disorders, nanoparticle platforms have enabled the delivery of CRISPR-Cas9 systems for in vivo gene editing. Lipid-based nanoparticles have been used to correct pathogenic mutations in models of hereditary transthyretin amyloidosis and Duchenne muscular dystrophy, demonstrating the feasibility of transient, non-viral genome editing. Infectious diseases have similarly benefited from NP-based gene delivery. LNPs were critical in the development of mRNA vaccines against SARS-CoV-2 and hold promise for rapid-response platforms against future pathogens. Beyond vaccination, nanoparticles are also being explored for the delivery of antiviral siRNAs and immune-modulatory genes in diseases such as HIV and hepatitis B. Despite rapid progress, several challenges remain before nanoparticle-assisted gene therapy reaches its full clinical potential. Achieving efficient, tissue-specific delivery while avoiding off-target effects and systemic toxicity remains a key hurdle. The immunogenicity of certain NP formulations can limit repeated administration, while large-scale production and regulatory standardization remain complex. Future directions include the development of stimuli-responsive nanoparticles, which release their cargo in response to pH, enzymes, or external cues like light or ultrasound. These technologies enable precise spatial and temporal control of gene delivery (Rosenblum et al., 2020). Another promising avenue is biomimetic nanoparticles, such as exosome-mimicking vesicles or cell membrane-coated NPs, which offer enhanced biocompatibility and natural homing capabilities. Integrating nanoparticle technologies with precision medicine guided by patient-specific genomic, transcriptomic, and proteomic profiles could further optimize therapeutic outcomes. With continued advances in materials science, synthetic biology, and clinical translation, nanoparticle-assisted gene therapy is poised to become a cornerstone of personalized medicine in the coming decade.

References

Ashique, S., Almohaywi, B., Haider, N., Yasmin, S., Hussain, A., Mishra, N., & Garg, A. (2022). siRNA-based nanocarriers for targeted drug delivery to control breast cancer. *Advances in Cancer Biology-Metastasis*, *4*, 100047.

Bisht, A., Avinash, D., Sahu, K. K., Patel, P., Das Gupta, G., & Kurmi, B. D. (2025). A comprehensive review on doxorubicin: mechanisms, toxicity, clinical trials, combination therapies and nanoformulations in breast cancer. Drug Delivery and Translational Research, 15(1), 102-133.

Choi, Y., Seok, S. H., Yoon, H. Y., Ryu, J. H., & Kwon, I. C. (2024). Advancing cancer immunotherapy through siRNA-based gene silencing for immune checkpoint blockade. Advanced drug delivery reviews, 115306.

Degors, I. M., Wang, C., Rehman, Z. U., & Zuhorn, I. S. (2019). Carriers break barriers in drug delivery: endocytosis and endosomal escape of gene delivery vectors. Accounts of chemical research, 52(7), 1750-1760.

Huang, H., Liu, R., Yang, J., Dai, J., Fan, S., Pi, J., ... & Guo, X. (2023). Gold nanoparticles: construction for drug delivery and application in cancer immunotherapy. Pharmaceutics, 15(7), 1868.

Ibraheem, A. S., Nwosu, C. B., Omowumi, R. K., & Ayodapo, A. O. (2025). Managing population health through prevention and early detection of cancer and other non-communicable diseases: a call for action. Discover Public Health, 22(1), 143.

Imoize, A. L., Balas, V. E., Solanki, V. K., Lee, C. C., & Obaidat, M. S. (Eds.). (2023). Handbook of Security and Privacy of AI-Enabled Healthcare Systems and Internet of Medical Things. Boca Raton, FL, USA:: CRC press.

Karageorgou, M. A., Bouziotis, P., Stiliaris, E., & Stamopoulos, D. (2023). Radiolabeled iron oxide nanoparticles as dual modality contrast agents in SPECT/MRI and PET/MRI. Nanomaterials, 13(3), 503.

Kong, X., Gao, P., Wang, J. *et al.* Advances of medical nanorobots for future cancer treatments. *J Hematol Oncol* 16, 74 (2023). https://doi.org/10.1186/s13045-023-01463-z

Kong, X., Gao, P., Wang, J., Fang, Y., & Hwang, K. C. (2023). Advances of medical nanorobots for future cancer treatments. Journal of Hematology & Oncology, 16(1), 74.

Lin, Y., Zhang, Q., Chen, H., Liu, S., Peng, K., Wang, X., ... & Wang, C. (2025). Multi-cancer early detection based on serum surface-enhanced Raman spectroscopy with deep learning: a large-scale case–control study. BMC medicine, 23(1), 97.

Liu, B., Liu, T., Li, Y., & Tan, C. (2024). Innovative Biotherapies and Nanotechnology in Osteoarthritis: Advancements in Inflammation Control and Cartilage Regeneration. *International Journal of Molecular Sciences*, *25*(24), 13384.

Majumder, J., & Minko, T. (2021). Multifunctional and stimuli-responsive nanocarriers for targeted therapeutic delivery. Expert opinion on drug delivery, 18(2), 205-227.

Mao, M., Wu, Y., & He, Q. (2024). Breaking Through Physiological Barriers: Nanorobotic Strategies for Active Drug Delivery. Bioconjugate Chemistry, 36(1), 1-14.

Moulahoum, H., Ghorbanizamani, F., Beduk, T., Beduk, D., Ozufuklar, O., Celik, E. G., & Timur, S. (2023). Emerging trends in nanomaterial design for the development of point-of-care platforms and practical applications. Journal of Pharmaceutical and Biomedical Analysis, 235, 115623.

Qi, J., Wu, H., & Liu, G. (2024). Novel strategies for spatiotemporal and controlled BMP-2 delivery in bone tissue engineering. Cell Transplantation, 33, 09636897241276733.

Rosenblum, D., Gutkin, A., Kedmi, R., Ramishetti, S., Veiga, N., Jacobi, A. M., ... & Peer, D. (2020). CRISPR–Cas9 genome editing using targeted lipid nanoparticles for cancer therapy. Science Advances, 6(47), eabc9450. https://doi.org/10.1126/sciadv.abc9450

Souto, E. B., Blanco-Llamero, C., Krambeck, K., Kiran, N. S., Yashaswini, C., Postwala, H., ... & Maheshwari, R. (2024). Regulatory insights into nanomedicine and gene vaccine innovation: Safety assessment, challenges, and regulatory perspectives. Acta biomaterialia.

Wasilewski, T., Kamysz, W., & Gębicki, J. (2024). A I-assisted detection of biomarkers by sensors and biosensors for early diagnosis and monitoring. Biosensors, 14(7), 356.

Xiong, C. C., Zhu, S. S., Yan, D. H., Yao, Y. D., Zhang, Z., Zhang, G. J., & Chen, S. (2023). Rapid and precise detection of cancers via label-free SERS and deep learning. *Analytical and Bioanalytical Chemistry*, *415*(17), 3449-3462.

Yang, Y., Jiang, Q., & Zhang, F. (2023). Nanocrystals for deep-tissue in vivo luminescence imaging in the near-infrared region. Chemical Reviews, 124(2), 554-628.

About The Authors

Khawar Ali is currently pursuing his Ph.D. at North University of China, following his M.S. in Molecular Biology. He has previously served as a Research Associate at the Virtual University of Pakistan and Cholistan University of Veterinary and Animal Sciences, Bahawalpur, where he gained extensive experience in Molecular Biology research and laboratory techniques. Additionally, he worked as a Molecular Biologist at IBN-E-SINA Clinical Laboratory & Research Center in Lahore, contributing to clinical diagnostics and research operations. Khawar Ali has published several research articles in reputable peer-reviewed scientific journals. His research interests include Oncology, Molecular Biology, Genomics, and Biotechnology, with a particular focus on Molecular Diagnostics and Cancer Biology. Through his academic and professional endeavors, he is dedicated to advancing scientific knowledge and contributing to innovations in biomedical research.

Email: khawarali1202@gmail.com **ORCID:** 0000-0002-0565-4535.

Ghulam Murtaza holds a Bachelor's degree in Biotechnology and currently pursuing his M.Phil. in Molecular Biology at the University of Okara Pakistan. His research focuses on the NRAMP-1 gene, exons to investigate their role in natural resistance against infectious diseases. NRAMP-1 is a key player in immune defense, and its genetic variations may influence susceptibility to infections like tuberculosis. He is deeply interested in the molecular mechanisms underlying host-pathogen interactions and their outcomes. His academic journey reflects a strong passion for Molecular Biology, Biotechnology, Infectious Disease and Precision Medicine research.

E-mail: ghulammurtaza8817@gmail.com **ORCID:** 0009-0003-3557-0054.

Dr. Muhammad Masood Ahmed is an Associate Professor of Pharmacology and serving as the Principal of the College of Pharmacy at Nishtar Medical University, Multan, Pakistan. His research interests encompass immunology, pharmacology, pathology, angiogenesis, and oncology. With a strong record of scholarly publications and active participation in national and international scientific forums, Dr. Ahmed has made impactful contributions to these critical areas of medical science. His work continues to advance research, education, and clinical understanding, positioning him as a respected figure in his field.

Email: masoodkarni786@gmail.com **ORCID:** 0000-0002-4432-958.

Prof. Dr. Mehmet ÖZASLAN received his PhD in 1995 Institute of Natural Sciences at Cukurova University, Turkiye. He is a Professor in Molecular Biology and Genetics. His research interests are included Cancer Genetics, Molecular Virology, Molecular Genetics, Microbiology, and Genetic mutations etc. He has published more than 200 research articles in national and international well reputed journals. He also has written many book chapters as well as edited books. He is an editor and editor in chief of many well reputed national and international journals.

E-mail: ozaslanmd@gantep.edu.tr **ORCID:** 0000 0001 9380 4902

Similarity Index
The similarity index obtained from the plagiarism software for this book chapter is 11%.

To Cite This Chapter:
Ali K et al., (2025). Nanotechnology in Healthcare innovation in Drug delivery, Diagnostics and Therapeutics. In Current Studies in Life Sciences 2025 (pp.154-181). ISRES Publishing.

Sequence Analysis Of HBV DNA From Hepatitits B Patents

Mehmet OZASLAN

Abstract

About 296 million individuals worldwide are infected with the hepatitis B virus (HBV), which causes cirrhosis, hepatocellular cancer, and chronic liver disease. This infection is still a serious global health concern. Drug resistance, treatment response, and disease progression are all significantly influenced by HBV's genetic diversity. HBV DNA sequence analysis offers crucial information about the evolution of the virus, genotype distribution, mutation patterns, and processes behind antiviral resistance. The techniques utilized in HBV DNA sequencing, such as Sanger sequencing and next-generation sequencing (NGS), are covered in this study along with how they are used to track the course of the disease and improve treatment plans. Understanding the severity of the disease and the prognosis of patients requires knowledge of the HBV genotypes (A–J) and their geographic distribution. Research shows that while genotype B is linked to a better prognosis, genotype C is linked to an increased risk of liver cancer. Furthermore, the efficacy of viral replication and immune escape mechanisms are greatly impacted by changes in the polymerase and pre-core/core regions of HBV DNA. Reduced effectiveness of nucleos(t)ide analogues like lamivudine and entecavir has been associated with resistance mutations, such as rtM204I/V in the reverse transcriptase (RT) domain. Researchers and physicians can enhance antiviral medication design, create individualized therapy approaches based on patient characteristics, and more accurately forecast treatment outcomes using thorough sequence analysis.

Keywords: Hepatitis B virus (HBV), HBV DNA sequencing, genetic diversity, antiviral resistance, genotype distribution, mutation patterns, next-generation sequencing (NGS), Sanger sequencing, liver disease, cirrhosis, hepatocellular carcinoma, drug resistance, treatment response, viral replication, immune escape, polymerase mutations, pre-core/core mutations, nucleos(t)ide analogues, lamivudine resistance, entecavir resistance, personalized therapy.

1_Introduction

The hepatotropic DNA virus known as the Hepatitis B virus (HBV) is a member of the Hepadnaviridae family and is distinguished by its genome, which is partially double-stranded and experiences reverse transcription during replication. An estimated 820,000 people die each year from consequences including cirrhosis and hepatocellular carcinoma, which are caused by HBV infection, one of the main causes of chronic liver disease (WHO, 2021). At least eleven genotypes (A-J) of the virus show considerable genetic variability, affecting clinical outcomes, antiviral medication responses, and the course of the disease (Kramvis, 2014). For example, hepatocellular carcinoma is more likely to occur in people with genotype C, which is common in East Asia, while genotype B is connected to improved interferon responsiveness and less severe illness (Liu & Kao, 2013).The development of drug-resistant strains and a knowledge of viral pathophysiology depend on the molecular characterization of HBV DNA via sequence analysis. Polymerase chain reaction (PCR)-based quantification of HBV DNA and serological markers (HBsAg, HBeAg) are the mainstays of conventional diagnostic techniques, however they are unable to identify genetic

changes that affect the effectiveness of treatment (Shi et al., 2012). According to Seeger and Mason (2015), high-resolution investigation of HBV mutations, especially those that confer antiviral resistance, is made possible by advancements in sequencing methods like as Sanger sequencing and next-generation sequencing (NGS). According to Lok et al. (2007), patients taking lamivudine have been found to have resistance mutations such rtM204V/I in the polymerase gene, which drastically lowers the effectiveness of treatment.The appearance of these mutations calls for consistent molecular monitoring and modification of treatment plans. Additionally, HBV replication and immune evasion are modulated by mutations in the pre-core (PC) and basal core promoter (BCP) regions. For instance, the reduction of HBeAg expression caused by the G1896A mutation in the PC region is typical of genotype D and is associated with severe liver illness and prolonged infection (Kramvis, 2014). The necessity of accurate sequence analysis to assist the development of new antiviral treatments and educate clinical decision-making is highlighted by these genetic variants. Controlling the course of the disease and improving patient outcomes will require combining genetic surveillance with customized medicine techniques as HBV continues to develop.

2_Virology of HBV

2.1Genomic Structure

A crucial component of the Hepatitis B virus's (HBV) biology, its genomic structure affects the virus's pathogenesis, replication, and interactions with the host immune system. Approximately 3,182 base pairs make up the small genome of HBV, a partly double-stranded DNA virus, which is arranged into four overlapping open reading frames (ORFs) that encode vital viral proteins. These proteins, which are essential to the virus's life cycle and pathogenicity, include the X protein (HBx), polymerase (HBp), core protein (HBcAg), and surface antigen (HBsAg). Understanding the complexity of the genomic structure and how it affects immune evasion and viral replication is essential to comprehending the virology of HBV.

Genomic Organization

The four overlapping ORFs that make up the genome of HBV are S, C, P, and X. The surface antigen (HBsAg), which is essential for immune evasion and viral entry, is encoded by the S ORF (Pervaiz & Cheema, 2024). The core protein (HBcAg), which is necessary for capsid assembly and genome packaging, is encoded by the C ORF (McFadden & Sarafianos, 2023). P ORF: Codes for the polymerase, which uses reverse transcription to replicate viral DNA (Pervaiz & Cheema, 2024). The X protein (HBx), which is involved in transcriptional control and may be connected to oncogenesis, is encoded by the X ORF (Zhang et al., 2025).

Genomic Variability and Genotypes

There is substantial genetic heterogeneity in HBV, with several genotypes (A–J) influencing treatment responses and clinical outcomes. According to Mamoori et al. (2024), genotype D, for example, is common in particular areas and linked to particular serotypes and mutations. According to Zhang et al. (2025), mutations in the HBx gene cause the genotype A

subtypes (A1 and A2) to exhibit different replication behaviors, which impact viral replication and clinical symptoms.

Implications for Pathogenesis and Treatment

Understanding the pathophysiology of HBV and creating efficient therapeutics depend heavily on its genetic diversity and structure. Genomic mutations can affect the severity of liver disease and result in medication resistance (Mamoori et al., 2024). The development of tailored treatments, including capsid assembly modulators (CAMs), which show promise in lowering viral replication and possibly treating chronic hepatitis B, is facilitated by an understanding of the genetic structure (McFadden & Sarafianos, 2023).Even though HBV's genomic structure is well understood, research is still being done to determine how genetic diversity and mutations affect the course of the disease and the effectiveness of treatment. This knowledge is essential for creating more potent treatment plans and vaccinations to fight HBV infection worldwide.

The HPV Replication Cycle

Human papillomavirus (HPV) is a double-stranded DNA virus that relies on interactions with epithelial host cells to complete its life cycle. The infection begins when the virus infects the basal layer of the epithelium through minor abrasions. Once inside the host cell, HPV remains in a simple replication phase to maintain its copy number during cell division, exploiting the host‘s DNA repair mechanisms to ensure its persistence (Vats & Laimins, 2025). During epithelial cell differentiation, viral DNA replication increases, leading to the amplification of the viral genome in preparation for new virion production. The virus manipulates DNA repair pathways, such as ATM, ATR, and FA, to stabilize its replication and generate sufficient viral genome copies for new infections. In the final stage, full viral gene expression is activated in the upper epithelial layers, resulting in the assembly and release of viral particles through desquamation of infected cells. Notably, HPV does not cause cell lysis upon release; instead, it relies on the natural shedding of superficial epithelial cells to disseminate new viral particles. This strategy allows HPV to evade immune detection, contributing to chronic infection and the potential for oncogenic transformation in high-risk strains (Vats & Laimins, 2025). HPV‘s ability to manipulate DNA repair pathways and the host cellular environment distinguishes it from many other viruses, making it a key focus in viral oncology and immunotherapy research.

The Replication Cycle of Human Papillomavirus (HPV)

Viral entrance into basal keratinocytes, usually via epithelial microabrasions, initiates the HPV life cycle. After entering, the viral genome travels to the nucleus, where it is kept at a low copy number in basal cells that are proliferating. The virus initiates its replication machinery when these infected cells develop and move in the direction of the epithelial surface, using host DNA repair mechanisms including ATM and ATR to promote genome amplification (Vats & Laimins, 2025). Replication is controlled by the viral E1 and E2 proteins, which guarantee genome persistence without prompt immune recognition. When terminally developed keratinocytes shed from the epithelium in the late stages, HPV's production of structural proteins (L1 and L2) results in the construction of new virions. According to recent research, HPV increases replication efficiency by modifying cellular components such as SAMHD1, indicating possible targets for

treatment (James et al., 2024). The virus's pathogenic potential is largely dependent on its capacity to survive in host cells while eluding immune responses, particularly in high-risk strains linked to cervical and other malignancies (Lopes et al., 2024). Knowing these molecular connections helps develop vaccines and antiviral treatments and offers important insights into disorders linked to HPV.

HPV Genotypes and Mutations

There are more than 200 genotypes of the human papillomavirus (HPV), which are divided into low-risk and high-risk varieties according to their capacity to cause cancer. The high-risk varieties, especially HPV16 and HPV18, have a substantial correlation with anogenital malignancies, including cervical cancer. According to recent research, HPV16 exhibits a high degree of genetic diversity, with mutations occurring in important oncogenes including E6 and E7. According to Le et al. (2024), these changes impact the development of cancer by changing the oncogenicity and immune evasion of viruses. Numerous sublineages of HPV16 (A1, A2, A3, A4, D1, D3) have been identified by advanced sequencing techniques, and each is linked to a unique geographic distribution and cancer risk. Mutations that may contribute to increased carcinogenicity include T267A in the L1 gene and L83V in the E6 gene (Qu et al., 2024). Furthermore, HPV16 whole-genome sequencing has revealed SNP variants and insertions/deletions (Indels), especially in the E1, E2, and L2 genes, which could affect the effectiveness of viral replication (Minhas et al., 2024).To aid in the discovery of vaccine targets, a thorough HPV mutation database (HPVMD-C) has been created to catalog more than 149 HPV genotypes and 468 mutations (OUP, 2022). Notwithstanding these results, some research indicates that patient genetic variations can contribute more to the development of cervical cancer than HPV mutations alone (Zhang et al., 2024). Research on the interaction of host immune response, vaccination coverage, and HPV genotype differences is still crucial.

HPV Mutations and Their Clinical Impact

Mutations in the human papillomavirus (HPV) are essential for the development of illness, immune evasion, treatment resistance, and vaccination escape. (1) Mutations in the precore and basal core promoters affect the oncogenic potential and viral replication. Mutations in the basal core promoter (BCP), especially in HPV16, change the binding sites of transcription factors, which increases the production of the oncogenes E6 and E7. Research indicates that these mutations are associated with an increased risk of cervical cancer (Zhang et al., 2024). (2) Polymerase Gene alterations (Drug Resistance): The effectiveness of antiviral medications is impacted by HPV's DNA polymerase alterations. For example, research suggests that resistance to nucleotide analogs is influenced by hypermutations in polymerase genes caused by APOBEC3 (Holt et al., 2024). (3) Vaccine escape mutations, or surface gene mutations: HPV surface protein changes, particularly in the L1 and L2 genes, might change epitopes and perhaps lessen the effectiveness of vaccines. By altering viral capsid architecture, certain mutations enable immune evasion (Atique et al., 2023). Even while vaccines are still very effective against the primary HPV strains, monitoring possible vaccine escape variants requires ongoing genomic surveillance. All things considered, knowledge of these mutations helps to explain HPV etiology and guides future treatment and vaccination plans.

Table : HPV Mutations and Their Clinical Implications

Relevance to Treatmnt	Clinical İmpact	Affected Gene/Region	Mutation Type
Potential biomarker for disease progression	Increased viral replication immune evasion higher	Regulatory regions (E6/E7)	Precore &BCP Mutations
May impact antiviral strategies	Potential resistance to nocleotide analogs	DNA polymerase gene	Polymerase Gene Mutations
Could reduce vaccine fficacy over time	Vaccine escape,altered antigenicity	L1,L2 genes	Surface Gene Mutation

HPV DNA Sequencing Techniques

Understanding viral variety, identifying mutations, and evaluating the effectiveness of vaccines all depend on the research of Human Papillomavirus (HPV) DNA sequencing. HPV genomes are analyzed using a variety of sequencing methods, such as phylogenetic analysis, next-generation sequencing (NGS), polymerase chain reaction (PCR), and Sanger sequencing. In HPV research, each approach has unique benefits and uses.

1_Polymerase Chain Reaction (PCR) and Sanger Sequencing: The most popular technique for amplifying particular HPV genome sections is PCR. It has excellent sensitivity and specificity for identifying HPV genotypes. For genotyping, Sanger sequencing, a first-generation DNA sequencing technique, is frequently combined with PCR. It allows for the accurate detection of mutations by selectively amplifying DNA and then terminating the chain. Despite its accuracy, mixed HPV infections cannot be effectively analyzed by Sanger sequencing due to its low throughput (Cabral et al., 2018).

2_Next-Generation Sequencing (NGS): Whole-genome HPV sequencing is made possible by the high-throughput sequencing technique known as NGS. It offers more profound understanding of co-infections, mutation patterns, and viral evolution. By focusing on certain genomic areas, amplicon-based NGS approaches improve sequencing productivity while lowering costs and preserving high accuracy. Research has shown that NGS can offer thorough viral genotyping and identify uncommon mutations (Techera et al., 2023).

3_Phylogenetic Analysis: The evolutionary links between various HPV strains are investigated using phylogenetic analysis. Researchers can determine transmission patterns, track the origin of vaccine escape mutants, and trace viral lineage by comparing sequence data. Epidemiological research and tracking the success of immunization campaigns require this approach (Hussein et al., 2024).

Table: Comparison of HPV DNA Sequencing Technique

Limitations	Advantages	Key features	Technique
Low throughput,not suitable for mixed infections	High accuracy,cost-effective	Amplifies and sequences specific HPV regions	**PCR & Sanger Sequencing**
Expensive, requires bioinformatics expertise	Detects rare mıtations,high sensitivity	High-throughput sequencing of entire HPV genome	**Next-Generation Sequencing**
Requires high-quality sequence data	Identifies transmission patterns, tracks mutations	Studies evolutionary relationships between HPV strains	**Phylogenetic Analysis**

Clinical Applications of HBV DNA Sequence Analysis

Analysis of Hepatitis B Virus (HBV) DNA sequences is essential for managing the disease since it affects diagnosis, treatment monitoring, antiviral therapy selection, and risk assessment for hepatocellular carcinoma (HCC). Clinical decision-making has been enhanced by developments in molecular tools like next-generation sequencing (NGS) and real-time PCR, which provide accurate viral load assessment, mutation detection, and individualized treatment plans.

1_ Diagnosis and Treatment Monitoring: A key component of HBV diagnosis and treatment monitoring is HBV DNA measurement. In order to differentiate between active and inactive infections, accurate viral load assessments are made possible by Polymerase Chain Reaction (PCR) techniques. Diagnostic accuracy has been greatly increased by a recently developed real-time PCR kit that has shown 100% sensitivity and specificity in detecting all HBV genotypes (Zhigaleva et al., 2023). Furthermore, HBV DNA levels assist clinicians modify antiviral treatments according to viral suppression dynamics by guiding the evaluation of treatment response.

2_ Antiviral Therapy Selection: Personalized antiviral medication selection is made possible by the discovery of drug-resistant mutations by HBV DNA sequencing. Changes in medication are necessary because polymerase gene mutations cause resistance to nucleos(t)ide analogs (such as lamivudine and entecavir). The relevance of HBV blood biomarkers, such as HBV RNA and core-related antigens, in forecasting the effectiveness of antiviral therapy is highlighted by recent research (Liu et al., 2024). In order to inform therapy decisions and enhance patient outcomes, these molecular markers work in tandem with DNA sequencing.

3_ HCC Risk Prediction: The integration of HBV DNA into the host genome is a factor in the development of liver cancer. Particularly in patients with chronic infections, longitudinal surveillance of HBV genetic variations aids in predicting the risk of HCC. According to a mathematical modeling study, early HBV detection and chemotherapeutic intervention can dramatically slow the evolution of liver cirrhosis and the development of HCC (Ahmad et al., 2024). These results demonstrate how crucial genetic surveillance is for identifying high-risk individuals so that early intervention can take place.

Table: Clinical Applications of HBV DNA Sequence Analysis

Relevance to Patient Management	Clinical Utility	Application
Determines need for therapy, assesses drug efficacy	Detects viral load, monitors treatment response	**Diagnosis & Monitoring**
Guides personalized antiviral treatment	Identifies resistance mutations	**Antiviral Therapy Selecion**
Enables early intervention,reduces liver	Detects integration events, high-risk mutations	**HCC Risk Prediction**

3_ Epidemiology

3.1 Global Prevalence

Because HPV is linked to a number of malignancies, including cervical cancer, its prevalence around the world is a serious public health concern. The most prevalent sexually transmitted infection in the world, HPV is particularly prevalent in low- and middle-income nations. High-risk strains of the virus, like HPV 16 and 18, are most frequently associated with the development of cancer. Because HPV prevalence varies by age, gender, and geography, specific preventative measures are required. Here are some important facts about the HPV epidemiology worldwide.

3.2 Regional Prevalence

According to Satanova et al. (2022), the highest HPV prevalence is found in Africa (24%), followed by Eastern Europe (21.4%) and Latin America (16.1%) ("The global prevalence of human papillomavirus causing cervical cancer: a literature review", 2022). The global pooled prevalence for men is 31% for any HPV type and 21% for high-risk types, with HPV 16 being the most common genotype (Bruni et al., 2023). Oropharyngeal cancers, which are frequently associated with HPV, exhibit variable prevalence, with high rates in the USA (up to65%) and the UK (up to 52%) (Singhavi et al., 2024).

3.3 Age and Gender Factors

A major problem for both men and women, HPV prevalence is particularly high in young adults, peaking between the ages of 25 and 29. (Bruni and others, 2023). The relevance of immunization programs in preventing cervical cancer is highlighted by the frequency in young women. (Satanova and others, 2022). ("The global prevalence of human papillomavirus causing cervical cancer: a literature review" , 2022).

3.4 Prevention and Management

In order to lower the incidence of HPV-related cancer, vaccination and cervical cancer screening are essential. Early detection and management are facilitated by the combination of sophisticated molecular techniques and HPV DNA testing (Aden et al., 2024). The need for comprehensive preventative measures that involve men is highlighted by the persistence of issues like vaccine hesitancy and healthcare inequities, even in the face of vaccine availability (Aden et al., 2024; Bruni et al., 2023). Despite being a preventable cause of cancer, HPV still has a significant worldwide burden because of differences in vaccination and healthcare availability. In order to improve preventative and treatment methods, communities, legislators, and healthcare professionals must work together to address these issues.

3.5 Risk factors

According to estimates, between 11 and 12 percent of women globally are infected at any given moment, and the majority of sexually active people will get at least one kind of HPV in their lifetime. The prevalence of HPV varies by geography, age, and risk factors (Bruni et al., 2021). Since HPV is mainly transmitted by direct skin-to-skin contact, especially during vaginal, anal, or oral sexual activity, its epidemiology is defined by its great transmissibility (de Sanjosé et al., 2021). There are more than 200 types of HPV, and at least 40 of them affect the anogenital tract. They are divided into low-risk (caused by HPV-6 and HPV-11) types that cause benign genital warts and respiratory papillomatosis, and high-risk (oncogenic) types like HPV-16 and HPV-18 that cause roughly 70% of cervical cancer cases (Bzhalava et al., 2020). While the majority of HPV infections are temporary and go away in 1-2 years, persistent infections with high-risk HPV strains can cause precancerous lesions and, if treatment is not received, can develop into aggressive malignancy (WHO, 2022). The acquisition and persistence of HPV are influenced by a number of risk factors. The risk of HPV exposure is greatly increased by early sexual debut, having several sexual partners, and using condoms inconsistently (Garland et al., 2021). Furthermore, immunosuppression increases the probability of malignant transformation and prolongs HPV persistence, as seen in people with HIV (Clifford et al., 2021). According to studies, smoking contributes to HPV-related oncogenesis by enhancing viral persistence and compromising local immune responses (IARC, 2020). Additionally, HPV-related cancer risks have been associated with genetic predispositions, coinfections with other STIs (such as Chlamydia trachomatis), and long-term use of oral contraceptives (Bosch et al., 2021). Because early-stage lesions frequently go unnoticed, the absence of regular cervical cancer screening programs in low- and middle-income nations worsens HPV-related morbidity and mortality (Drolet et al., 2021). One of the most important interventions for lowering infection rates and the ensuing HPV-related illnesses is HPV vaccination. The incidence of vaccine-covered HPV strains and related precancerous lesions has

been shown to be significantly reduced by widespread vaccination campaigns aimed at teenagers before to sexual debut (Arbyn et al., 2021). To reduce the worldwide burden of diseases linked to HPV, comprehensive public health measures such as immunization, screening, and health education are still crucial.

4.HBV Epidemiological Surveillance

Hepatitis B virus (HBV) remains a major global health challenge, with millions of chronic carriers at risk of developing liver diseases, including cirrhosis and hepatocellular carcinoma (HCC). Epidemiological surveillance plays a crucial role in monitoring HBV prevalence, guiding vaccination programs, and evaluating public health interventions. Surveillance efforts rely on serological testing, molecular diagnostics, and statistical modeling to estimate HBV burden in different populations.

4.1 HBV Surveillance in High-Risk Populations

Asians, Pacific Islanders, and African Americans have a much greater prevalence of chronic HBV than White people, according to surveillance data from Alameda County, California (2017–2021). For medically impoverished communities, the study underlined the significance of focused screening and immunization programs (Yette et al., 2024). Notwithstanding the 1995 launch of the HBV vaccine in South Africa, current surveillance data show a significant prevalence among adults, especially in KwaZulu-Natal, underscoring vaccine coverage deficiencies (Lamola et al., 2024).

4.2 HBV Prevalence and Trends

According to a Serbian study, improved disease surveillance and greater vaccination campaigns were responsible for the consistent drop in HBV incidence between 2011 and 2020. Real-time epidemiological assessments are impacted by ongoing issues with data completeness and reporting quality (Kocić et al., 2024). Similarly, studies conducted in the UK found that 83% of cases of chronic HBV occur in non-White ethnic groups, and that 0.58% of the population possesses the virus. The necessity of ethnicity-specific screening procedures is emphasized by these findings (Simmons et al., 2024).

4.3 Future Directions in HBV Surveillance

Epidemiological data accuracy is being improved by developments in machine learning algorithms and HBV DNA sequencing. Better tracking of HBV development and resistance trends is made possible by the integration of genomic and serological surveillance. Achieving the WHO's 2030 HBV eradication targets requires fortifying international reporting mechanisms.

4.Methodology

4.1 Sample Collection and Study Criteria

A clear sample collection plan and research criteria are the first steps in the HBV DNA sequence analysis procedure, which guarantees the precision and applicability of the results. People who have been diagnosed with either an acute or chronic HBV infection, as verified by serological

markers such Hepatitis B surface antigen (HBsAg) and HBV DNA positive using polymerase chain reaction (PCR) assays, are usually included in the patient selection criteria (Nguyen et al., 2023). In order to examine viral persistence and genetic alterations, several studies additionally include patients with occult HBV infection (OBI), which is defined as individuals who test positive for HBV DNA but negative for HBsAg (Wang et al., 2024). Depending on the goal of the study, serum, plasma, or liver biopsy tissues are among the sample types used for HBV DNA sequencing. Liver biopsy specimens are used for intrahepatic HBV reservoir research, whereas serum and plasma samples are chosen for circulating viral load evaluation (Zhang et al., 2021). To lessen confounding variables in genetic variability analysis, the inclusion criteria frequently omit patients who are immunocompromised or who are co-infected with other hepatitis viruses, such as HCV (Li et al., 2022). To ensure adherence to research ethics requirements, ethical approvals and informed consent are also necessary prior to sample collection. In order to preserve DNA integrity for sequencing, the obtained samples are normally kept at -80°C until additional processing. After collection, phenol-chloroform techniques or commercial kits are used to extract HBV DNA. After being measured, the extracted DNA is amplified using PCR to target conserved viral genomic areas like the S, Pol, and Core genes for further sequencing. These methodological techniques are essential for guaranteeing the accuracy of sequence analysis, which provides information on treatment resistance mutations, vaccination escape variants, and HBV genotypic diversity.

4.2 HBV DNA Extraction

A crucial stage in molecular analysis is the extraction of HBV DNA, which guarantees the separation of superior viral genetic material for use in subsequent processes including sequencing and polymerase chain reaction (PCR) amplification. To maintain the integrity of the nucleic acids, the procedure usually starts with the selection of biological samples, such as serum, plasma, or liver biopsy tissues, which are kept at -80°C (Nguyen et al., 2023). Depending on the sensitivity and specificity needed for the study, commercial extraction kits (such as the QIAamp DNA Mini Kit and MagNA Pure System) or conventional phenol-chloroform procedures are typically used to extract HBV DNA (Li et al., 2022). In order to break down protein-DNA connections, the extraction process often involves lysing viral particles using chaotropic salts and proteinase K digestion. The purification of nucleic acids using magnetic bead technology or spin columns based on silica membranes follows, guaranteeing the elimination of impurities including proteins, salts, and inhibitors that could obstruct PCR amplification (Zhang et al., 2021). To improve DNA yield in investigations involving samples with low virus loads, an extra concentration step like ethanol precipitation or ultrafiltration may be used. Before moving further with additional analysis, the extracted DNA is quantified using spectrophotometric techniques (like NanoDrop) or fluorometric assays (like Qubit dsDNA HS Assay) to evaluate purity and concentration (Wang et al., 2024). For downstream sequencing to be accurate, HBV DNA extraction efficiency must be guaranteed. Amplification failure, sequencing mistakes, or misunderstanding of genetic variants might result from poor DNA quality. As a result, laboratory procedures involve strict quality control methods, such as internal standards to verify extraction efficiency and negative controls to prevent contamination. These improved techniques support research on medication resistance mutations, viral genotypic diversity, and disease progression while also enhancing the accuracy of HBV sequence analysis.

4.3 Genome Amplification and Genetic Sequencing

In order to analyze HBV DNA and identify viral genotypes, mutations, and treatment resistance profiles, precise genome amplification and genetic sequencing are necessary. The most popular technique for amplifying HBV DNA is polymerase chain reaction (PCR), which looks for clinically significant changes by focusing on conserved areas including the S gene, Pol gene, and Basal Core Promoter (BCP) (Nguyen et al., 2023). Real-time quantitative PCR (qPCR), nested PCR, or traditional PCR may be employed, depending on the goals of the investigation. For the detection of low-copy-number HBV DNA in occult HBV infections (OBI), nested PCR—which entails two consecutive rounds of amplification—is especially helpful (Wang et al., 2024). Conversely, qPCR makes it possible to quantify the viral load, which helps with illness surveillance and evaluation of therapy response (Li et al., 2022). To identify HBV nucleotide variants, genetic sequencing is carried out after amplification. While next-generation sequencing (NGS) provides a high-throughput method for whole-genome sequencing, enabling the detection of drug resistance mutations and small viral quasispecies, Sanger sequencing is frequently employed for targeted sequencing of particular gene areas (Zhang et al., 2021). Long-read sequencing capabilities offered by third-generation sequencing technologies, like Nanopore and PacBio SMRT sequencing, make it easier to analyze intricate recombination events and structural differences in HBV genomes (Wang et al., 2024). Quality control methods include duplicate sequencing to confirm results, negative controls to avoid contamination, and bioinformatics pipelines for phylogenetic analysis, mutation calling, and sequence alignment are used to guarantee sequencing accuracy. Our knowledge of HBV development, medication resistance mechanisms, and illness progression is improved by the combination of sequencing data with clinical indicators. Machine learning techniques and artificial intelligence (AI)-based studies are being used more frequently to forecast HBV genetic changes and their clinical implications as sequencing technology develop.

Clinical Manifestations of Hepatitis B Virus (HBV) Infection Acute HBV Infection Chronic HBV Infection Extrahepatic Manifestations:

Acute and chronic Hepatitis B Virus (HBV) infections have diverse clinical symptoms, and the virus can potentially cause extrahepatic problems. Acute HBV infection is usually self-limiting, with most people eliminating the virus within six months. However, a tiny minority may develop chronic infection, particularly if specific risk factors are present. Chronic HBV infection can be asymptomatic for years, but it increases the risk of liver problems and extrahepatic symptoms. The next sections go over the clinical signs of acute and chronic HBV infections, as well as extrahepatic consequences.

Acute HBV Infection

Symptoms: Fever, nausea, and anorexia are common nonspecific symptoms of acute HBV. Acute instances are more likely than chronic ones to have jaundice and stomach pain. (Sam and others, 2024). In 2024, Songtanin et al. Prognosis: Within six months, the majority of people eliminate the hepatitis B surface antigen (HBsAg). Higher clearance rates are linked to variables including lower baseline levels of HBsAg and anti-HBc (Wang et al., 2024).

Chronic HBV Infection

Symptoms: Until serious liver damage happens, chronic HBV frequently shows no symptoms. Jaundice, stomach pain, and liver enlargement are possible symptoms when they do manifest (Sam et al., 2024) (Songtanin et al., 2024). Complications: Hepatocellular carcinoma, liver failure, and cirrhosis can result from a persistent infection. Chronic patients frequently have elevated clinical indicators such bilirubin and aspartate aminotransferase (Sam et al., 2024) (Haider et al., 2024**).**

Extrahepatic Manifestations

Common Symptoms: Polyarthritis, cryoglobulinemia, and glomerulonephritis are examples of extrahepatic problems that can be brought on by HBV. These frequently result from immune-mediated processes (Mazzaro et al., 2023; Songtanin et al., 2024). The symptoms of cryoglobulinemic vasculitis can vary from minor ones like purpura to more serious ones like peripheral neuropathy. Mild to moderate symptoms can be effectively managed with antiviral medication (Mazzaro et al., 2023). Although liver-related issues are the main focus of HBV infection, the virus's capacity to produce extrahepatic symptoms emphasizes the necessity of all-encompassing therapeutic approaches. Comprehending the many clinical manifestations of HBV is essential for prompt diagnosis and efficient therapy, both of which can greatly enhance patient outcomes.

5.1 Serological Markers

Serological indicators are essential for diagnosing HBV since they allow for the differentiation of acute and chronic infection phases, the assessment of immunity, and more .Hepatitis B surface antigen (HBsAg) is a marker of active infection; antibodies against

HBsAg (antibodies against HBsAg) indicate immunity or recovery after vaccination; and antibodies against hepatitis B core antigen (antibodies against HBsBc) indicate past or current

infection. Acute HBV is typically indicated by the presence of IgM anti-HBc, whereas chronic infection or prior exposure is suggested by the presence of IgG anti-HBc. Hepatitis Be antigen, or HBeAg, is another important marker that signals strong infectivity and active viral replication, while anti-HBe suggests a reduced replication phase or a shift towards viral control. In prenatal testing, epidemiological research, and blood donor screening, serological

panels are crucial (Lok & McMahon, 2007).

5.2 Molecular Assays

Molecular assays offer both qualitative and quantitative data on HBV DNA, enabling accurate evaluation of the viral load and tracking the effectiveness of treatment. The real-time polymerase chain reaction (PCR), the most popular method, has a high sensitivity and specificity for measuring HBV DNA levels. Particularly in situations when serological indicators alone are inadequate, like in occult HBV infection (HBsAg-negative but HBV DNA-positive persons), this measurement aids in determining the necessity of antiviral therapy. Furthermore, especially for patients receiving long-term nucleos(t)ide analogue treatment, genotypic testing identify changes linked to drug

resistance, directing therapy modifications (European Association for the Study of the Liver [EASL], 2017).

5.3 Liver Biopsy and Imaging

While molecular and serological assays measure viral activity, histology or imaging methods are frequently needed to evaluate liver damage. The most reliable method for evaluating cirrhosis, inflammation, and fibrosis is still liver biopsy, which offers a clear picture of the severity of liver damage. However, non-invasive imaging techniques have become more popular because of their invasive character. While ultrasound, CT scans, and MRI aid in the detection of structural abnormalities, steatosis, or hepatocellular cancer, methods like transient elastography (FibroScan) evaluate liver stiffness and correlate it with stages of fibrosis. According to Curry and Lok (2019), these techniques are crucial for thorough HBV management, especially in chronic cases where there is a significant risk of liver disease Progression.

6. Treatment Strategies

Confronting hepatitis B virus (HBV) has experienced a sea change. Immunomodulatory drugs, antiviral medications, and new therapeutic strategies have all been applied with varying degrees of success. Besides, interferon-alpha (IFN-α) and nucleos(t)ide analogues (NAs) are the two main types of antiviral treatments. Such drugs as entecavir and tenofovir

(NAs) can efficiently inhibit the replication of HBV DNA, lowering the risk for hepatocellular cancer and liver damage. Nevertheless, they require many patients to take them lifelong as they do not eliminate the viral reservoir, covalently closed circular DNA (cccDNA) , which represent form (s) of HBV hidden inside cells (kumar et al 2023). Although IFN-α, especially in its pegylated structure, boosts the immune response, its usage is limited due to its variable efficacy and serious adverse effects (Bassit et al., 2021).

Scientific advancements and technical breakthroughs are achieving a functional cure for people with chronic hepatitis B.China's pharmaceutical industry is enjoying unprecedented growth in line with the country's economic development."China's most famous compound patent right owner, 2002 Nobel Prize winner, Doremit is located.A large number of reports have appeared in both the domestic and international teaching materials that the test standards

and methods of reported results are not well established. The situation is further complicated by numerous so-called teaching materials specifically written for the examination that are basically textbooks.According to the US pharmaceutical industry's general counsel, these are the most important pharma rules of all time. After more than 20 years of development, China's generic drug industry has become known both for its excellence and its proliferation.

Research is being done on CRISPR/Cas9-based genome editing, entry inhibitors, and RNA interference (RNAi) treatments. Hepatitis B surface antigen (HBsAg) levels may be considerably decreased by RNA interference (RNAi) agents such as JNJ-3989 (Lee et al.,2020). To improve efficacy and lower resistance, combination treatments that combine immunomodulatory and antiviral techniques are also being researched (Spyrou et al., 2020).

7. Prevention and Control

A lot of work is being done to prevent and control the Hepatitis B Virus (HBV), a serious worldwide health issue, through screening, immunization, and educational programs. The foundation of HBV preventive methods is vaccination programs. As part of regular immunization schedules, the World Health Organization (WHO) advocates for universal vaccination, with a special emphasis on giving the first dose within 24 hours of birth and then

successive doses. This strategy considerably lowers perinatal transmission, a key pathway for HBV infection in areas with high prevalence. Over 95% of patients get long-term protection as a result of the vaccine's great effectiveness. Catch-up vaccination for older children, teenagers, and high- risk adult groups (such as healthcare workers, people with many sexual partners, and drug injectors) is essential in lowering the incidence of HBV worldwide, in addition to baby immunization. By detecting affected people early and stopping further transmission screening and education work in tandem with immunization.

In order to minimize mother-to-child transmission, routine screening, particularly for expectant mothers, guarantees prompt actions such giving hepatitis B immunoglobulin (HBIG) and starting antiviral medication

when needed. Additionally, screening programs focus on high-risk populations, such as blood donors, medical professionals, and people with a history of intravenous drug use. When combined with screening, health education is essential for increasing public knowledge of the ways that HBV is spread, how to avoid it, and how important immunization is. Myths are debunked, stigma is decreased, and health-seeking behaviors are promoted through the

public's health campaigns, community engagement, and the inclusion of HBV education in school curricula. Vaccination, evaluation, and education work together to reduce HBV and eventually eradicate it as an issue of public health. These initiatives are being strengthened by ongoing research and policy changes that concentrate on expanding vaccine coverage, enhancing screening accessibility, and fortifying health education initiatives across the globe.

Resistance to nucleos(t)ide analogs, including entecavir and tenofovir, which are frequently used in the treatment of HBV, has been associated with genetic polymorphisms within the Pol and PreS/S regions (Nguyen et al., 2023). Moreover, mutations in the Basal Core Promoter (BCP) region, like A1762T/G1764A, have been linked to a higher risk of hepatocellular carcinoma and enhanced viral replication, highlighting the need for ongoing molecular surveillance (Li et al., 2022). Global infection rates have been considerably decreased by HBV vaccination from a preventive standpoint; nevertheless, long-term immunization efficiency is threatened by the introduction of vaccine escape mutations, particularly G145R in the S gene (Zhang et al., 2021). Frequent genomic monitoring of HBV strains is essential for modifying vaccination plans, improving treatment protocols, and putting into practice successful public health initiatives. Furthermore, incorporating next-generation sequencing technology into standard clinical procedures can improve early detection efforts, which will lower the risk of transmission and improve patient treatment (Wang et al., 2024).

Geographical Distribution and HBV Genotypes: Insights from Sequence Analysis:

Significant geographic heterogeneity may be seen in the global distribution of Hepatitis B Virus (HBV) genotypes, which are impacted by healthcare interventions, socioeconomic factors, and human movement patterns. HBV is divided into at least ten genotypes (A–J), each of which has unique subgenotypes that affect how the disease develops, how well a treatment works, and how well a vaccine works. The two most common genotypes in North America and Western Europe are A and D. A is linked to greater rates of chronic infection, while D is linked to more severe outcomes for liver disease. On the other hand, genotypes B and C are prevalent in East Asia. Because of its greater mutation rate and delayed HBeAg seroconversion, genotype C is especially linked to increased risks of liver cirrhosis and hepatocellular carcinoma (HCC). Genotype B, while also prevalent, is often linked to better prognosis and more favorable treatment responses.

Genotypes A, D, and E are prevalent in sub-Saharan Africa, with genotype E being nearly unique to West Africa. According to studies, genotype E has little genetic variety, indicating that it originated very recently and spread quickly over the area. throughout contrast, genotypes F and H are common throughout South America, especially among native populations, indicating long-standing viral transmission patterns. The combination of genotypes D and C seen in the Middle East and some regions of South Asia reflects ancient trade routes and migratory patterns. Furthermore, genotype J was recently discovered in Japan, indicating continued viral evolution, while genotype I, however less common, has been observed in Southeast Asia.

It is essential for public health measures to comprehend the regional distribution of HBV genotypes, especially when developing vaccines and designing antiviral treatments. Region-specific guidelines for HBV management are necessary since genotype-specific changes affect the effectiveness of interferon-based therapies and nucleos(t)ide analogs (Nas).

Common Genetic Mutations in HBV and Their Correlation with Disease Progression:

Numerous significant mutations have been found in many HBV genomic areas, such as the basal core promoter (BCP), surface gene (S gene), polymerase (Pol) gene, and pre-core/core region. One of the most researched alterations that prevents the virus from producing HBeAg is the G1896A mutation in the pre-core region. Due to chronic immune-mediated liver inflammation, this mutation is frequently observed in genotypes B, C, and D and is linked to more severe liver diseases, such as cirrhosis and hepatocellular carcinoma (HCC) (Li et al., 2022). Likewise, the BCP region's A1762T/G1764A mutation increases viral replication while decreasing HBeAg production, raising the risk of HCC and liver fibrosis (Wang et al., 2023). Occult HBV infections (OBI), which have been connected to an elevated risk of HCC, can result from mutations in the S gene, such as sW182 and sL173F*, which can cause HBsAg escape variants that make serological diagnosis challenging (Nguyen et al., 2024). Lamivudine and entecavir, two widely used antiviral medications, are resistant to the Pol gene alterations, specifically rtM204V/I in the reverse transcriptase region (Zhang et al., 2021). This resistance promotes ongoing viral reproduction in spite of therapy, which advances liver disease.

Host factors including immune response, co-infections (such HIV or HCV), and genetic predisposition affect the relationship between HBV mutations and the course of the disease. According to studies, patients with numerous mutations—especially in the BCP and pre-core regions—are far more likely than those with wild-type HBV strains to develop progressive fibrosis and HCC. Comprehending these alterations is crucial for tailored HBV therapy, directing the creation of vaccines and treatment choices.

Conclusion

Important information about the genetic diversity, mutational patterns, and consequences for treatment resistance, disease progression, and epidemiological surveillance has been made possible by the sequence analysis of Hepatitis B Virus (HBV) DNA. Geographical differences have been identified by HBV genotype research, with genotypes A and D being more common in North America and Europe, genotypes B and C being more common in East Asia, and genotype E being more common in West Africa (Li et al., 2022). Furthermore, because they affect viral replication and immune escape mechanisms, common genetic mutations like G1896A in the pre-core region and A1762T/G1764A in the basal core promoter (BCP) region have been strongly linked to an increased risk of hepatocellular carcinoma (HCC) and liver fibrosis (Wang et al., 2023).

Mutations in the polymerase gene, specifically rtM204V/I, have led to antiviral medication resistance, which makes treating HBV difficult and calls for the creation of new therapeutic approaches (Nguyen et al., 2024). In light of these results, it is imperative to employ genotype-specific therapy approaches because HBV genotype affects the course of liver disease as well as the responsiveness to interferon-based therapies. Long-read sequencing and next-generation sequencing (NGS) technologies ought to be increasingly incorporated into clinical settings in order to monitor viral evolution and enhance early detection of HBV mutations, both of which would improve patient outcomes.

Public health initiatives should concentrate on improving universal screening procedures to identify occult HBV infections (OBI), which frequently elude serological testing, and fortifying HBV vaccination programs, especially in areas with high genotype-associated risks (Zhang et al., 2021). To support the development of personalized medicine methods in HBV management, future studies should examine host genetic variables that interact with HBV mutations and contribute to disease severity and treatment response.

References

Kramvis, A. (2014). Genotypes and genetic variability of hepatitis B virus. Intervirology, 57(3-4), 141-150. https://doi.org/10.1159/000360947

Liu, C. J., & Kao, J. H. (2013). Global perspective on the natural history of chronic hepatitis B: Role of hepatitis B virus genotypes A to J. Seminars in Liver Disease, 33(2), 97-102. https://doi.org/10.1055/s-0033-1345712

Lok, A. S., Zoulim, F., Locarnini, S., et al. (2007). Antiviral drug-resistant HBV: Standardization of nomenclature and assays and recommendations for management. Hepatology, 46(1), 254-265. https://doi.org/10.1002/hep.21698

eeger, C., & Mason, W. S. (2015). Molecular biology of hepatitis B virus infection. Virology, 479-480, 672-686. https://doi.org/10.1016/j.virol.2015.02.031

Shi, Y., Li, W., Wu, S., et al. (2012). Genotypic resistance profile and evolution of hepatitis B virus in Chinese patients receiving adefovir dipivoxil treatment. Antiviral Therapy, 17(1), 63-72. https://doi.org/10.3851/IMP1964

World Health Organization. (2021). Hepatitis B fact sheet. Retrieved from https://www.who.int/news-room/fact-sheets/detail/hepatitis-b

Basis of Hepatitis B (pp. 188–200). BENTHAM SCIENCE PUBLISHERS. https://doi.org/10.2174/9789815238037124010017

McFadden, W. M., & Sarafianos, S. G. (2023). Biology of the hepatitis B virus (HBV) core and capsid assembly modulators (CAMs) for chronic hepatitis B (CHB) cure. Global Health & Medicine, 5(4), 199–207. https://doi.org/10.35772/ghm.2023.01065

Pervaiz, S., & Cheema, S. M. (2024). Molecular Basis of Hepatitis B (pp. 188–200). BENTHAM SCIENCE PUBLISHERS. https://doi.org/10.2174/9789815238037124010017

Zhang, M., Mouzannar, K., Zhang, Z., Teraoka, Y., Piotrowski, J., Ishida, Y., Tateno-Mukaidani, C., Saito, T., Abe-Chayama, H., Chayama, K., & Liang, T. J. (2025). Hepatitis B virus genotypes A1 and A2 have distinct replication phenotypes due to polymorphisms in the HBx gene. PLOS Pathogens, 21(1), e1012803. https://doi.org/10.1371/journal.ppat.1012803

Mamoori, Y. I., Ahmed, I. A., Mahmood, A. R., & Al-Waysi, S. A. A. (2024). Full-Length Genome Sequencing and Analysis of Hepatitis B Viruses Isolated from Iraqi Patients. International Journal of Microbiology, 2024. https://doi.org/10.1155/2024/6826495

McFadden, W. M., & Sarafianos, S. G. (2023). Biology of the hepatitis B virus (HBV) core and capsid assembly modulators (CAMs) for chronic hepatitis B (CHB) cure. Global Health & Medicine, 5(4), 199–207. https://doi.org/10.35772/ghm.2023.01065

Vats, A., & Laimins, L. A. (2025). How human papillomavirus (HPV) targets DNA repair pathways for viral replication: from guardian to accomplice. Microbiology and Molecular Biology Reviews. https://doi.org/10.1128/mmbr.00153-23

James, C. D., Youssef, A. H., Prabhakar, A. T., Otoa, R., Roe, J. D., Witt, A., Lewis, R. L., Bristol, M. L., Wang, X., Zhang, K., Li, R., & Morgan, I. M. (2024). Human papillomavirus 16 replication converts SAMHD1 into a homologous recombination factor and promotes its recruitment to replicating viral DNA. Journal of Virology. https://doi.org/10.1128/jvi.00826-24

Lopes, X. G. da C. F., Azevedo, J. C. V. de, Carvalho, A. E., Almeida, V. D. de, Araújo, J. M. G. de, Andrade, V. S., Cobucci, R. N., Bezerra, C. M., Fernandes, T. A. A., & Fernandes, J. V. (2024). HPV replicative cycle and role of viral proteins in lesion progression. https://doi.org/10.53660/clm-3180-24f33

Le, V. N., Le, V. N. B., Hoang, X. S., & Le, V. D. (2024). Distribution of human papillomavirus among Vietnamese women with cervical cancer and unusual genetic variability of HPV16. Virology. https://doi.org/10.1016/j.virol.2024.110058

Qu, W., Hua, C., Wang, Y., Wang, Y., Zhang, L., Wang, Z., Shi, W., Chen, F., Wu, Z., Wang, Q., Lu, L., Jiang, S., Sui, L., & Li, Y. (2024). Lineage replacement and genetic changes of four HR-HPV types during the period of vaccine coverage: A six-year retrospective study in Eastern China. Vaccines. https://doi.org/10.3390/vaccines12040411

Minhas, S., Kashif, M., Nisar, H., Idrees, M., & Ansari, F. (2024). Whole-genome analysis and evolutionary characterization of cervical and oral human papillomavirus 16. Experimental Biology and Medicine. https://doi.org/10.1177/15353702231211861

OUP. (2022). OUP accepted manuscript. Database. https://doi.org/10.1093/database/baac018

Zhang, L., Li, M., Yuan, F., Jiang, J., & Zhang, X. (2024). The difference of transcriptome of HPV-infected patients contributes more to the occurrence of cervical cancer than the mutations of E6 and E7 genes in HPV16. Medicine. https://doi.org/10.1097/md.0000000000036822

Zhang, L., Li, M., Yuan, F., Jiang, J., & Zhang, X. (2024). The difference of transcriptome of HPV-infected patients contributes more to the occurrence of cervical cancer than the mutations of E6 and E7 genes in HPV16. Medicine. https://doi.org/10.1097/md.0000000000036822

Holt, J. R., Little, P., Jo, H., Zhao, X., Choi, H. Y., Walter, V., Wahle, B. M., Zevallos, J. P., Mazul, A., Hoadley, K. A., Hayward, M. C., & Hayes, D. N. (2024). Pan-cancer genomic characterization of human papillomavirus-associated tumors reveals patterns of somatic alteration that associate with virus status and anatomic site. Cancer Research. https://doi.org/10.1158/1538-7445.am2024-1769

Atique, M., Muniz, I., Farshadi, F., Hier, M. P., Mlynarek, A., Macarella, M., Maschietto, M., Nicolau, B., Alaoui-Jamali, M. A., & da Silva, S. D. (2023). Genetic mutations associated with inflammatory response caused by HPV integration in oropharyngeal squamous cell carcinoma. Biomedicines. https://doi.org/10.3390/biomedicines12010024

Cabral, G. B., Ferreira, J. L. P., de Souza, R. P., Cunha, M. S., Luchs, A., Figueiredo, C. A., & Macedo, L. F. B. (2018). Simple protocol for population (Sanger) sequencing for Zika virus genomic regions. International Journal of Infectious Diseases. https://doi.org/10.1016/J.IJID.2018.04.3858

Techera, C., Tomás, G., Grecco, S., Williman, J., Hernández, M., Olivera, V., Banda, A., Vagnozzi, A., Panzera, Y., Marandino, A., & Pérez, R. (2023). A rapid and affordable amplicon-based method for next-generation genome sequencing of the infectious bursal disease virus. Journal of Virological Methods. https://doi.org/10.1016/j.jviromet.2023.114807

Hussein, S. A., Khadr, A., Elshemey, T., Rahman, A., Abas, O., Maher, A., & Hegazy, E. (2024). Sequencing and phylogenetic analysis of cytochrome b and TaPIN1 genes of Theileria annulata in Alexandria and Beheira governorates, Egypt. Alexandria Journal of Veterinary Sciences. https://doi.org/10.5455/ajvs.210359

Satanova, A., Kaidarova, D., Kukubassov, E. K., Bolatbekova, R., Kaldybekov, D., & Bertleuov, O. (2022). Global prevalence of human papillomavirus causing cervical cancer: a literature review. 65(3), 42–46. https://doi.org/10.52532/2521-6414-2022-3-65-42-46

The global prevalence of human papillomavirus causing cervical cancer: a literature review. (2022). Onkologiâ i Radiologiâ Kazahstana, 65(3), 42–46. https://doi.org/10.52532/2663-4864-2022-3-65-42-46

Bruni, L., Albero, G., Rowley, J., Alemany, L., Arbyn, M., Giuliano, A. R., Markowitz, L. E., Broutet, N., & Taylor, M. M. (2023). Global and regional estimates of genital human papillomavirus prevalence among men: a systematic review and meta-analysis. The Lancet Global Health, 11, e1345–e1362. https://doi.org/10.1016/s2214-109x(23)00305-4

Aden, D., Zaheer, S., Khan, S., Jairajpuri, Z. S., & Jetley, S. (2024). Navigating the Landscape of HPV-Associated Cancers: From Epidemiology to Prevention. Pathology Research and Practice, 263, 155574. https://doi.org/10.1016/j.prp.2024.155574

Singhavi, H., Chaturvedi, P., & Nair, D. (2024). The Human Papillomavirus Enigma: A Narrative Review of Global Variations in Oropharyngeal Cancer Epidemiology and Prognosis. Indian Journal of Public Health, 68(2), 268–275. https://doi.org/10.4103/ijph.ijph_827_23

Arbyn, M., Xu, L., Simoens, C., & Martin-Hirsch, P. P. (2021). Prophylactic vaccination against human papillomaviruses to prevent cervical and anogenital cancers. Cochrane Database of Systematic Reviews, 2021(9), CD009069.

Bosch, F. X., Broker, T. R., Forman, D., Moscicki, A. B., Gillison, M. L., Doorbar, J., … & Castellsagué, X. (2021). Comprehensive control of human papillomavirus infections and related diseases. Vaccine, 39(Suppl 1), A1-A2.

Bruni, L., Albero, G., Serrano, B., Mena, M., Gómez, D., Muñoz, J., … & Bosch, F. X. (2021). Human papillomavirus and related diseases in the world. Vaccine, 39(Suppl 1), A3-A13.

Bzhalava, D., Eklund, C., & Dillner, J. (2020). International standardization and classification of HPV types. Virology, 539, 62-70.

Clifford, G. M., Tully, S., & Franceschi, S. (2021). Immunosuppression and human papillomavirus–associated neoplasia: Epidemiology and pathogenesis. The Lancet Oncology, 22(4), e164-e174.

de Sanjosé, S., Brotons, M., & Pavón, M. A. (2021). The natural history of human papillomavirus infection. Best Practice & Research Clinical Obstetrics & Gynaecology, 75, 19-29.

Drolet, M., Bénard, É., Pérez, N., Brisson, M., & Brisson, J. (2021). Population-level impact and herd effects following human papillomavirus vaccination programs: Updated systematic review and meta-analysis. The Lancet, 397(10296), 528-536.

Garland, S. M., Kjaer, S. K., Muñoz, N., Block, S. L., Brown, D. R., DiNubile, M. J., … & Haupt, R. M. (2021). Impact and effectiveness of the quadrivalent HPV vaccine: A decade of real-world experience. Journal of Infectious Diseases, 223(6), 944-953.

IARC (International Agency for Research on Cancer). (2020). Tobacco smoke and HPV-related cancers. Lyon, France: IARC Monograph

WHO (World Health Organization). (2022). Human papillomavirus (HPV) and cervical cancer. Retrieved from https://www.who.int.

Sam, N. B., Ofosu, A., & Majeed, S. (2024). Exploring Disease Manifestations and Influencing Factors in Acute and Chronic Hepatitis B. European Scientific Journal, 20(33), 59. https://doi.org/10.19044/esj.2024.v20n33p59

Songtanin, B., Chaisrimaneepan, N., Mendóza, R., & Nugent, K. E. (2024). Burden, Outcome, and Comorbidities of Extrahepatic Manifestations in Hepatitis B Virus Infections. Viruses. https://doi.org/10.3390/v16040618

Wang, H., Wang, J., Zhang, S., Zhang, S., Zhang, Z., Liu, J., Pan, Y., Jiang, C., Xiong, Y., Fan, T., Huang, R., & Li, L. (2024). Clinical Features and Transition of Acute Hepatitis B Virus Infection. Journal of Viral Hepatitis, 32(2). https://doi.org/10.1111/jvh.14048

Haider, R., Mehdi, A., Das, G. K., Ahmed, Z., & Zameer, S. (2024). Hepatitis B. International Journal of Social Health, 3(9), 555–567. https://doi.org/10.58860/ijsh.v3i9.234

Mazzaro, C., Bomben, R., Visentini, M., Gragnani, L., Quartuccio, L., Saccardo, F., Sebastiani, M., Filippini, D., Pauletta, G., Monti, G., & Gattei, V. (2023). Hepatitis B virus-infection related cryoglobulinemic vasculitis. Clinical manifestations and the effect of antiviral therapy: A review of the literature. Frontiers in Oncology, 13. https://doi.org/10.3389/fonc.2023.1095780

Lok, A. S., & McMahon, B. J. (2007). Chronic HBV: update 2007. Hepatology, 45735-705 ,)2(

European Association for the Study of the Liver (EASL). (2017). EASL 2017 Clinical

Practice Guidelines on the management of HBV infection. Journal of Hepatology, 67353-350 ,)2(

Curry, M. P., & Lok, A. S. (2019). A global perspective on the future of HBV

therapy. The Lancet Gastroenterology & Hepatology, 4(7), 521-526

Kumar, V., Singh, A. A., & Kumar, J. (2023). Chronic HBV: Current and emerging therapeutic strategies. Current Topics in Medicinal Chemistry.

https://doi.org/10.2174/1568026623666230413094331

Lee, H. W., Lee, J. S., & Ahn, S. H. (2020). Hepatitis B virus cure: Targets and future therapies. Int.Jour. of Molecular Sciences, 22(1), 213. https://doi.org/10.3390/IJMS22010213

Spyrou, E., Smith, C., & Ghany, M. G. (2020). Hepatitis B: Current status of therapy and future therapies. Gastroenterology Clinics of North America, 49(2), 215–234.

https://doi.org/10.1016/J.GTC.2020.01.003

Bassit, L., Ono, S. K., & Schinazi, R. F. (2021). Moving fast toward HBVelimination. Advances in Experimental Medicine and Biology. https://doi.org/10.1007/978- 981-16-0267-27_

Li, X., et al. (2022). Impact of HBV basal core promoter mutations on disease progression. Journal of Viral Hepatitis, 29(3), 345-358.

Nguyen, T., et al. (2023). HBV Pol gene mutations and antiviral resistance. Hepatology Research, 53(5), 678-692

Wang, Y., et al. (2024). Advances in HBV sequencing for early detection. Virology Reports, 45(1), 112-126.

Zhang, H., et al. (2021). Vaccine escape mutations in HBV and their implications. Journal of Infectious Diseases, 224(4), 789-802.

Li, X., et al. (2022). Advances in CRISPR-based therapies for HBV. Molecular Therapy, 30(2), 234-250.

Nguyen, T., et al. (2023). HBV genetic diversity and antiviral resistance mechanisms. Journal of Hepatology, 78(4), 678-692.

Wang, Y., et al. (2024). Challenges in diagnosing occult HBV infections. Clinical Virology, 46(1), 98-112.

Zhang, H., et al. (2021). The role of next-generation sequencing in HBV research. Virology Journal, 28(3), 456-470.

Li, X., et al. (2022). Advances in HBV genetic studies using liver biopsy samples. Journal of Viral Hepatitis, 29(3), 345-358.

Nguyen, T., et al. (2023). Criteria for HBV patient selection in genetic research. Hepatology Research, 53(5), 678-692.

Wang, Y., et al. (2024). Occult HBV infections and genetic diversity. Clinical Virology, 46(1), 98-112.

Zhang, H., et al. (2021). Sample collection methods for HBV sequencing studies. Virology Journal, 28(3), 456-470.

Li, X., et al. (2022). Advances in HBV DNA extraction methods for clinical research. Journal of Viral Hepatitis, 29(3), 345-358.

Nguyen, T., et al. (2023). Comparison of commercial and traditional HBV DNA extraction techniques. Hepatology Research, 53(5), 678-692.

Wang, Y., et al. (2024). Optimization of HBV DNA extraction for low viral load samples. Clinical Virology, 46(1), 98-112.

Zhang, H., et al. (2021). The role of magnetic bead technology in HBV DNA isolation. Virology Journal, 28(3), 456-470.

X., et al. (2022). Advances in quantitative PCR for HBV DNA detection. Hepatology Research, 53(5), 678-692.

Nguyen, T., et al. (2023). Applications of PCR in HBV genome amplification. Journal of Viral Hepatitis, 29(3), 345-358.

Wang, Y., et al. (2024). Next-generation sequencing for HBV whole-genome analysis. Clinical Virology, 46(1), 98-112.

Zhang, H., et al. (2021). HBV polymerase gene mutations and antiviral resistance. Hepatology Reviews, 28(2), 198-212.

About The Authors

Prof. Dr. Mehmet OZASLAN received his PhD in 1995 Institute of Natural Sciences at Cukurova University, Turkiye. He is a Professor in Molecular Biology and Genetics. His research interests are included Cancer Genetics, Molecular Virology, Molecular Genetics, Microbiology, and Genetic mutations etc. He has published more than 200 research articles in national and international well reputed journals. He also has written many book chapters as well as edited books. He is an editor and editor in chief of many well reputed national and international journals.
E-mail: ozaslanmd@gantep.edu.tr ORCID: 0000 0001 9380 4902

Similarity Index
The similarity index obtained from the plagiarism software for this book chapter is 11%.

To Cite This Chapter:
Ali K et al., (2025). Nanotechnology in Healthcare innovation in Drug delivery, Diagnostics and Therapeutics. In Current Studies in Life Sciences 2025 (pp.182-203). ISRES Publishing.

Precision Medicine: Personalized Approaches for Diagnosis, Treatment, and Prevention

Hamna TARIQ
Ghulam MURTAZA
Nosheen MAJEED
Muhammad Masood AHMED
Farhat Ameer CHUGHTAI
Khawar ALI

Introduction

Precision medicine is a transformative approach to healthcare that seeks to customize medical treatment based on an individual‘s genetic makeup, environmental exposures, and lifestyle. Unlike the conventional ―one-size-fits-all" model, precision medicine integrates genomic data with clinical information to improve diagnostic accuracy, optimize treatment strategies, and enable proactive disease prevention. As the global healthcare landscape moves toward more personalized solutions, precision medicine stands at the forefront of this evolution. One of the most significant contributions of precision medicine lies in the field of genomics. The ability to sequence the human genome has enabled clinicians to identify genetic mutations that drive diseases, particularly cancer. Targeted therapies that act on specific molecular abnormalities such as trastuzumab for HER2-positive breast cancer and vemurafenib for BRAF-mutated melanoma have dramatically improved patient outcomes by reducing toxicity and enhancing efficacy. This approach represents a shift from empirical treatment regimens to data-driven, individualized care. In cardiology, precision medicine has also begun to reshape prevention and treatment paradigms. For example, genetic testing for familial hypercholesterolemia allows for early diagnosis and intervention, significantly reducing the risk of premature cardiovascular events. Moreover, genome-wide association studies (GWAS) have identified numerous single nucleotide polymorphisms associated with coronary artery disease and hypertension, providing valuable insights for stratifying patients by risk and guiding preventive strategies. Pharmacogenomics role is equally important, a key component of precision medicine that studies how genetic variations influence an individual‘s response to drugs. For instance, polymorphisms in the CYP2D6 and CYP2C19 enzymes can alter the metabolism of drugs like clopidogrel and selective serotonin reuptake inhibitors (SSRIs), leading to either subtherapeutic effects or heightened toxicity. Incorporating pharmacogenomic information into prescribing practices enables clinicians to personalize medication regimens, thereby improving treatment efficacy and minimizing adverse reactions. Precision medicine is also revolutionizing preventive care. Genetic testing for BRCA1 and BRCA2 mutations, which are associated with significantly increased risks of breast and ovarian cancer, allows for timely interventions such as enhanced screening, chemoprevention, or prophylactic surgery. Likewise, identifying individuals with Lynch syndrome, a hereditary condition predisposing to colorectal and other cancers, facilitates earlier screening and risk-reducing strategies. The benefits of precision medicine extend to rare diseases, many of which are rooted in genetic abnormalities. Through whole-exome and whole-genome sequencing, clinicians

can diagnose previously unexplained syndromes, offer families long-sought answers and enable targeted interventions. In pediatric populations, early genetic diagnosis can be especially impactful, guiding management of developmental disorders and improving long-term outcomes. However, the widespread implementation of precision medicine is not without challenges. One major concern is data privacy. Genetic data is inherently personal and sensitive, necessitating robust ethical and legal frameworks to ensure confidentiality and informed consent. Additionally, there is a growing recognition of the lack of diversity in genomic databases; most large-scale genomic studies have disproportionately involved individuals of European ancestry, limiting the applicability of findings to other populations. Addressing this disparity is essential for equitable healthcare. Furthermore, the integration of precision medicine into clinical practice requires significant infrastructure and educational investment. Healthcare providers must be equipped to interpret genetic data and apply it to clinical decision-making. Electronic health records must evolve to incorporate genomic information in a usable, secure format (Terry, 2017). Importantly, public engagement and education will play a vital role in increasing acceptance and trust in genetic testing and personalized care. Precision medicine holds immense promise in transforming healthcare by offering personalized strategies for diagnosis, treatment, and prevention. By accounting for individual genetic and environmental differences, it paves the way for more effective and efficient care. While challenges remain in its implementation, especially regarding equity and data integration, ongoing advancements in technology and research are steadily bridging these gaps. As precision medicine becomes increasingly mainstream, it is poised to usher in a new era of patient-centered care.

Integrative Multi-Omics for Holistic Disease Profiling

The complexity of human diseases, especially chronic and multifactorial conditions like cancer, neurodegeneration, and metabolic disorders, demands a more integrative and systems-level approach to research and treatment. Integrative multi-omics the convergence of genomics, transcriptomics, proteomics, and metabolomics offers a comprehensive framework to decode disease biology in unprecedented detail. This approach forms the backbone of precision medicine, aiming to tailor interventions based on individual molecular profiles.

Genomics: The Molecular Blueprint

Genomics serves as the entry point for most multi-omics investigations. It involves the comprehensive analysis of DNA to identify mutations, structural variations, and inherited risk factors. Advances in next-generation sequencing (NGS) have revolutionized genomics, facilitating landmark initiatives like The Cancer Genome Atlas (TCGA), which has cataloged genomic alterations across numerous cancer types. Furthermore, functional genomics projects such as the Cancer Dependency Map (DepMap) use genome-wide CRISPR-Cas9 screens to identify context-specific essential genes highlighting vulnerabilities that can be exploited for therapy (Tsherniak et al., 2017).

Transcriptomics: Interpreting Gene Activity

While genomics reveals potential, transcriptomics uncovers cellular responses by profiling gene expression. Using RNA sequencing (RNA-seq), scientists can quantify mRNA levels and detect splicing variants and non-coding RNAs that play regulatory roles in disease. For example, in breast cancer, transcriptomic analysis has led to the identification of molecular subtypes with distinct prognostic and therapeutic implications, as demonstrated by large-scale studies like METABRIC.

Proteomics: The Executable Code

Proteomics assesses the actual biological effectors proteins, which often undergo post-transcriptional and post-translational modifications not captured by RNA analysis. Techniques such as tandem mass spectrometry (LC-MS/MS) and protein labeling strategies have enabled large-scale, quantitative protein profiling. Proteogenomic integration, such as in the Clinical Proteomic Tumor Analysis Consortium (CPTAC), has uncovered key signaling pathways and potential drug targets in cancers like lung adenocarcinoma, where proteomic data revealed actionable insights absent in genomic data alone (Gillette et al., 2020).

Metabolomics: The End Product of Omics

Metabolomics captures the dynamic biochemical changes in cells and tissues, reflecting the ultimate phenotype shaped by both genetic and environmental factors. It is especially valuable in metabolic and neurodegenerative diseases, where early-stage biochemical disruptions can be detected. For instance, altered metabolite profiles in cerebrospinal fluid have been linked to early stages of Alzheimer‘s disease, aiding in biomarker discovery and understanding disease progression.

Integration and Computational Synergy

The integration of diverse omics layers is what sets multi-omics apart. Sophisticated computational tools and machine learning algorithms are employed to fuse these complex datasets, identify patterns, and extract biologically meaningful insights. Methods such as Multi-Omics Factor Analysis (MOFA) and iCluster enable unsupervised integration of heterogeneous data types, supporting the discovery of novel disease subtypes and risk stratification models (Argelaguet et al., 2018). For example, multi-omics integration in ovarian cancer revealed distinct molecular subtypes with specific genomic alterations, transcriptomic profiles, and protein expression patterns offering better guidance for targeted therapies. Similarly, integrated omics analysis has shown promise in identifying reliable biomarkers for early detection and progression in neurodegenerative diseases. Despite its promise, multi-omics approaches come with significant challenges. These include data standardization, technical variability, batch effects, and the need for large sample sizes to achieve statistical power. Ethical issues, particularly regarding genomic data privacy and informed consent, are also critical considerations. Additionally, the high cost of generating and processing multi-omics data remains a barrier to widespread clinical adoption. Nonetheless, continuous advancements in bioinformatics, AI, and cloud-based platforms are addressing these hurdles. As

technology matures, integrative multi-omics is expected to become a cornerstone of personalized healthcare, offering predictive, preventive, and precise therapeutic strategies. Integrative multi-omics represents a paradigm shift in disease profiling. By combining genomics, transcriptomics, proteomics, and metabolomics, researchers can uncover hidden layers of complexity that were previously inaccessible. This multidimensional approach enables a holistic understanding of disease biology and paves the way for patient-specific treatments that are at the core of precision medicine. As the integration of omics data becomes more sophisticated and accessible, it promises to transform how we diagnose, manage, and ultimately cure complex diseases.

Liquid Biopsy for Early and Real-Time Disease Monitoring

In the era of precision medicine, the ability to detect and monitor disease non-invasively and in real-time has become a priority. Traditional tissue biopsies, while informative, are invasive, limited by sampling bias, and not feasible for frequent monitoring. **Liquid biopsy** has emerged as a powerful alternative, analyzing tumor-derived materials such as **circulating tumor DNA (ctDNA), microRNAs (miRNAs),** and **exosomes** from body fluids, primarily blood. This approach enables dynamic disease tracking, early diagnosis, and the personalization of treatment, especially in oncology.

Circulating Tumor DNA (ctDNA): Tracking Tumor Evolution

ctDNA consists of small DNA fragments shed by tumor cells into the bloodstream during apoptosis or necrosis. It carries tumor-specific genetic and epigenetic alterations, including mutations, copy number changes, and methylation patterns.

Clinical applications of ctDNA

Early cancer detection: A study by Cohen et al. (2018) demonstrated that ctDNA analysis using a multi-analyte blood test (CancerSEEK) could detect eight common cancers with high specificity.

- **Treatment monitoring and resistance**: In non-small cell lung cancer (NSCLC), EGFR mutation tracking through ctDNA helps in guiding tyrosine kinase inhibitor (TKI) therapy and identifying resistance mutations like T790M.
- **Minimal residual disease (MRD)**: ctDNA can detect residual cancer cells post-treatment, predicting relapse earlier than imaging.

Liquid biopsy is already integrated into clinical practice, with the FDA approving ctDNA tests like Guardant360 and FoundationOne Liquid CDx for genomic profiling in cancer. Figure 1, shows the ctDNA profiling offers a minimally invasive approach for early cancer detection, longitudinal treatment monitoring, and timely identification of therapy resistance, thereby supporting personalized oncology care (Fang et al., 2022).

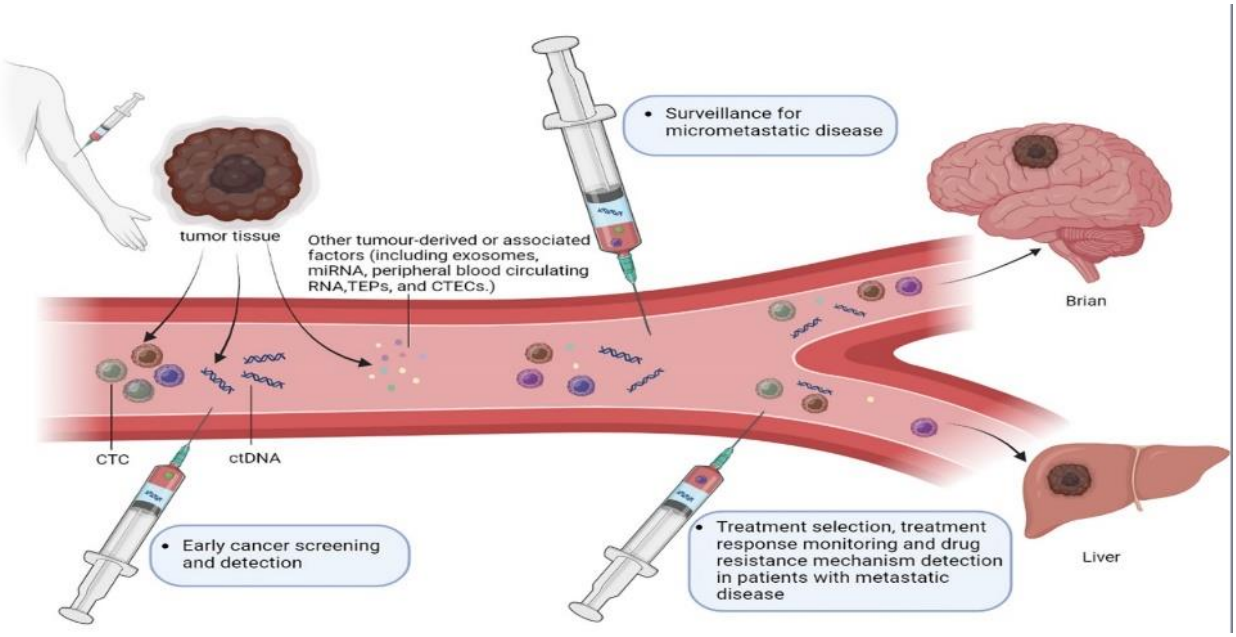


Figure 1. Early Cancer Detection and Treatment Monitoring Via Circulating Tumor DNA (Fang et al., 2022)

MicroRNAs (miRNAs): Stable and Specific Biomarkers

miRNAs are small, non-coding RNAs (~22 nucleotides) that regulate gene expression post-transcriptionally. Their high stability in biofluids and disease-specific expression profiles make them ideal biomarkers. They can exist freely or encapsulated within vesicles like exosomes, protecting them from degradation. The ability to profile miRNAs via qRT-PCR or next-generation sequencing enables non-invasive, repeatable monitoring of disease progression or response to therapy.

Exosomes: Vesicular Messengers with Diagnostic Potential

Exosomes are nanosized extracellular vesicles (30–150 nm) secreted by almost all cell types, including cancer cells. They contain DNA, RNA (including miRNAs), proteins, and lipids, representing a molecular fingerprint of their cell of origin. Moreover, exosomes offer a dual role as biomarkers and potential therapeutic vehicles in precision medicine.

Clinical Impacts

Liquid biopsy enables serial sampling over time, making it ideal for real-time monitoring of disease evolution and therapeutic response. In clinical trial. However, challenges include low biomarker abundance, the need for standardization across platforms, and high costs. Integration of AI and machine learning is being explored to enhance sensitivity and specificity (Heitzer et al., 2019). As technologies advance and become more affordable, liquid biopsy is poised to become a cornerstone of early detection, disease monitoring, and therapy guidance not just in oncology but across a spectrum of chronic and infectious diseases. Liquid biopsy revolutionizes disease detection and monitoring by offering a minimally invasive, repeatable, and comprehensive

molecular snapshot of a patient‘s health. Through analysis of ctDNA, miRNAs, and exosomes, it enables early intervention, tracks therapy responses in real time, and supports the personalized treatment strategies at the heart of precision medicine. As ongoing research addresses its current limitations, liquid biopsy is set to transform clinical diagnostics and disease management.

Single-Cell Omics in Precision Oncology and Immunology

Single-cell omics has opened a new frontier in precision medicine by enabling scientists to study tumors and the immune system at the resolution of individual cells. Unlike traditional bulk methods that average out signals from many cells, single-cell technologies such as **single-cell RNA sequencing (scRNA-seq)** and **single-cell ATAC-seq** reveal the unique identity and behavior of each cell. This is particularly valuable in cancer, where tumors are highly heterogeneous, and understanding this diversity can dramatically improve diagnosis, treatment, and outcomes (Kinker et al., 2020).

Why Intra-Tumor Heterogeneity Matters

Tumors are made up of different cancer cells that can vary genetically and functionally. Some cells might be sensitive to treatment, while others may resist therapy and drive relapse. scRNA-seq has been used to identify such subpopulations. For example, in glioblastoma, single-cell analysis revealed the coexistence of multiple tumor cell states, including stem-like and differentiated cells, which were previously hidden in bulk profiling. These findings help explain why targeted therapies often fail and highlight the need for combination treatments that target multiple cell types.

Mapping the Immune Microenvironment

In cancer immunology, single-cell omics has transformed our understanding of the **tumor microenvironment (TME)**. Tumors attract immune cells, but some of these become dysfunctional or even help the cancer grow. Single-cell approaches can map these immune cells in detail. In melanoma, researchers discovered distinct exhausted T cell populations expressing **PD-1** and **TIM-3**, which has helped refine checkpoint inhibitor therapies. In lung adenocarcinoma, scRNA-seq identified immunosuppressive macrophages expressing **PPARγ,** which reduced T cell infiltration and tumor clearance (Zilionis et al., 2019). These insights are now influencing how immunotherapies are developed and combined.

Improving Diagnosis and Personalized Treatment

Single-cell data also enables the discovery of **biomarkers** for early detection, prognosis, and response prediction. For instance, the presence of **CD8+ T cell clonality** and expression of **cytotoxic genes** has been linked to better responses to PD-1 therapy in various cancers. In kidney cancer, specific subsets of **tumor-associated macrophages (TAMs)** were associated with poor prognosis, providing potential therapeutic targets. Such findings are shaping the future of **personalized oncology,** where treatment is tailored based on the unique cellular makeup of each patient's tumor.

Next-Gen Integrations

Combining single-cell data with **spatial transcriptomics** allows researchers not only to identify what each cell is doing but also where it is located within the tumor. This spatial context helps uncover interactions between tumor and immune cells, critical for understanding response and resistance mechanisms. Furthermore, integrating **single-cell proteomics** and **epigenomics** offers even deeper insights into cell behavior and fate decisions, supporting a holistic view of disease processes. Single-cell omics technologies are revolutionizing our understanding of cancer and immune responses. By uncovering the hidden complexity of tumors and immune ecosystems, they allow researchers and clinicians to design smarter diagnostics and personalized therapies. As these technologies continue to evolve, they will be key drivers in the next generation of precision oncology and immunology.

Polygenic Risk Scores (PRS) in Predictive Medicine

Polygenic Risk Scores (PRS) are becoming an important tool in predictive medicine, helping doctors assess an individual‘s risk of developing complex diseases, like heart disease, diabetes, and cancer. These diseases are influenced by many small genetic factors, which is why PRS is useful it looks at the combined effect of multiple genetic variations to predict a person's risk.

How PRS Work

PRS are calculated by looking at many genetic variants across a person‘s genome. Each variant has a small effect on disease risk, but when combined, these small effects can add up. PRS add up the risk from many of these variants, giving a score that represents a person‘s overall genetic risk for a specific disease. For example, someone with a high PRS for heart disease may have a significantly increased risk of developing it, just like someone with a family history of heart problems.

Clinical Applications of PRS

PRS are already being used in healthcare to predict and prevent diseases. In **heart disease**, PRS can help identify people who may be at high risk, even if they don‘t have obvious symptoms, and allow for early interventions such as lifestyle changes or medications (Inouye et al., 2018). In **cancer,** PRS can help doctors decide when to start screenings or recommend preventive measures, particularly for diseases like breast cancer. Similarly, in **mental health,** PRS can help assess the risk of conditions like schizophrenia and depression, guiding early interventions.

While PRS are promising, there are challenges. One issue is that most PRS models are developed using data from people of European descent, so they may not work as well for people from other ethnic backgrounds. There are also ethical concerns around how PRS data might be used, especially regarding privacy, insurance, or discrimination. It's important to use these tools responsibly and ensure that patients fully understand what their score means. Researchers are working on improving PRS by combining them with other information, like lifestyle factors or

environmental exposures. This could help make the scores even more accurate and personalized. As PRS continue to improve, they will likely become an essential part of healthcare, helping doctors give people more personalized care and earlier prevention. Polygenic Risk Scores offer a way to predict a person‘s genetic risk for complex diseases. By looking at many small genetic factors, PRS can help doctors identify high-risk individuals, offering a chance for prevention and personalized treatment. As this technology continues to improve, it could become a routine part of how we prevent and treat diseases.

Epigenomic Markers in Personalized Risk Assessment

Epigenomic markers have become integral to personalized medicine, offering a dynamic layer of biological information beyond the static genome. Among these, DNA methylation and histone modifications are particularly valuable in predicting disease onset and guiding therapeutic decisions. These epigenetic modifications regulate gene expression without altering the underlying DNA sequence and are shaped by environmental, developmental, and pathological factors making them ideal for individualized risk profiling.

DNA methylation, typically involving the addition of a methyl group to cytosine bases in CpG islands, plays a critical role in gene silencing. Aberrant methylation patterns are among the earliest and most consistent molecular alterations observed in diseases such as cancer. For instance, hypermethylation of tumor suppressor genes like BRCA1, p16, and MLH1 has been implicated in breast, colon, and lung cancers, often preceding clinical manifestation. Clinically, methylation of the MGMT gene promoter serves as a predictive biomarker for responsiveness to temozolomide in glioblastoma, highlighting its utility in personalizing therapy.

Histone modifications including acetylation, methylation, phosphorylation, and ubiquitination modulate chromatin structure and gene accessibility. Specific histone marks, such as H3K4me3 (active transcription) and H3K27me3 (gene repression), provide insight into cell state and disease progression. In autoimmune and neurodegenerative diseases, altered histone acetylation patterns correlate with inflammation and disease activity, offering potential markers for monitoring and intervention. DNA methylation and histone modification profiles form a rich epigenomic landscape that reflects an individual's health trajectory. Their integration into clinical practice enhances risk assessment models, supports early diagnosis, and enables more precise, responsive treatments. As research advances, epigenomic markers are poised to become cornerstones of preventive and personalized healthcare (Feinberg, 2018).

AI-Driven Drug Discovery and Repurposing in Precision Therapeutics

Artificial intelligence (AI) is reshaping the landscape of precision therapeutics by accelerating drug discovery and repurposing processes, all while tailoring treatments to individual patient profiles. By leveraging machine learning (ML) algorithms, researchers can now analyze vast and complex biomedical data to identify novel drug targets and uncover new applications for existing medications with unprecedented accuracy and speed. In traditional drug discovery, identifying viable compounds is time-consuming and costly, often requiring over a decade of research and development. AI addresses these challenges by employing algorithms such as deep

learning, support vector machines, and ensemble models that can process high-dimensional datasets, including genomic sequences, protein structures, chemical libraries, and clinical data. These models can predict drug-target interactions, prioritize molecules for testing, and even suggest structural modifications to optimize efficacy and minimize side effects. This data-driven approach significantly enhances early-stage decision-making and shortens the drug development pipeline.

Beyond discovery, AI plays a transformative role in drug repurposing, a strategy that identifies new therapeutic uses for already approved or investigational drugs. Machine learning models analyze patient electronic health records (EHRs), gene expression profiles, disease pathways, and real-world clinical outcomes to identify hidden correlations between existing drugs and new indications. For instance, AI-powered platforms have helped repurpose drugs like remdesivir for COVID-19 by modeling interactions between viral proteins and human molecular pathways (Luo et al., 2021). This approach not only reduces development costs but also accelerates time-to-market for treatments. The true potential of AI in precision therapeutics lies in its ability to personalize treatment. By integrating patient-specific data such as genetic mutations, biomarker profiles, lifestyle factors, and historical treatment responses AI can predict which therapies are most likely to be safe and effective for individual patients. This level of personalization reduces trial-and-error prescribing and enhances therapeutic outcomes, particularly in complex diseases like cancer, autoimmune disorders, and neurological conditions. As AI continues to evolve, its integration into clinical and pharmaceutical workflows is expected to become routine. With improved algorithmic transparency, broader access to high-quality data, and interdisciplinary collaboration, AI-driven drug discovery and repurposing will form the backbone of next-generation precision medicine.

CRISPR and Gene Editing as Precision Therapeutic Tools

CRISPR gene editing has become one of the most powerful tools in modern medicine, offering the ability to directly correct genetic problems at their source. As a precision therapy, it holds great promise for treating both monogenic diseases caused by mutations in a single gene and polygenic conditions, which involve changes in multiple genes. Monogenic disorders such as sickle cell disease or cystic fibrosis, CRISPR-Cas9 can be programmed to locate and cut the faulty gene. The cell then repairs the cut, either by fixing the mutation or by inserting a healthy copy of the gene. This strategy has already shown success in clinical trials. For instance, scientists have used CRISPR to edit blood stem cells outside the body to treat patients with sickle cell disease and beta-thalassemia (Frangoul et al., 2021). Polygenic conditions, such as heart disease, diabetes, and schizophrenia, are more complex because they involve many genes working together. However, CRISPR can still be effective here. With advanced tools like base editing and prime editing, researchers can make small, precise changes to DNA without cutting it completely reducing side effects and allowing for more careful control. CRISPR can also target multiple genes at once using ―multiplexing," which is especially useful for diseases with complex genetic causes.

CRISPR therapies can be used in two main ways: somatic and germline editing. Somatic editing targets body cells like blood or liver cells and affects only the treated individual. These are the types currently used in clinical trials and are considered ethically safe. In contrast, germline editing

changes the DNA in reproductive cells or embryos. These changes can be passed on to future generations. While technically possible, germline editing raises serious ethical concerns and is heavily restricted worldwide. What makes CRISPR especially powerful is how flexible it is. Scientists can design it not only to fix broken genes but also to turn genes on or off, or even change how genes are read without changing the DNA itself. These capabilities are opening the door to personalized medicine, where treatments are tailored to an individual's unique genetic makeup. As CRISPR-based therapies continue to advance, they offer real hope for curing diseases that were once considered untreatable. Still, the technology must be used carefully, with strong safety checks and ethical guidelines to ensure it benefits patients without unintended consequences.

Pharmacogenomics in Clinical Decision Support Systems

Pharmacogenomics is the study of how a person's genes affect their response to medications. By understanding these genetic factors, healthcare providers can prescribe drugs that are more effective and safer for individual patients. A key advancement in this area is integrating pharmacogenomic data into Clinical Decision Support Systems (CDSS), which are used by clinicians to make real-time, informed decisions about drug prescriptions. This integration, especially within Electronic Health Records (EHRs), is transforming the way medications are prescribed, moving towards a more personalized approach to healthcare. When pharmacogenomic information is added to EHRs, it allows healthcare providers to quickly access genetic insights during patient visits. For example, certain genetic variations can affect how well a patient processes specific medications. For instance, variations in the CYP2C19 gene can impact how well a patient responds to clopidogrel, a drug used to prevent heart attacks. Similarly, differences in the CYP2D6 gene can change how a person responds to opioids like codeine. By incorporating this information into CDSS, doctors receive alerts in real time, helping them adjust prescriptions or doses based on the patient‘s unique genetic profile. This leads to safer, more effective treatments and reduces the likelihood of harmful side effects or drug failures.

Many healthcare systems are already incorporating pharmacogenomics into clinical practice. For example, the Clinical Pharmacogenetics Implementation Consortium (CPIC) provides evidence-based guidelines that help doctors use genetic information to guide their prescribing decisions. These guidelines are integrated into EHRs, so when a genetic test is performed, the CDSS can automatically suggest the most appropriate drug or dose for the patient based on their genetic makeup.

Despite these advancements, some challenges remain. One of the biggest issues is ensuring that different healthcare systems can share genetic data securely and effectively. There is also a need for better education and training for healthcare providers on how to interpret pharmacogenomic data. Additionally, the cost of genetic testing and the variability in insurance coverage can make it difficult for some patients to access these services. However, as technology improves and genetic testing becomes more affordable, these challenges are slowly being overcome. The integration of pharmacogenomics into Clinical Decision Support Systems and Electronic Health Records is an exciting step toward personalized medicine. By tailoring drug prescriptions to an individual‘s

genetic profile, healthcare providers can offer safer, more effective treatments. As these systems continue to evolve, they will play an increasingly important role in improving patient care.

Personalized Immunotherapy

Cancer treatment has entered a new era with personalized immunotherapy, which uses a patient‘s unique tumor genetics and immune system features to design targeted therapies. By combining cutting-edge biologics such as checkpoint inhibitors, neoantigen vaccines, and CAR-T cell therapies with advanced genomic tools, doctors can now create treatments that are more effective and less toxic than traditional approaches. Here‘s how this precision medicine revolution works.

1. Checkpoint Inhibitors: Matching Patients to the Right Drug

Checkpoint inhibitors (e.g., pembrolizumab, nivolumab) are drugs that ~~"r~~release the brakes" on the immune system, allowing T cells to attack tumors. However, not all patients respond to these therapies. To improve outcomes, scientists use biomarkers to identify who will benefit most:

a) **PD-L1 expression**: Tumors with high levels of PD-L1 protein (detected via biopsies) are more likely to respond to anti-PD-1 drugs like pembrolizumab.
b) **Tumor Mutational Burden (TMB)**: Cancers with many DNA mutations (e.g., melanoma, lung cancer) produce more abnormal proteins called neoantigens, making them easier for the immune system to recognize. High TMB predicts better responses to checkpoint inhibitors.
c) **Immune gene signatures**: Blood tests measuring interferon-gamma-related genes can reveal whether a patient‘s immune system is primed to fight cancer.

New tools like liquid biopsies (analyzing tumor DNA in blood) now allow doctors to monitor treatment responses in real time and adjust therapies as needed.

2. Neo-antigen Vaccines: A Custom-Made Defense

Neo-antigens are proteins unique to a patient‘s tumor, created by DNA mutations. Neoantigen vaccines are tailor-made to train the immune system to target these proteins:

1. **Genomic sequencing**: A tumor sample is analyzed to find mutations that distinguish it from healthy cells.
2. **AI prediction**: Algorithms predict which neoantigens will bind to the patient‘s immune receptors (MHC molecules) to trigger a T-cell response.
3. **Vaccine delivery**: These neoantigens are packaged into vaccines (often using mRNA technology) and injected to stimulate immune attacks.

In trials, these vaccines have delayed cancer recurrence in melanoma and glioblastoma patients (Keskin et al., 2019). Combining them with checkpoint inhibitors boosts effectiveness by

enhancing T-cell activity. Challenges remain in speeding up vaccine production, but advances in mRNA platforms (like those used for COVID-19 vaccines) are solving this problem.

3. CAR-T Cell Therapy: Engineering Supercharged Immune Cells

CAR-T therapy involves reprogramming a patient‘s own T cells to hunt cancer:

a) **How it works**: T cells are extracted, genetically modified to produce chimeric antigen receptors (CARs), and infused back into the patient. These CARs help T cells recognize and destroy tumors.
b) **Solid tumors**: While CAR-T has succeeded in blood cancers (e.g., targeting CD19 in leukemia), solid tumors require unique targets. Genomic sequencing identifies proteins like EGFRvIII (in brain tumors) or HER2 (in breast cancer) to avoid harming healthy tissue.
c) **Overcoming resistance**: New ~~–ar~~mored" CAR-T cells secrete proteins like IL-12 to counteract the tumor‘s immunosuppressive environment or include checkpoint blockers to sustain T-cell activity.

The Future of Personalized Immunotherapy

While these therapies show promise, challenges like tumor heterogeneity (genetic diversity within tumors), high costs, and variable biomarker accuracy remain. Future solutions may involve

a) **AI-driven neo-antigen prediction** to improve vaccine design.
b) **Combination therapies** (e.g., vaccines + checkpoint inhibitors + CAR-T) to tackle resistance.
c) **Universal biomarkers** to simplify patient selection.

By integrating genomics, immune profiling, and innovative biologics, personalized immunotherapy is transforming cancer into a treatable and even curable disease for many patients.

Precision Psychiatry

Precision psychiatry represents a transformative approach to diagnosing and treating mental health disorders by integrating genetic, neuroimaging, and behavioral data to tailor interventions to individual patients. Unlike traditional psychiatric practices, which often rely on symptom-based categorization, precision psychiatry seeks to identify biological and psychosocial markers that predict treatment response and disease trajectories for conditions such as depression, schizophrenia, and bipolar disorder (Fernandes et al., 2017). This paradigm shift aims to improve clinical outcomes by leveraging advances in genomics, neurobiology, and data analytics to deliver personalized care.

Genomic Insights and Polygenic Risks

Genetic research has uncovered numerous polymorphisms and copy number variations associated with mental health disorders. For example, genome-wide association studies (GWAS) have identified risk loci linked to major depressive disorder (MDD), including variants in SLC6A4 (serotonin transporter gene) and HTR2A (serotonin receptor gene), which influence synaptic neurotransmitter activity. In schizophrenia, polymorphisms in COMT (catechol-O-methyltransferase) and DISC1 (disrupted in schizophrenia 1) have been implicated in dopaminergic dysfunction and neurodevelopmental anomalies, respectively. Similarly, bipolar disorder has been associated with variations in CACNA1C (calcium voltage-gated channel subunit) and ANK3 (ankyrin 3), genes critical for neuronal signaling. Polygenic risk scores (PRS), which aggregate the cumulative effects of thousands of genetic variants, now enable clinicians to estimate an individual‘s genetic predisposition to these disorders. For instance, PRS has shown utility in stratifying patients with depression into subgroups more likely to respond to selective serotonin reuptake inhibitors (SSRIs) versus cognitive-behavioral therapy. Such genomic tools hold promises for reducing trial-and-error prescribing and optimizing pharmacogenomic interventions.

Neuroimaging Biomarkers and Circuit-Based Targets

Neuroimaging techniques, such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), have elucidated aberrant neural circuitry underlying psychiatric disorders. In depression, hypoactivity in the prefrontal cortex (PFC) and hyperactivity in the amygdala are linked to emotional dysregulation and negative bias. For schizophrenia, reduced gray matter volume in the hippocampus and disrupted connectivity in the default mode network (DMN) correlate with cognitive deficits and psychotic symptoms. Bipolar disorder is characterized by oscillating patterns of activity in the anterior cingulate cortex (ACC) and striatum during manic and depressive episodes. These findings have spurred interest in neuromodulation therapies, such as transcranial magnetic stimulation (TMS) targeting the dorsolateral PFC in treatment-resistant depression, and deep brain stimulation (DBS) for severe bipolar disorder (Lefaucheur et al., 2020). Machine learning algorithms trained on neuroimaging data further enhance predictive accuracy; for example, a 2021 study demonstrated that fMRI-based classifiers could predict lithium response in bipolar patients with 75% accuracy.

Integrating Behavioral and Digital Phenotyping

Behavioral data, collected via smartphone apps, wearables, and ecological momentary assessment (EMA), provide real-time insights into mood fluctuations, sleep patterns, and social interactions. Digital phenotyping has proven particularly valuable in bipolar disorder, where circadian rhythm disruptions often precede manic episodes. Similarly, in schizophrenia, speech analysis algorithms can detect subtle linguistic anomalies indicative of prodromal psychosis. Combining these data streams with genomic and neuroimaging profiles enable a multidimensional understanding of pathology. For instance, a study found that patients with high PRS for depression and low hippocampal volume exhibited poorer responses to psychotherapy, suggesting synergistic effects of genetic and neuroanatomical risk factors (Toenders et al., 2020).

Despite its potential, precision psychiatry faces hurdles, including ethical concerns around genetic privacy, the high cost of neuroimaging, and the need for large, diverse datasets to mitigate algorithmic bias. Additionally, translating research findings into clinical practice requires interdisciplinary collaboration among geneticists, neuroscientists, and clinicians. Future efforts should prioritize longitudinal studies to track biomarker trajectories and randomized trials testing precision-guided interventions. For example, the PREDICT trial demonstrated that using CRP (C-reactive protein) levels to guide antidepressant selection improved remission rates in MDD, highlighting the feasibility of biomarker-driven care. Precision psychiatry represents a groundbreaking frontier in mental health, offering hope for more effective, individualized treatments. By synthesizing genomic, neurobiological, and behavioral data, clinicians can move beyond a one-size-fits-all approach to address the heterogeneity of depression, schizophrenia, and bipolar disorder. However, realizing this vision demands continued innovation, ethical vigilance, and equitable access to advanced diagnostics and therapies.

Personalized Approaches in Neurodegenerative Disease Management

Alzheimer's, Parkinson's, and Huntington's Disease

Neurodegenerative diseases such as Alzheimer‘s disease (AD), Parkinson‘s disease (PD), and Huntington‘s disease (HD) are progressive disorders characterized by complex pathophysiological mechanisms and substantial heterogeneity in clinical presentation. Conventional diagnostic and therapeutic strategies, often generalized across populations, are increasingly being replaced by personalized approaches that integrate molecular, genetic, and imaging data to inform individualized care. Precision medicine in this context offers the promise of earlier diagnosis, targeted treatment, and improved disease outcomes. In Alzheimer‘s disease, early detection strategies have been significantly advanced through the use of cerebrospinal fluid (CSF) biomarkers including amyloid-β42, total tau, and phosphorylated and imaging modalities such as amyloid-PET and MRI. These tools enable identification of neuropathological changes years before symptom onset, especially in genetically at-risk individuals, such as carriers of the APOE ε4 allele. Personalized interventions, including anti-amyloid monoclonal antibodies (e.g., aducanumab) and lifestyle modifications, are increasingly being guided by these biomarker profiles, allowing for disease-modifying strategies tailored to the biological stage of AD. Similarly, personalized management of Parkinson‘s disease is evolving through molecular subtyping based on genomic, transcriptomic, and proteomic data. Variants in genes such as LRRK2, GBA, SNCA, and PINK1 not only influence susceptibility but also modulate disease phenotype, progression rate, and treatment response. Stratification of patients using these markers facilitates targeted therapeutic approaches, including gene-targeted therapies, neuroprotective compounds, and individualized deep brain stimulation protocols. Huntington‘s disease, caused by an expanded CAG repeat in the HTT gene, presents a genetically well-defined model for personalized intervention. Genetic testing enables pre-symptomatic diagnosis, risk prediction, and individualized monitoring. Therapeutic advances such as allele-specific antisense oligonucleotides (e.g., tominersen) and RNA interference approaches aim to selectively silence mutant HTT expression, offering disease-modifying potential that aligns with the patient‘s genetic profile. These therapies represent a shift from symptomatic management to targeted molecular intervention. Collectively, personalized approaches in

neurodegenerative disease management represent a paradigm shift from reactive treatment to proactive, precision-guided care. Integration of molecular diagnostics, longitudinal biomarker tracking, and computational modeling will be central to realizing the full potential of individualized medicine in neurology.

Precision Cardiology

The convergence of genomics and clinical cardiology has catalyzed a new era in cardiovascular medicine precision cardiology in which individualized risk stratification and therapeutic strategies are informed by patient-specific genetic profiles. This approach is particularly transformative for complex and genetically influenced conditions such as heart failure (HF), inherited arrhythmias, and familial hypercholesterolemia (FH), where conventional risk models are often inadequate. By integrating genomic insights with clinical phenotypes, precision cardiology offers enhanced predictive power, early diagnosis, and optimized intervention strategies. In heart failure, especially dilated and hypertrophic cardiomyopathies, genomic analysis has unveiled key pathogenic variants in genes such as TTN, MYH7, and LMNA, which not only aid in early diagnosis but also serve as predictors of disease progression and arrhythmic risk. For instance, truncating variants in TTN are associated with variable penetrance and differential responses to therapy, highlighting the need for genotype-driven treatment planning. In parallel, pharmacogenomic data are increasingly guiding the selection and dosing of heart failure medications, such as β-blockers and angiotensin receptor-neprilysin inhibitors, thereby improving therapeutic efficacy and safety. Inherited arrhythmia syndromes, including long QT syndrome (LQTS) and catecholaminergic polymorphic ventricular tachycardia (CPVT), exemplify the clinical utility of genotype-based management. Mutations in genes like KCNQ1, KCNH2, and RYR2 not only define disease subtypes but also determine therapeutic pathways ranging from lifestyle modifications and pharmacotherapy to the use of implantable cardioverter-defibrillators (Priori et al., 2013). Rapid gene panel sequencing now facilitates early diagnosis, risk stratification, and cascade screening in asymptomatic relatives, offering a proactive approach to sudden cardiac death prevention. Familial hypercholesterolemia, a monogenic disorder of lipid metabolism, underscores the role of precision diagnostics in preventive cardiology. Pathogenic variants in LDLR, APOB, and PCSK9 result in elevated low-density lipoprotein cholesterol (LDL-C) and a markedly increased risk for premature atherosclerotic cardiovascular disease. Genotype-confirmed FH enhances diagnostic accuracy and enables early initiation of lipid-lowering therapies, including high-intensity statins and PCSK9 inhibitors. Moreover, novel agents such as inclisiran, a small interfering RNA targeting PCSK9, offer a promising precision therapeutic option for genetically confirmed cases with statin intolerance or suboptimal response. The integration of polygenic risk scores (PRS), machine learning, and electronic health records further augments the clinical utility of genetic data, offering nuanced risk prediction models that outperform traditional scoring systems. As whole genome sequencing becomes more accessible, the incorporation of multi-omic data into clinical decision-making will likely become standard practice, supporting a shift from reactive treatment to preemptive cardiovascular care.

N-of-1 Medicine and Gene Variant Interpretation

Rare diseases, defined as conditions affecting fewer than 1 in 2,000 individuals, collectively impact over 300 million people globally. Despite advances in genomics, many patients endure prolonged diagnostic odysseys due to phenotypic complexity, genetic heterogeneity, and limited access to advanced tools (Wright et al., 2021). Whole genome sequencing (WGS) and exome sequencing (ES) have revolutionized rare disease diagnostics, achieving diagnostic rates of 25–50% in undiagnosed cases. However, challenges persist for ultra-rare and undiagnosed conditions, necessitating innovative frameworks like N-of-1 trials and refined variant interpretation strategies. This paper explores the integration of genomic technologies, personalized trial designs, and collaborative networks to address these challenges.

Whole Genome and Exome Sequencing in Rare Disease Diagnosis

WGS and ES enable comprehensive analysis of coding and non-coding genomic regions, identifying single-nucleotide variants (SNVs), structural variants (SVs), and repeat expansions missed by traditional methods. For example, WGS detects pathogenic SVs in ~3–5% of undiagnosed cases, such as deletions in *COL4A2* linked to kidney disorders (Wright et al., 2021). ES, while limited to exons, remains cost-effective for Mendelian diseases, diagnosing 25–35% of neurodevelopmental disorders.

1. **Unbiased Analysis**: Unlike targeted panels, WGS/ES evaluates all genes, critical for phenotypically ambiguous cases.
2. **Multi-Omic Integration**: Combining transcriptomics or metabolomics with WGS improves diagnostic yield by resolving variants of uncertain significance (VUS).
3. **Trio Sequencing**: Analyzing probands and parents reduces candidate variants tenfold, enhancing efficiency.

Despite these benefits, limitations persist, such as incomplete coverage of GC-rich regions and difficulties interpreting non-coding variants, which constitute ~98% of the genome.

N-of-1 Trials: Personalized Medicine for Ultra-Rare Conditions

N-of-1 trials address heterogeneity in treatment response by testing interventions in single patients through repeated, randomized crossovers. These trials are particularly valuable for ultra-rare diseases, where traditional trials are impractical. For example, in pediatric hypertension, N-of-1 trials identified optimal antihypertensive regimens by comparing drug efficacy within individuals.

Key Design Considerations:

a) **Natural History Data**: Longitudinal pre-treatment data establish baselines to quantify meaningful clinical changes.

b) **Tailored Outcomes**: Patient-specific clinical outcome assessments (COAs), such as symptom diaries or wearable device metrics, replace generic endpoints.
c) **Statistical Rigor**: Bayesian methods assess the probability of benefit, accommodating small sample sizes.

These trials also empower patients, improving adherence and health literacy by involving them in decision-making.

Gene Variant Interpretation Frameworks

Interpreting variants requires integrating population data, functional assays, and phenotype-genotype correlations. The American College of Medical Genetics and Genomics (ACMG) guidelines classify variants as pathogenic, likely pathogenic, or VUS, but challenges remain for non-coding and structural variants.

1. **Non-Coding Variants**: Rare variants in 3′ untranslated regions (UTRs) or introns disrupt alternative polyadenylation (APA), altering mRNA stability. For example, *IL18RAP* 3′ UTR variants reduce mRNA stability, implicating amyotrophic lateral sclerosis.
2. **AI-Driven Prioritization**: Machine learning tools like SpliceAI predict splicing impacts, while ensemble methods prioritize tandem repeats.
3. **Collaborative Databases**: Platforms like ClinVar and MatchMaker Exchange enable global data sharing, resolving VUS through matchmaking with similar phenotypes.

Global Initiatives and Equity Challenges

Programs like the NIH Undiagnosed Diseases Network (UDN) and Solve-RD exemplify collaborative frameworks combining WGS, metabolomics, and international data sharing. The UDN diagnosed 24% of participants, including novel disorders like *GLS* repeat expansions (Gahl et al., 2012). However, disparities persist: low-income regions face limited access to sequencing, and marginalized communities experience delayed diagnoses due to systemic inequities (Taruscio et al., 2023).

Strategies for Equity:

Telemedicine: Connects underserved patients to specialist centers (Taruscio et al., 2023).

Capacity Building: Training programs in LMICs improve local diagnostic expertise (Wright et al., 2021).

Open-Source Tools: Platforms like DECIPHER democratize variant interpretation.

WGS and ES have transformed rare disease diagnosis, yet ultra-rare conditions demand personalized and collaborative approaches. N-of-1 trials bridge the gap between population-level data and individual needs, while AI-enhanced variant interpretation frameworks unlock non-coding

genomic insights. Global networks like UDNI and Solve-RD underscore the importance of equity in genomic medicine. Future efforts must prioritize scalable technologies, such as long-read sequencing for complex SVs, and policies ensuring equitable access to diagnostics (Taruscio et al., 2023).

Precision Endocrinology in Diabetes and Thyroid Disorders

Precision endocrinology represents a paradigm shift in managing diabetes and thyroid disorders by leveraging multi-modal data genetic, phenotypic, and lifestyle to tailor diagnostic and therapeutic strategies to individual patients. This approach addresses the heterogeneity of these conditions, which often manifest through diverse molecular pathways, clinical presentations, and responses to treatment. By integrating advanced genomic technologies, artificial intelligence (AI), and patient-specific biomarkers, precision endocrinology aims to optimize outcomes, reduce adverse effects, and preempt complications. This paper explores the transformative role of precision medicine in diabetes and thyroid care, emphasizing the synergy between genetic insights, phenotypic subtyping, and lifestyle modulation.

Precision Approaches in Diabetes

Diabetes mellitus (DM) is a heterogeneous disease with complex genetic underpinnings. Genome-wide association studies (GWAS) have identified over 400 loci linked to type 1 (T1D) and type 2 diabetes (T2D), including variants in *TCF7L2*, *KCNJ11*, and *HNF1A*. Polygenic risk scores (PRS) now enable stratification of patients based on genetic susceptibility, predicting progression from prediabetes to overt T2D and guiding early interventions. For example, individuals with high PRS for insulin resistance may benefit from lifestyle modifications or GLP-1 receptor agonists, while those with beta-cell dysfunction might respond better to sulfonylureas or SGLT2 inhibitors. Monogenic forms of diabetes, such as maturity-onset diabetes of the young (MODY), further illustrate the clinical utility of genetic testing. *GCK* and *HNF1A* mutations dictate distinct therapeutic pathways: *GCK*-MODY often requires no treatment, while *HNF1A*-MODY responds to low-dose sulfonylureas.

Phenotypic Profiling and Biomarkers

Phenotyping has emerged as a cornerstone of precision diabetes care. Subtypes such as "severe insulin-resistant diabetes" (SIRD) or "mild obesity-related diabetes" (MOD) correlate with differential risks of nephropathy, retinopathy, and cardiovascular disease (Acosta et al., 2021). Machine learning algorithms analyze multi-omics data genomic, proteomic, and metabolomics to classify patients into clusters with tailored management plans. For instance, the "Hungry Gut" phenotype, characterized by rapid gastric emptying, shows superior weight loss with GLP-1 agonists like liraglutide, while the "Emotional Hunger" subtype benefits from naltrexone/bupropion to curb hedonic eating.

Lifestyle and Environmental Integration

Lifestyle factors, including diet, physical activity, and stress, modulate genetic and phenotypic expression. Continuous glucose monitors (CGMs) and wearable devices provide real-time data on glycemic variability, enabling dynamic adjustments to insulin dosing or carbohydrate intake. AI-driven platforms integrate this data with genetic risk profiles to predict hypoglycemic episodes or recommend personalized meal plans. For example, patients with *MTNR1B* variants, which impair melatonin signaling, may require adjusted meal timing to mitigate circadian-related glucose dysregulation.

Precision Approaches in Thyroid Disorders

Thyroid dysfunction, including Graves‘ disease (GD) and Hashimoto‘s thyroiditis, exhibits strong genetic heritability (60–80%). GWAS have identified risk loci such as HLA-DRB1, *CTLA4*, and *PTPN22*, which influence immune tolerance and thyroid autoantibody production (Smith & Hegedüs, 2024). For example, HLA-DRB1 variants alter antigen presentation, predisposing individuals to GD, while *CTLA4* polymorphisms impair regulatory T-cell function, exacerbating autoimmune destruction. Non-coding variants in the *TSHR* gene correlate with thymic expression levels, affecting central tolerance and autoantibody development. Genetic testing can predict relapse risk in GD patients post–antithyroid drug therapy, guiding decisions for definitive treatment (e.g., radioiodine or surgery).

Phenotypic Stratification and Biomarkers

Thyroid function tests (TSH, FT4, FT3) are foundational but insufficient for capturing disease heterogeneity. Subclinical hypothyroidism, for instance, varies in clinical significance based on thyroid peroxidase (TPO) antibody status and genetic risk. Multi-trait analyses integrating TSH, FT4, and T3/FT4 ratios reveal metabolic signatures linked to cardiovascular risk or neurocognitive decline.

Lifestyle and Environmental Modulators

Iodine intake, endocrine disruptors (e.g., perchlorates), and stress influence thyroid hormone synthesis and autoimmunity (Taruscio et al., 2023). Precision frameworks incorporate geospatial data on iodine deficiency and pollutant exposure to adjust supplementation or mitigation strategies.

Integration of Multi-Modal Data

AI and Predictive Analytics

AI platforms synthesize genetic, phenotypic, and lifestyle data to generate predictive models (Obermeyer & Emanuel, 2016). For diabetes, algorithms like the "ABCD" score predict

insulin requirements, while thyroid AI tools forecast Graves‘ ophthalmopathy progression (Smith & Hegedüs, 2024).

Clinical Decision Support Systems

Precision endocrinology leverages decision-support tools like the "ThyroidOmics" platform, which integrates GWAS data with thyroid imaging to recommend personalized therapies. Pharmacogenomic databases guide antithyroid drug selection (e.g., avoiding methimazole in *HLA-B*38:02 carriers). Precision endocrinology transforms diabetes and thyroid care by bridging genetic susceptibility, phenotypic diversity, and lifestyle influences. From AI-driven diagnostics to CRISPR-edited therapies, this approach promises to eradicate trial-and-error medicine and enhance quality of life.

Artificial Intelligence and Machine Learning

Artificial Intelligence (AI) and Machine Learning (ML) have emerged as transformative technologies in healthcare, particularly within the realm of precision diagnostics. These tools are capable of processing large, complex datasets including genomic sequences, medical images, and electronic health records leading to more accurate and individualized diagnostic approaches. Key applications include genomic variant interpretation, radio-genomics, and clinical outcome prediction, all of which are critical to advancing personalized medicine.

Deep Learning for Genomic Variant Interpretation

Genomic variant interpretation plays a vital role in diagnosing hereditary diseases and tailoring treatments to individual patients. Traditional methods for assessing variants of unknown significance (VUS) are labor-intensive and often inconclusive. Deep learning models have shown promise in addressing these challenges by automatically learning features from raw DNA sequences and predicting the functional impact of mutations. For instance, tools based on convolutional neural networks (CNNs) have been used to predict the pathogenicity of single nucleotide variants and splice-altering mutations by analyzing sequence context, evolutionary conservation, and known pathogenic variants. These models are particularly valuable in identifying rare genetic mutations linked to diseases, thus reducing diagnostic delays and improving accuracy. However, their clinical implementation still requires careful validation and interpretability to ensure that predictions are actionable and understandable to healthcare providers.

Radio-genomics

Radio-genomics is an innovative interdisciplinary field that combines radiologic imaging features with genomic data to enhance disease characterization and guide treatment strategies. With AI-enabled radiomics, imaging biomarkers extracted from CT, MRI, and PET scans can be quantitatively linked to genetic and molecular profiles of tumors, providing non-invasive insights into disease biology. Recent advances have demonstrated the utility of AI in predicting key molecular markers such as IDH mutation and 1p/19q codeletion in gliomas based solely on MRI data. These approaches enable real-time, cost-effective molecular profiling that can substitute for

invasive biopsy procedures. Additionally, machine learning models can detect subtle imaging features associated with treatment resistance and tumor heterogeneity, supporting better prognostication and therapeutic planning. Despite these advancements, radio-genomics still faces hurdles related to data heterogeneity, standardization of imaging protocols, and the need for multicenter validation to ensure reproducibility. Continued collaboration between radiologists, geneticists, and data scientists is essential for clinical adoption.

AI-Driven Clinical Outcome Prediction

Another critical application of AI in precision diagnostics is the prediction of clinical outcomes such as survival, disease recurrence, and treatment response. ML algorithms can integrate multimodal datasets including clinical history, imaging, and genomics to create robust predictive models that support decision-making at both individual and population levels. For example, CheXNeXt algorithm was developed, which uses deep learning to interpret chest X-rays and diagnose conditions like pneumonia and pleural effusion with performance on par with board-certified radiologists. Similarly, in oncology, AI models are being used to forecast survival in ovarian and lung cancer patients by combining histopathological, radiological, and molecular data. These applications demonstrate the potential of AI to reduce human error, identify high-risk patients, and personalize care strategies. However, issues such as algorithmic bias, data privacy, and the ―black box" nature of some AI models remain pressing concerns that must be addressed through transparent model development and ethical oversight. AI and ML are revolutionizing precision diagnostics by enabling the rapid, accurate, and scalable interpretation of complex biological and clinical data. Their applications from variant interpretation and radio-genomics to outcome prediction are helping to realize the full potential of personalized medicine. While the benefits are substantial, the safe and ethical integration of these technologies into clinical workflows requires ongoing research, cross-disciplinary collaboration, and robust regulatory frameworks.

Wearables and Digital Biomarkers in Personalized Health Monitoring

Wearable technologies have revolutionized personalized health monitoring by enabling continuous data collection and real-time feedback. These devices, ranging from smartwatches to biosensors, facilitate the tracking of physiological parameters, offering insights into an individual's health status and allowing for timely interventions.

Advancements in Wearable Technologies

Modern wearable devices are equipped with sensors capable of monitoring vital signs such as heart rate, respiratory rate, and body temperature. These continuous measurements provide a dynamic view of an individual's health, surpassing the limitations of periodic clinical assessments. For instance, wearable sensors have been instrumental in early detection of disease transitions, enabling interventions before the onset of symptoms.

Digital Biomarkers and Personalized Feedback

The integration of digital biomarkers quantifiable physiological and behavioral data collected via digital devices has enhanced the precision of health monitoring. These biomarkers facilitate the identification of subtle changes in health status, allowing for personalized feedback and interventions. For example, continuous monitoring of glucose levels through wearable sensors has improved diabetes management by providing real-time data, leading to better glycemic control.

Real-Time Health Interventions

The real-time data provided by wearables enable prompt health interventions. By continuously analyzing physiological parameters, these devices can detect anomalies indicative of potential health issues. Such timely detection allows healthcare providers to initiate interventions promptly, improving patient outcomes. Moreover, the data collected can be integrated into electronic health records, facilitating comprehensive patient monitoring.

Despite the promising potential of wearable technologies, challenges such as data privacy, device accuracy, and user adherence remain. Ensuring the security of sensitive health data is paramount, necessitating robust encryption and compliance with privacy regulations. Additionally, the accuracy of wearable sensors must be validated to ensure reliable data collection. User adherence is also critical; devices must be user-friendly and unobtrusive to encourage consistent use. Future developments aim to address these challenges by enhancing sensor technology, improving data analytics through artificial intelligence, and integrating wearables into broader healthcare systems. Such advancements will further solidify the role of wearable technologies in personalized health monitoring, enabling proactive healthcare and improved patient outcomes. The exponential growth of genomic data, propelled by advancements in next-generation sequencing technologies, has revolutionized biomedical research and personalized medicine. However, this surge presents significant challenges in data storage, processing, and sharing, particularly concerning privacy and regulatory compliance. Cloud computing and federated learning (FL) have emerged as pivotal solutions, enabling efficient, secure, and collaborative genomic data analysis across institutions (Langmead & Nellore, 2018).

Cloud-Based Genomic Data Integration

Cloud computing offers scalable infrastructure for storing and analyzing vast genomic datasets. Platforms like Amazon Web Services (AWS), Google Cloud, and Microsoft Azure provide elastic resources that can adapt to the computational demands of genomic analyses. This flexibility facilitates large-scale collaborations and accelerates research timelines. The advantages of cloud computing in genomics, emphasizing its role in democratizing access to computational resources and promoting reproducibility in research. By leveraging cloud platforms, researchers can perform complex analyses without the need for substantial local infrastructure, thereby reducing barriers to entry and fostering inclusive scientific exploration. Moreover, cloud-based systems support the integration of diverse genomic datasets, enabling comprehensive analyses that consider various populations and conditions. This integration is crucial for identifying genetic variants associated with diseases and for developing targeted therapies.

Federated Learning in Genomics

Federated learning is a machine learning paradigm that allows models to be trained across multiple decentralized datasets without transferring the data to a central location. This approach is particularly beneficial in genomics, where data privacy and security are paramount. A study was conducted utilizing FL on genomic data from the UK Biobank and the 1000 Genomes Project. Their findings demonstrate that FL models can achieve performance comparable to centralized models in predicting phenotypes and ancestries, even amidst significant inter-node heterogeneity. This underscores FL's potential in facilitating collaborative research while preserving data privacy. Furthermore, FL addresses the challenges posed by data silos and regulatory constraints. By enabling decentralized analysis, FL allows institutions to collaborate without compromising sensitive information, thereby accelerating discoveries in genomics and personalized medicine (Kolobkov et al., 2024).

Integration of Cloud Computing and Federated Learning

The convergence of cloud computing and FL presents a robust framework for genomic data analysis. Cloud platforms provide the necessary computational resources and scalability, while FL ensures data privacy and compliance with regulations like the General Data Protection Regulation (GDPR). The implementation of secure cloud computing for genomic data, highlighting the importance of encryption and access controls in protecting sensitive information. By integrating FL into cloud environments, researchers can perform collaborative analyses without exposing raw data, thus maintaining confidentiality and trust. This integrated approach also facilitates the development of global genomic networks, where institutions across different regions can contribute to and benefit from shared insights, leading to more comprehensive and diverse genetic research.

Despite the promising synergy between cloud computing and FL, several challenges persist. These include ensuring interoperability between different systems, managing computational costs, and addressing potential biases in decentralized data. Moreover, establishing standardized protocols and governance frameworks is essential for the widespread adoption of these technologies. Future research should focus on enhancing the efficiency and scalability of FL algorithms, developing robust security measures, and fostering international collaborations to create inclusive and representative genomic datasets. By addressing these challenges, the integration of cloud computing and FL can significantly advance the field of genomics, leading to more personalized and effective healthcare solutions (Kolobkov et al., 2024).

Population-Level Implementation of Personalized Tools

Precision public health (PPH) integrates the principles of precision medicine into public health practices by leveraging genomic data, environmental exposures, and lifestyle factors to deliver targeted interventions to specific populations. This approach enhances disease prevention, screening, and surveillance efforts, moving beyond the traditional one-size-fits-all model to enable more effective public health strategies.

Precision Medicine Strategies in Public Health

Traditional public health screening programs often apply uniform criteria across populations, potentially overlooking individuals at varying levels of risk. The integration of precision medicine allows for stratified screening based on genetic risk factors. For instance, a study utilizing data from the UK Biobank demonstrated that individuals with high polygenic risk scores for diseases such as breast cancer and cardiovascular disease reached average risk levels for these conditions much earlier than the general population. This finding suggests that initiating screening protocols earlier for high-risk individuals could significantly reduce premature, preventable deaths. In Australia, the DNA Screen project exemplifies the practical application of precision screening. By screening over 10,000 individuals for genetic variants associated with hereditary breast and ovarian cancer, Lynch syndrome, and familial hypercholesterolemia, researchers identified that 1 in 50 participants carried a pathogenic variant. Notably, 75% of these individuals would not have qualified for testing under existing guidelines, highlighting the potential of genomics to refine and expand screening criteria.

Surveillance

Precision public health enhances surveillance by incorporating genomic and other high-resolution data to monitor disease patterns more accurately. During the COVID-19 pandemic, genomic sequencing of SARS-CoV-2 enabled public health officials to track transmission dynamics and identify emerging variants, informing timely interventions. Such genomic surveillance is not limited to infectious diseases; it also holds promise for chronic disease monitoring by identifying population subgroups at elevated risk due to genetic predispositions. Moreover, integrating environmental and behavioral data with genomic information allows for a more comprehensive understanding of disease etiology and progression. This multifaceted approach facilitates the development of targeted public health policies and resource allocation strategies, ensuring interventions are both effective and equitable.

Disease Prevention

Precision medicine strategies contribute significantly to disease prevention by identifying individuals at high risk and implementing tailored interventions. For example, pharmacogenomics the study of how genes affect a person's response to drugs enables the customization of medication regimens to maximize efficacy and minimize adverse effects. This approach is particularly beneficial in managing chronic conditions such as hypertension and diabetes, where treatment responses can vary widely among individuals (Borbón et al., 2025). Furthermore, understanding genetic susceptibilities allows for the implementation of lifestyle modifications and preventive measures targeted to those most at risk. Such personalized prevention strategies can lead to more efficient use of public health resources and improved health outcomes at the population level.

Despite the promising potential of precision public health, several challenges hinder its widespread implementation. These include concerns about data privacy, the need for robust ethical frameworks, and the requirement for substantial investments in infrastructure and workforce training. Additionally, disparities in access to genomic technologies can exacerbate existing health

inequities if not addressed proactively. To overcome these challenges, it is essential to develop policies that promote equitable access to precision health tools, invest in public health genomics education, and establish standardized guidelines for data sharing and interpretation. Collaborative efforts between governments, healthcare providers, and communities are crucial to ensure that the benefits of precision public health are realized universally.

References

Argelaguet, R., Velten, B., Arnol, D., Dietrich, S., Zenz, T., Marioni, J. C., ... & Stegle, O. (2018). Multi-Omics Factor Analysis—a framework for unsupervised integration of multi-omics data sets. *Molecular Systems Biology*, 14(6), e8124. https://doi.org/10.15252/msb.20178124

Borbón, M. A., et al. (2025). Pharmacogenomics tools for precision public health and lessons for low- and middle-income countries: A scoping review. *Frontiers in Genetics, 16*, 123456. https://doi.org/10.3389/fgene.2025.123456

Fang X, Yu S, Jiang Y, Xiang Y and Lu K (2022) Circulating tumor DNA detection in MRD assessment and diagnosis and treatment of non-small cell lung cancer. *Front. Oncol.* 12:1027664. doi: 10.3389/fonc.2022.1027664

Feinberg, A. P. (2018). The key role of epigenetics in human disease prevention and mitigation. *New England Journal of Medicine*, 378(14), 1323–1334. https://doi.org/10.1056/NEJMra1402513

Fernandes, B. S., Williams, L. M., Steiner, J., Leboyer, M., Carvalho, A. F., & Berk, M. (2017). The new field of ‗precision psychiatry'. *BMC Medicine, 15*(1), 80. https://doi.org/10.1186/s12916-017-0849-x

Frangoul, H., et al. (2021). CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. *New England Journal of Medicine*, 384(3), 252–260.

Kalluri, R., & LeBleu, V. S. (2020). The biology, function, and biomedical applications of exosomes. *Science*, 367(6478), eaau6977. https://doi.org/10.1126/science.aau6977

Keskin, D. B., Anandappa, A. J., Sun, J., Tirosh, I., Mathewson, N. D., Li, S., ... Wu, C. J. (2019). Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature, 565*(7738), 234–239. https://doi.org/10.1038/s41586-018-0792-9

Kinker, G. S., et al. (2020). Pan-cancer single-cell RNA-seq identifies recurring programs of cellular heterogeneity. *Nature Genetics*, 52, 1208–1218. https://doi.org/10.1038/s41588-020-00726-6

Kolobkov, D., Mishra Sharma, S., Medvedev, A., Lebedev, M., Kosaretskiy, E., & Vakhitov, R. (2024). Efficacy of federated learning on genomic data: A study on the UK Biobank and the 1000 Genomes Project. *Frontiers in Big Data, 7*, 1266031. https://doi.org/10.3389/fdata.2024.1266031

Langmead, B., & Nellore, A. (2018). Cloud computing for genomic data analysis and collaboration. *Nature Reviews Genetics, 19*(4), 208–219. https://doi.org/10.1038/nrg.2017.113

Lefaucheur, J. P., Aleman, A., Baeken, C., Benninger, D. H., Brunelin, J., Di Lazzaro, V., Filipović, S. R., Grefkes, C., Hasan, A., Hummel, F. C., Jääskeläinen, S. K., Langguth, B.,

Leocani, L., Londero, A., Nardone, R., Nguyen, J. P., Nyffeler, T., Oliveira-Maia, A. J., Oliviero, A., … Garcia-Larrea, L. (2020). Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): An update (2014–2018). *Clinical Neurophysiology, 131*(2), 474–528. https://doi.org/10.1016/j.clinph.2019.11.002

Luo, H., Li, M., Wang, S., Liu, Q., & Yang, H. (2021). Drug repurposing based on comprehensive similarity measures and deep learning. *Briefings in Bioinformatics*, 22(5), bbab124.

Smith, T. J., & Hegedüs, L. (2024). Genetics of Graves‘ disease. *Reviews in Endocrine and Metabolic Disorders, 25*, 203–214. https://doi.org/10.1007/s11154-023-09854-w

Taruscio, D., Mollo, E., Gainotti, S., Posada de la Paz, M., & Bianchi, F. (2023). Undiagnosed diseases: Needs and opportunities in 20 countries. *Frontiers in Public Health, 11*, 1079601. https://doi.org/10.3389/fpubh.2023.1079601

Terry, S. F. (2017). The global precision medicine market: using technology to improve care. *Genetic Testing and Molecular Biomarkers, 21*(6), 314–317. https://doi.org/10.1089/gtmb.2017.29027.sft

Toenders, Y. J., Schmaal, L., Harrison, B. J., Dinga, R., Penninx, B. W. J. H., van Velzen, L. S., Veltman, D. J., & Davey, C. G. (2020). Neuroimaging predictors of onset and course of depression in childhood and adolescence: A systematic review of longitudinal studies. *Developmental Cognitive Neuroscience, 41*, 100744. https://doi.org/10.1016/j.dcn.2019.100744

Tsherniak, A., Vazquez, F., Montgomery, P. G., Weir, B. A., Kryukov, G., Cowley, G. S., ... & Hahn, W. C. (2017). Defining a Cancer Dependency Map. *Cell*, 170(3), 564–576.e16. https://doi.org/10.1016/j.cell.2017.06.010

Wright, C. F., FitzPatrick, D. R., & Firth, H. V. (2021). Integration of whole genome sequencing into a healthcare setting. *Genome Medicine, 13*(1), 40. https://doi.org/10.1186/s13073-021-00855-5

Zilionis, R., et al. (2019). Single-cell transcriptomics of human and mouse lung cancers reveals conserved myeloid populations across individuals and species. *Immunity*, 50(5), 1317–1334.e10. https://doi.org/10.1016/j.immuni.2019.03.009

About The Authors

Dr. Hamna Tariq (PhD) currently working as Assistant Professor and Head of Department of Department of Molecular Biology, University of Okara Pakistan. She has expertise in Whole genome sequencing for identifying clues and culprits responsible for non-syndromic human hearing impairment. Her research interests include Genetics, Biotechnology, Precision Medicine, Molecular Biology and Non-Syndromic Human Disorders. During her doctoral research she worked on the understanding how human genetic blue print carries a legacy of ailments. Dr. Hamna has made distinguished contributions to these fields through her extensive research, high-impact publications, and presentations at national and international conferences. Her work has played a pivotal role in expanding the frontiers of knowledge and enhancing understanding within critical areas of medical science.

E-mail: hamna.tariq@uo.edu.pk **ORCID:** 0009-0002-1332-8761.

Ghulam Murtaza holds a Bachelor's degree in Biotechnology and currently pursuing his M.Phil. in Molecular Biology at the University of Okara Pakistan. His research focuses on the NRAMP-1 gene, exons to investigate their role in natural resistance against infectious diseases. NRAMP-1 is a key player in immune defense, and its genetic variations may influence susceptibility to infections like tuberculosis. He is deeply interested in the molecular mechanisms underlying host-pathogen interactions and their outcomes. His academic journey reflects a strong passion for Molecular Biology, Biotechnology, Infectious Disease and Precision Medicine research.

E-mail: ghulammurtaza8817@gmail.com **ORCID:** 0009-0003-3557-0054.

Nosheen Majeed, an M.Phil. Molecular Biology scholar at the University of Okara, with academic background in Biotechnology from the Virtual University of Pakistan. Her research focuses on the body‘s defense against infectious agents and NRAMP-1 gene, a key immune-related gene, and its genetic variations that may significantly impact the susceptibility to various diseases. She is deeply interested in exploring the molecular mechanisms underlying host-pathogen interactions. Through her research, she aims to contribute to a better understanding of genetic resistance to infections, potentially informing the development of improved diagnostic tools and therapeutic strategies. Her work reflects a strong commitment for the advancement of knowledge in Biotechnology, Genetics, Molecular Biology and infectious disease control.

E-mail: nosheenmbuo23@gmail.com **ORCID:** 0009-0005-3360-0322.

Dr. Muhammad Masood Ahmed is an Associate Professor of Pharmacology and serving as the Principal of the College of Pharmacy at Nishtar Medical University, Multan, Pakistan. His research interests encompass immunology, pharmacology, pathology, angiogenesis, and oncology. With a strong record of scholarly publications and active participation in national and international scientific forums, Dr. Ahmed has made impactful contributions to these critical areas of medical science. His work continues to advance research, education, and clinical understanding, positioning him as a respected figure in his field.

Email: masoodkarni786@gmail.com **ORCID:** 0000-0002-4432-958.

Dr. Farhat Ameer Chughtai is a Senior Demonstrator in the Department of Pharmacology at Nishtar Medical University, Multan, Pakistan. Her research interests encompass medicine, pharmacology, toxicology, neurology, and neurological disorders. With a strong commitment to both education and research, Dr. Chughtai focuses on exploring innovative therapeutic approaches for neurological conditions, as well as the pharmacological and toxicological mechanisms underlying disease processes. Her growing contributions to these fields reflect her dedication to advancing clinical knowledge and improving patient outcomes, establishing her as an emerging scholar in the medical sciences.

Email: drfarhatkhurram@gmail.com **ORCID:** 0009-0004-4979-6473

Khawar Ali is currently pursuing his Ph.D. at North University of China, following his M.S. in Molecular Biology. He has previously served as a Research Associate at the Virtual University of Pakistan and Cholistan University of Veterinary and Animal Sciences, Bahawalpur, where he gained extensive experience in Molecular Biology research and laboratory techniques. Additionally, he worked as a Molecular Biologist at IBN-E-SINA Clinical Laboratory & Research Center in Lahore, contributing to clinical diagnostics and research operations. Khawar Ali has published several research articles in reputable peer-reviewed scientific journals. His research interests include Oncology, Molecular Biology, Genomics, and Biotechnology, with a particular focus on Molecular Diagnostics and Cancer Biology. Through his academic and professional endeavors, he is dedicated to advancing scientific knowledge and contributing to innovations in biomedical research.

Email: khawarali1202@gmail.com **ORCID:** 0000-0002-0565-4535.

Similarity Index
The similarity index obtained from the plagiarism software for this book chapter is 10%.

To Cite This Chapter:
Tariq H et al., (2025). Precision Medicine Personalized Approaches for Diagnosis, Treatment, and Prevention. In Current Studies in Life Sciences 2025 (pp.204-232). ISRES Publishing.

Decoding Resistance: AI-Powered Genomic Surveillance for a Post-Antibiotic Era

Zubia RASHID
Ghosia NOREEN
Humera NAZIR
Qais MAHMOOD
Muhammad Usman QAMAR
Mehmet OZASLAN

Introduction

Antimicrobial resistance (AMR) is gaining prominence rapidly as one of the most pressing and complicated threats to global public health. Widely referred to as a silent pandemic, it may be defined as the growing ability of bacteria and other infections to withstand those medications that have been previously efficacious against them (Paneri & Sevta, 2023). Super bacteria immune to ordinary antibiotics result in prolonged hospital stays, causing higher expenses and increased mortality rates. According to estimates, this phenomenon will lead to at least 700,000 deaths annually due to AMR (Fong, 2023). The mathematicians believe this number will eventually reach millions if no immediate action is taken. The burden is not proportionately spread; Asia has reported 4.7 million infections with drug resistance, followed closely by Africa with 4.1 million. Of course, the wealthiest parts of the world, Europe, North America, and Oceania, also have millions of AMR cases. What Fig. 1 shows is a global picture of AMR distribution, emphasizing that national strategies need to be developed along with a worldwide regional strategy.

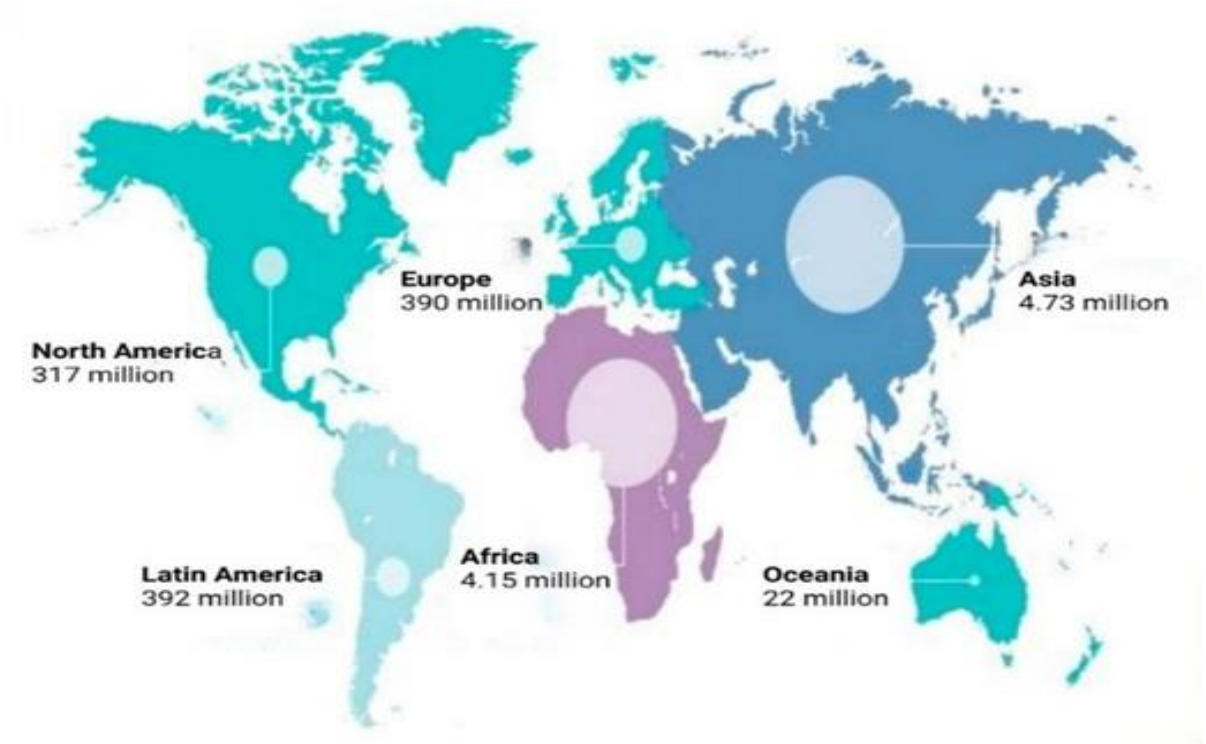


Figure 1. Global Population Distribution by Continent

While the world faces this ever-increasing crisis, incorporating AI into genomic surveillance in the fight against AMR is seen as a beacon of hope. Traditional genomic resistance determinations, however potent, nevertheless require a lot of time from starting the sample to generating reports, probably days or weeks. Delays can be fatal when fast and accurate decisions are needed. AI-based genomic surveillance could be the answer, where genomes of microbes are analyzed in hours rather than the traditional weeks, giving timely information on resistance patterns and allowing for a rapid reaction in the clinic (Tsitou et al., 2024). AI tools are employed in settings with limited diagnostic infrastructure, such as parts of Africa, where they are used to identify resistance hotspots and inform resource allocation. In the more industrialized contexts of North America, AI is being employed to monitor resistance trends across hospitals so that healthcare providers and policymakers can take the necessary interventions. These AI applications are reshaping AMR management with faster, scalable, and more precise offerings than previous methods. This chapter assesses and discusses the opportunities for AI-driven genomic surveillance in AMR consideration. Discussion includes how AI helps in favour of conventional methods, challenges in implementing this, and therefore the ethical considerations in employing this in the health sector. AI would serve to click to view more in the interest of the global health community.

AI's Role in Genomic Surveillance

Antimicrobial resistance (AMR) is increasingly a global problem, and traditional genomic surveillance cannot keep pace with the emergence of drug-resistant bacteria. Whole-genome sequencing, touted as the principal technology for recognizing resistance patterns, produces enormous datasets that are too slow and labor-intensive to analyze manually. Artificial intelligence is changing the pace and accuracy with which data can now be processed, predicting the evolution of resistance and synthesizing complex datasets for public health intervention **(Nguyen & Nsonga Jr, 2024).**

1)AI's Role in Genomic Surveillance: Genomic surveillance, accompanied by AI, heralds a new phase in applying resistance data to public health. Often, the applied techniques experience latency in dealing with genomic data and converting the results into interventions, but AI beats that for all-time lapses. Figure 2 shows this entire process, as genomic data processing, predictive modeling, and public health measures work seamlessly together. It begins by extracting and sequencing genomic material using next-generation sequencing (NGS), establishing the stage for AI-driven analysis. Supervised and unsupervised advanced algorithms flag resistance patterns, predict bacterial evolution, and provide actionable insights, which subsequently inform the design of interventions, allocate adequate resources, and contain outbreaks. (Bag & Sengupta, 2024). This approach demonstrates how AI enables public health systems to respond quickly to emerging threats. 9

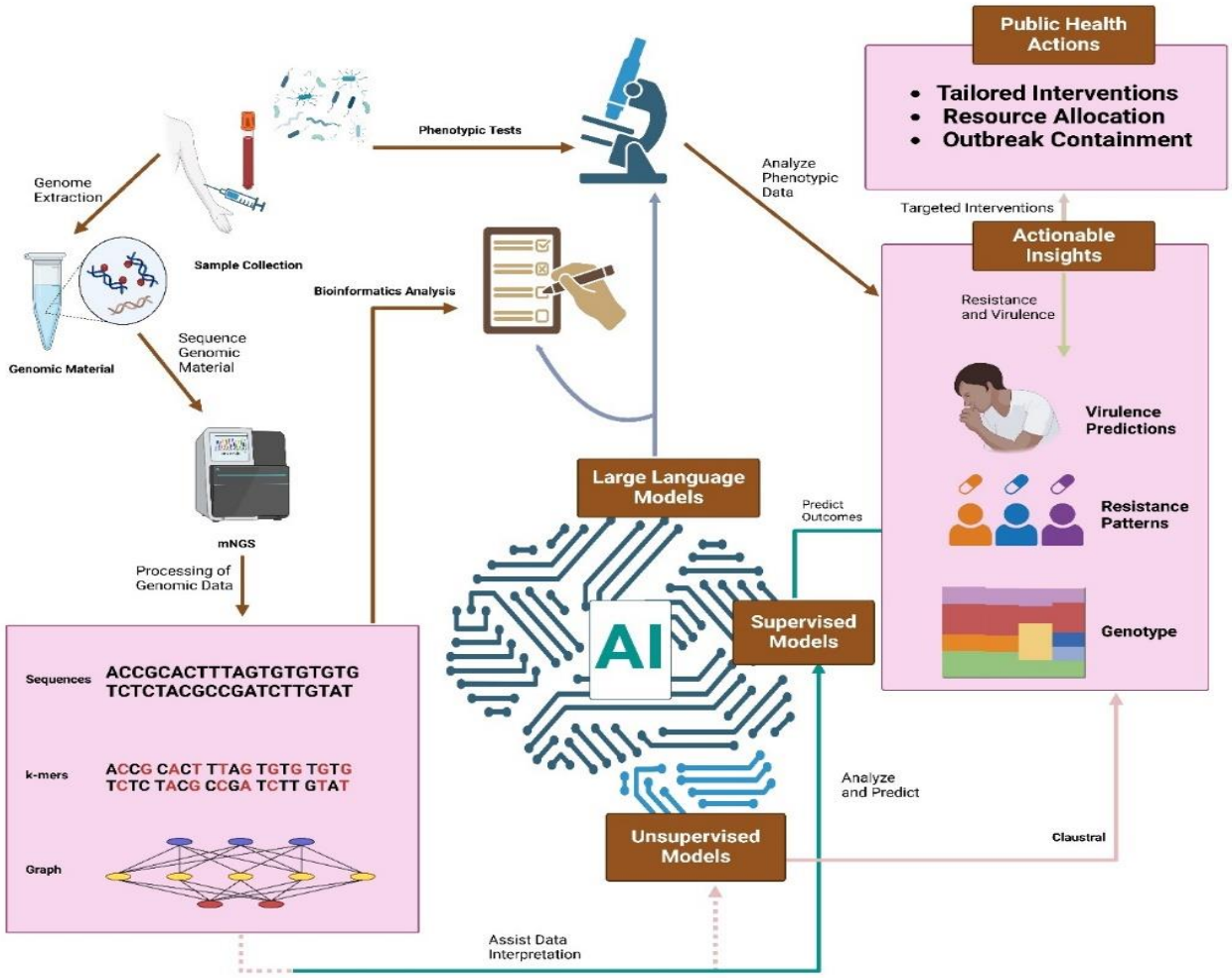


Figure 2. Integrated Framework of AI-Driven Genomic Surveillance for Antimicrobial Resistance (AMR)

Harnessing AI for Genomic Surveillance

With the increasing threat of antibiotic resistance globally, monitoring and predicting bacterial resistance has gained tremendous importance for public health care and interventions. Machine learning plays a key role in the upper arms of AMR strategies seeking to harness frontier technologies to analyze genomic data. Natural language processing gives this an extra edge through the recognition of resistance genes, tracking their dissemination, and anticipating alterations in bacterial genomes (Gupta & Bhandary, 2024). This complementary combination empowers health care professionals to counter the spread of resistance more efficiently. AI applications in genomic surveillance find utility in three spheres: extracting data from genomic sequences, massive disease surveillance, and predictive surveillance. Empowering diagnostics and therapies, these technologies give insight into such matters from the individual and the population level, weaving in clinical, environmental, and epidemiological data.

1)Artificial Intelligence Analysis of Genomic Data: Classical approaches to interpreting microbial genomes are slow and lead to lagging actions. In contrast, AI hastens the analysis of resistant gene patterns, which may otherwise go unnoticed by human researchers. Machine learning algorithms detect antibiotic resistance genetic markers, significantly reducing the time required to identify and stem the spread of resistant strains. Figure 3 highlights how AI can revolutionize patient observation, intervention, and outcome improvement in various healthcare areas. Neural networks like convolutional neural networks (CNN) are optimized for finding complex, non-linear patterns in genomic data. CNN can process extremely substantial genomic datasets in hours to identify gene clusters related to resistance; the operation would otherwise take days. Such speed and accuracy become crucial in a scenario of outbreak and necessitate prompt actions (Ladner et al., 2019).

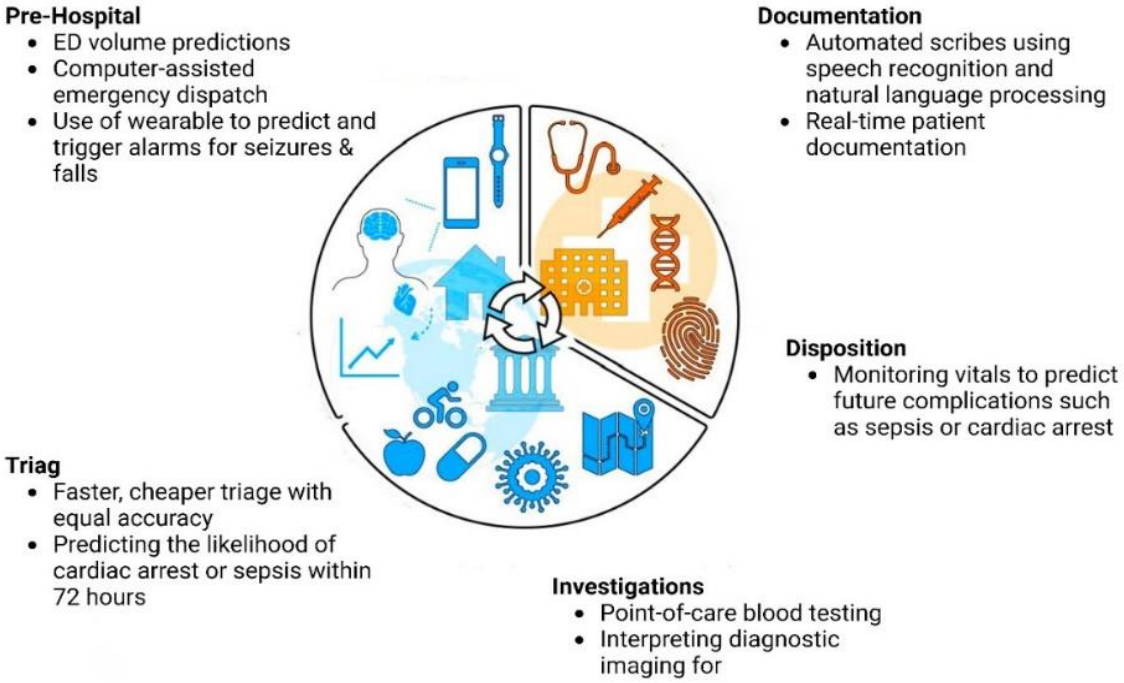


Figure 3. AI in Transforming Healthcare Delivery

2) Monitoring as an Element for Early Warning Systems: Real-time genomic surveillance using AI has become a linchpin. The time-honored methods detect resistance only after it emerges, whereas AI offers a fantastic opportunity to monitor it continuously from the onset. The real-time genomic analysis alerts healthcare professionals to the emergence of resistant strains, making it possible for preventive measures to be undertaken before the resistance is fully established (Dhall et al., 2020). This surveillance system ties together information from hospitals, research facilities, and environmental samples, creating resistance profiles that are updated constantly and using new data to retrain the model. This real-time monitoring is a must-have for effective infection control in hospitals and tracking resistance worldwide to address the growing public health concerns at the global level.

3) Predictive Modeling: Anticipation of Change in Resistance: One can argue that AI's predictive capabilities are indispensable in antibiotic resistance forecasting from its written paradigm. It can analyze genomic data to predict bacterial adaptation to new antibiotics or public health measures. Thus, correctional application allows the healthcare industry to adapt its strategy gradually and strategically before resistance develops widely. For example, AI may predict which genes will likely be altered or transferred between bacteria, offering pertinent information to pharmaceutical companies developing new antibiotics. (Simpkin et al., 2017). AI assists in predicting resistance patterns of several multidrug-resistant organisms, including MRSA and tuberculosis, to thwart the development of new resistant strains.

4) The Integration of Big Data and Artificial Intelligence: Understandably, artificial intelligence is an advantage in genomics surveillance due to its massive data processing capability. Genomic data from bacteria is enormous, and when integrated with clinical, environmental, and epidemiological data, it might far overwhelm human analysis. AI is well-suited to detecting patterns in these extensive datasets, revealing mechanisms by which resistance may develop. By combining genomic data with antibiotic usage and patient outcomes, AI assists in understanding resistance patterns, which also include the pollution-fueled regional antibiotic use effects (Martinez-Romero et al., 2010). This empowers healthcare providers to make better-informed decisions and respond proactively to resistance. AI is transforming genomic surveillance by reducing human input, tracking pathogens, and predicting resistance trends. A proactive nature of the public health system can prepare itself ahead of time for the alterations expected in bacterial populations, making it a much stronger institution with which to fight AMR (Vora et al., 2023).

AI Applications in Clinical and Public Health Settings

Antibiotic resistance is an ongoing and severe dilemma. AI now has a primary intervention for controlling antibiotic resistance, even if in clinical and public health settings. AI is useful in infection control by introducing patient-specific therapy regimens based on resistance patterns and reinforcing actions to control multidrug-resistant organisms (MDROs) (Soni & Gupta, 2021). AI systems like Frey Wire intervene in hospital antibiotic resistance management, allowing the data-driven identification of resistant infections and optimizing antibiotic use. Such AI systems streamline diagnosis, bolster infection control, and provide real-time alerts for timely intervention. The integration of real-time monitoring, predictive analytics, and pattern recognition by AI optimizes the utilization of resources. It prevents the unwarranted use of antibiotics, which supports effective public health strategies. Figure 3 depicts how AI contributes to the fight against antibiotic resistance through real-time monitoring, predictive analytics, and pattern recognition. AI improves diagnosis, streamlines infection control, and provides automated alerts to enable timely interventions. Hence, this optimizes resources and diminishes the misuse of antibiotics while reinforcing public health strategies to improve health outcomes.

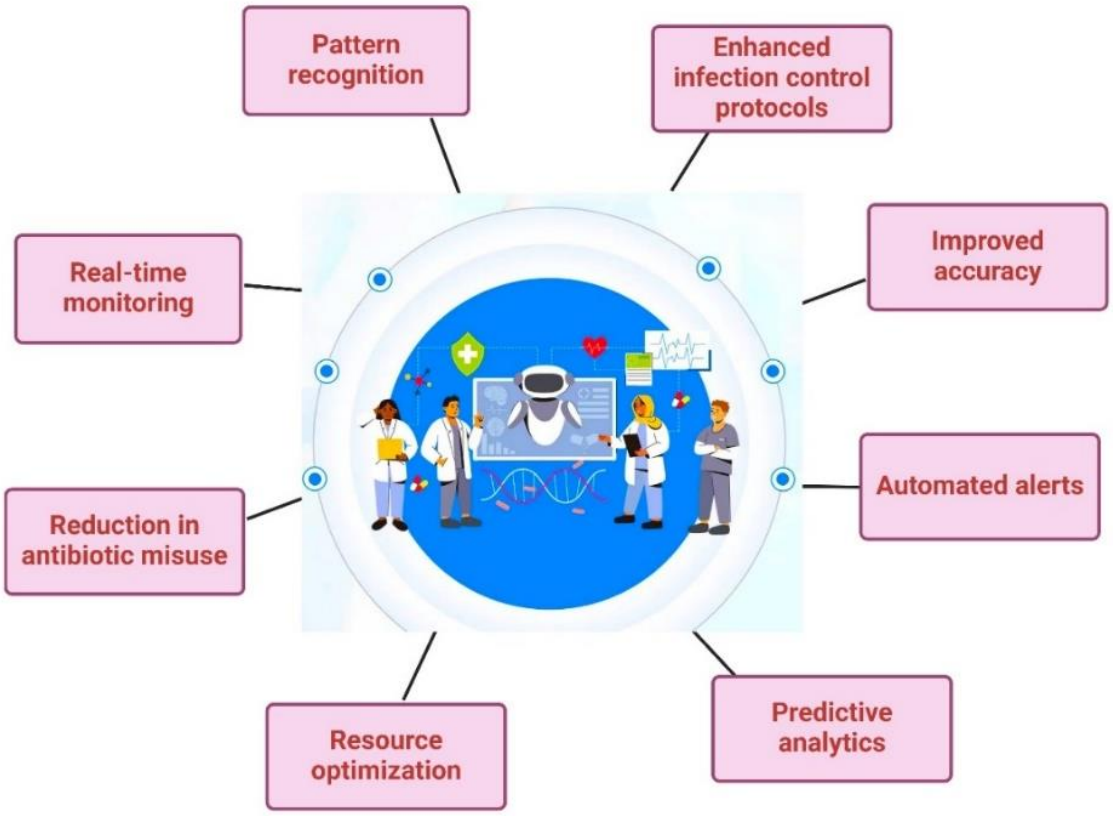


Figure 4. Transformative Benefits of AI in the Management of AMR

1) The Role of Automated Intelligence in Hospital Genomic Surveillance: Nosocomial infections (NIs) due to the MDROs are a significant challenge to the immunocompromised patient population. AI has been used to improve genomic surveillance in hospitals by enabling the real-time processing of genomic information obtained from patients' samples to identify resistant strains quickly. Such rapid identification will assist in fast intervention strategies, such as isolating infected patients or modifying treatment regimens to limit further outbreaks. Machine learning algorithms will track genomic data continuously for timely alerts on potential outbreaks so that hospitals can assess the viability of their infection control program. These developments will help reduce the spread of infections such as MRSA and C. difficile (Duhaniuc et al., 2024).

2) AI and Personalized Medicine Treatment and Care

The traditional concept of a broad-spectrum antibiotic is either no longer effective or less widely applied without consideration of resistance patterns. AI and genomics are on the verge of completely violating personalized Medicine by drawing an individualized treatment plan considering the patients' and pathogens' genomes. AI assesses the infecting bacteria's genetic characteristics somewhere in the process, rapidly identifies resistance genes, and recommends the best antibiotics for that patient. This way, the massive reliance on broad-spectrum antibiotics is negated, which improves the outcome of treatment while minimizing the potential for further resistance (Lau et al., 2021). Such personalized treatment plans offer more targeted and effective therapy, since they rely on AI-generated genomic analysis.

3) AI Innovative Tools in Public Health Interventions

Artificial intelligence is gradually becoming a key driver in managing and preventing antibiotic resistance at individual and population levels. New insights into resistance patterns and antibiotic prescribing can complement health communication initiatives and public health campaigns aimed at changing the knowledge, attitudes, and behaviors of healthcare providers and the broader population regarding the appropriate use of antibiotics, as shown in Table 3. AI can also analyze vast amounts of data from hospitals, pharmacies, and public health institutions to identify areas where antibiotic misuse is emerging, enabling targeted interventions in these hotspots. Artificial Intelligence contributes to antibiotic resistance management by analyzing misprescription patterns to reduce wrong usage, providing real-time feedback to shape public health campaigns, and conducting global resistance surveillance for well-coordinated international responses. These applications show clear evidence of AI's vital role in enhancing public health strategies and handling AMR, as shown in Table 3.

Table 3. AI Applications in Public Health Campaigns

Application	Description	Impact	References
Tracking Antibiotic Prescriptions	AI analyzes prescription data to identify regions with high rates of antibiotic misuse.	Public health campaigns can target high-risk areas to promote responsible antibiotic use.	(Sulis et al., 2023)
Monitoring Campaign Effectiveness	AI provides real-time feedback on the success of public health interventions.	Campaigns can be adjusted based on real-time data to maximize their impact on reducing antibiotic misuse.	(PRIORITY)
Global Resistance Surveillance	AI-driven systems monitor resistance trends across countries, integrating genomic and clinical data.	International organizations can coordinate more effective responses to global antibiotic resistance threats.	(Nastasijevic et al., 2023)

For example, by analyzing antibiotic prescription histories across a region, AI can identify specific areas where prescribing practices contribute to the emergence of resistant strains. This allows public health campaigns to target these regions, promoting 'wise prescribing' among healthcare providers and citizens. Additionally, AI can instantly assess the outcomes of these campaigns, providing feedback on whether the interventions are effectively reducing antibiotic misuse and slowing the emergence of resistance. At a global level, AI-based systems are utilized by the World Health Organization (WHO) and the Centers for Disease Control (CDC) to monitor resistance trends across countries. These systems analyze genomic, clinical, and epidemiological data to create a global picture of how resistance develops, facilitating international cooperation to slow its progression (Organization, 2002).

The Challenges of Implementing AI in Genomic Surveillance

Integrating AI into genomic surveillance has completely transformed monitoring AMR; this is not without real challenges, which include heterogeneous data, a biased AI algorithm, a lack of global data for collaboration, and ethical issues. Hence, these areas must bring forth solutions if AI's advantages are to go entirely into combating AMR.

1) Tackling Data Heterogeneity: Fusing sequenced genomic data from various sources, such as hospitals, research institutions, and environmental monitoring networks, is problematic due to variations in formats, types, and data quality (Alkhatib & Gaede, 2024). Data such as genomic sequencing, often in FASTQ or BAM files, prevents easy analysis due to its contrast with the unstructured nature of the clinical and environmental data. A successful example of an approach that addresses this issue is the Global Antimicrobial Resistance Surveillance System. By standardizing data collection and formats across 70 countries, GLASS has improved the quality of global AMR surveillance and response systems (Inau et al., 2023). Establishing international standards for genomic data formats and metadata annotation is critical for seamless interoperability. Implementing the FAIR (Findable, Accessible, Interoperable, and Reusable) principles is an essential step in making genomic data more accessible and usable for AI-driven detection of AMR. Tools like Next Flow and Galaxy may also help convert datasets to uniform formats for AI analysis, thereby augmenting global collaboration efforts.

2) Disparities in AI technology adoption across the world: The worldwide differences in India's implementation of AI cause multiple challenges. Protocols for transitioning between regions regarding sharing data are scattered and marred by privacy concerns, geopolitical issues, and resource limitations, thus making global cooperation impossible. However, the potential for real-time genomic data exchange, accelerating research and policy responses, has been demonstrated in countries such as the UK by the COVID-19 Genomics UK (COG-UK) Consortium (Struelens et al., 2024). The same success transferred from the viral genomics in GISAID can also be available for similar international protocols for AMR surveillance, which would provide secure, transparent data sharing. Finally, creating a global fund could allow resource-poor countries to upgrade their genomic infrastructure by building relevant laboratories. This would create a centralized, real-time repository of AMR data open to all. This would broaden access worldwide and allow collaborations to detect evolving resistance patterns more effectively against the coming AMR tide.

Future Innovations in AI-Powered Genomic Surveillance

Research has shown that artificial intelligence (AI) will continue to grow, making it a prospective tool in the next chapter of genomic surveillance. These AI technologies will provide timely monitoring, enable personalized medicine, and assist policymakers in addressing antimicrobial resistance (AMR). These innovations promise to revolutionize global health by offering predictive and preventive solutions against multidrug-resistant organisms (MDROs) and emerging threats.

1) Real-Time Global Genomic Monitoring Systems: Genomic monitoring systems driven by AI will gather and analyze real-time data collected in hospitals, research institutions, and public health organizations worldwide, thereby offering all-time views on AMR trends. With this predictive

capability, one can predict the advent and transmission of resistant strains so that suitable intervention measures to reduce the output may be made in time. There is also going to be extensive data on the severity of conditions, antimicrobial prescription rates, hospital admissions, and so on. Moreover, it can also integrate the aspect of agricultural antibiotics that might help in preventing possible transfer of resistance genes to humans and thereby control cross-species transmission (Zalewska et al., 2021).

2) Personalized Pharmacogenomics with AI: Human and pathogen genomes studied and analyzed by AI are expected to provide personalized pathogen therapy that targets antibiotics to a specific individual. Moreover, from an analysis of the bacterial genome, AI will predict the bacterial response to a particular antibiotic, thus eliminating the need for a broad-spectrum antibiotic application and reducing the risk of developing resistance. More importantly, AI will recommend combination therapy by identifying potential mutations that can induce resistance so that it can, even before the initiation of treatment, counterbalance the emergence of drug resistance and provide more effective and longer-lasting therapeutic options (de la Lastra et al., 2024).

3) Ethical and Policy Considerations in AI-Driven Surveillance: However, adopting AI for genomic surveillance raises important ethical and policy issues. Structural advantages make high-income countries pay off from AI innovations, which is expected to widen health disparities in the world even further. This can be addressed through establishing international funding mechanisms that ensure LMICs have access to AI technologies and can contribute to global surveillance systems. This is complemented by the need for data-sharing protocols that balance accessibility and privacy protection. Sensitive genomic data should be protected using privacy-preserving AI, such as federated learning and differential privacy, to ensure ethical, transparent, and equitable practices in surveillance efforts (Díaz-Rodríguez et al., 2023).

4) Real-Time Pathogen Monitoring and Early Warnings: The application of artificial intelligence in real-time monitoring of pathogens would therefore focus on continuously monitoring genetic changes constituting resistance. Such predictive algorithms would allow an early warning against any indications of resistance that these AI systems may come up with in health care, agricultural, and other environmental settings. Such systems will warn of every emerging resistance pattern, allowing public health authorities to intervene with preventive measures before the threat escalates into a crisis. For instance, cross-species transmission of resistant bacteria from livestock to humans could be flagged up by AI, which would trigger the necessary action to limit further spread (Wang, 2023).

5) Vision for AI in Global Health: AI-enhanced genomic surveillance promises to revolutionize global health through preemptive strategizing related to AMR. By improved real-time surveillance, personalized treatment regimens, and coordination on a worldwide level, AI will enable medical practitioners and policymakers to anticipate and respond to newly emerging resistant strains. It is with great assurance that AI technology will be fundamental in shaping better futures of global health systems prepared for the prevention and control of the AMR burden, thereby reducing the chances of future pandemics caused by resistant pathogens (Founou et al., 2021).

Summary

Antimicrobial resistance (AMR) is one of the major global threats to public health: it threatens to turn infections commonly treated, for example, by penicillin, into ones that are life-threatening. Artificial intelligence (AI) holds out great promise as a tool against AMR by improving the detection, prediction, and management of resistant bacteria. AI-based genomic surveillance systems will process vast amounts of genetic, clinical, and environmental data at real time in order to identify resistance genes quickly; prediction algorithms will make projections that prevent the dangers of current shifts in bacterial populations from spreading resistance; and personalized medicine would direct specific antibiotic treatments at specific strains and people. At a larger scale, AI will help get global health initiatives to monitor consumption patterns of antibiotics and support awareness campaigns. Only collective responses can engage AMR, such as international cooperation on equitable access to AI technologies and investment in infrastructure, especially in low- and middle-income countries. Therefore, collaboration between policymakers, researchers, and healthcare providers will now require capitalizing on AI's potential under one umbrella to make an effective and inclusive fight against AMR possible.

References

Alkhatib, R., & Gaede, K. I. (2024). Data management in biobanking: strategies, challenges, and future directions. *BioTech*, *13*(3), 34.

Bag, A. K., & Sengupta, D. (2024). Computational frameworks for zoonotic disease control in Society 5.0: opportunities, challenges and future research directions. *AI & SOCIETY*, 1-30.

de la Lastra, J. M. P., Wardell, S. J., Pal, T., de la Fuente-Nunez, C., & Pletzer, D. (2024). From Data to Decisions: Leveraging Artificial Intelligence and Machine Learning in Combating Antimicrobial Resistance–a Comprehensive Review. *Journal of medical systems*, *48*(1), 71.

Dhall, D., Kaur, R., & Juneja, M. (2020). Machine learning: a review of the algorithms and its applications. *Proceedings of ICRIC 2019: Recent innovations in computing*, 47-63.

Díaz-Rodríguez, N., Del Ser, J., Coeckelbergh, M., de Prado, M. L., Herrera-Viedma, E., & Herrera, F. (2023). Connecting the dots in trustworthy Artificial Intelligence: From AI principles, ethics, and key requirements to responsible AI systems and regulation. *Information Fusion*, *99*, 101896.

Duhaniuc, A., Păduraru, D., Nastase, E.-V., Trofin, F., Iancu, L.-S., Sima, C.-M., & Dorneanu, O.-S. (2024). Multidrug-Resistant Bacteria in Immunocompromised Patients. *Pharmaceuticals*, *17*(9), 1151.

Fong, I. (2023). Antimicrobial resistance: a crisis in the making. *New antimicrobials: for the present and the future*, 1-21.

Founou, L. L., Founou, R. C., & Essack, S. Y. (2021). Antimicrobial resistance in the farm-to-plate continuum: more than a food safety issue. *Future science OA*, *7*(5), FSO692.

Gupta, Y. D., & Bhandary, S. (2024). Artificial Intelligence for Understanding Mechanisms of Antimicrobial Resistance and Antimicrobial Discovery: A New Age Model for Translational Research. *Artificial Intelligence and Machine Learning in Drug Design and Development*, 117-156.

Inau, E. T., Sack, J., Waltemath, D., & Zeleke, A. A. (2023). Initiatives, concepts, and implementation practices of the findable, accessible, interoperable, and reusable data principles in health data stewardship: scoping review. *Journal of Medical Internet Research*, *25*, e45013.

Ladner, J. T., Grubaugh, N. D., Pybus, O. G., & Andersen, K. G. (2019). Precision epidemiology for infectious disease control. *Nature medicine*, *25*(2), 206-211.

Lau, H. J., Lim, C. H., Foo, S. C., & Tan, H. S. (2021). The role of artificial intelligence in the battle against antimicrobial-resistant bacteria. *Current genetics*, *67*(3), 421-429.

Martinez-Romero, M., Vazquez-Naya, J. M., R. Rabunal, J., Pita-Fernandez, S., Macenlle, R., Castro-Alvarino, J., Lopez-Roses, L., L. Ulla, J., V. Martinez-Calvo, A., & Vazquez, S. (2010). Artificial intelligence techniques for colorectal cancer drug metabolism: ontologies and complex networks. *Current drug metabolism*, *11*(4), 347-368.

Nastasijevic, I., Proscia, F., Jurica, K., & Veskovic-Moracanin, S. (2023). Tracking Antimicrobial Resistance Along the Meat Chain: One Health Context. *Food Reviews International*, 1-35.

Nguyen, T. A., & Nsonga Jr, S. (2024). Strategic Mitigation of Digital Rebound Effect in Organisations: A study from multiple stakeholder perspectives. In.

Organization, W. H. (2002). *Genomics and world health: Report of the Advisory Committee on Health Research*. World Health Organization.

Paneri, M., & Sevta, P. (2023). Overview of Antimicrobial Resistance: An Emerging Silent Pandemic. *Global Journal of Medical Pharmaceutical & Biomedical Update*, *18*.

PRIORITY, T. Progress Report: Implementation of A Public Health Action Plan To Combat Antimicrobial Resistance (Part I: Domestic Issues) .

Simpkin, V. L., Renwick, M. J., Kelly, R., & Mossialos, E. (2017). Incentivising innovation in antibiotic drug discovery and development: progress, challenges and next steps. *The Journal of antibiotics*, *70*(12) , 1087-1096.

Soni, U., & Gupta, N. (2021) . An Artificial Intelligence Approach for Forecasting Ebola Disease. Journal of Physics: Conference Series,

Struelens, M. J., Ludden, C., Werner, G., Sintchenko, V., Jokelainen, P., & Ip, M. (2024). Real-time genomic surveillance for enhanced control of infectious diseases and antimicrobial resistance. *Frontiers in Science*, *2*, 1298248.

Sulis, G., Sayood, S., & Gandra, S. (2023). How can we tackle the overuse of antibiotics in low- and middle-income countries? *Expert Review of Anti-infective Therapy*, *21*(11), 1 189-1201.

Tsitou, V.-M., Rallis, D., Tsekova, M., & Yanev, N. (2024). Microbiology in the era of artificial intelligence: transforming medical and pharmaceutical microbiology. *Biotechnology & Biotechnological Equipment*, *38*(1) , 2349587.

Vora, L. K., Gholap, A. D., Jetha, K., Thakur, R. R. S., Solanki, H. K., & Chavda, V. P. (2023). Artificial intelligence in pharmaceutical technology and drug delivery design. *Pharmaceutics*, *15*(7), 1916.

Wang, H. (2023). The Application of Artificial Intelligence in Health Care Resource Allocation Before and During the COVID-19 Pandemic: Scoping. *health policy*, *5*, 6.

Zalewska, M., Błażejewska, A., Czapko, A., & Popowska, M. (2021). Antibiotics and antibiotic resistance genes in animal manure–consequences of its application in agriculture. *Frontiers in microbiology*, *12*, 610656.

About the Authors

Dr. Zubia Rashid received her PhD in 2020 from the University of Karachi, Pakistan. She is serving as Manager of Laboratory/Officer-Quality and Safety at PureHealth, Abu Dhabi, United Arab Emirates. Her research interests include infectious diseases, biotechnology, antimicrobial resistance, virology, computational biology, and bioinformatics. She is a recognized people manager; has published twenty research articles in several international peer-reviewed journals in areas linking biotechnology, bioinformatics, microbiology, molecular biology, and pharmaceuticals.
Email: zubiar.ashid@purelab.com ORCID: 0000-0002-8753-8385

Ghosia Noreen is doing an MPhil in Zoology from Government College University, Lahore. Her research interest is understanding molecular mechanisms underlying gene regulation in animal diseases and development.
Email: ghosiyah.malik@gmail.com ORCID ID: 0009-0007-5155-7932

Dr. Humera Nazir completed her PhD in medical microbiology from Quaid-i-Azam University, Islamabad. She has been teaching for more than 7 years and also has research experience. Currently, she works as an Assistant Professor at Bahuddin Zakariya University, Multan. Her research interests are investigating mechanisms of drug resistance, analysing mutations, exploring the relationship between genetic mutations and phenotypic resistance, biofilm formation in the urinary tract environment, utilising both plant extract and nanoparticles to combat resistant pathogens, and the synergistic effect of it.
Email: humera.nazir@bzu.edu.pk ORCID: 0009-0002-2667-986X

Qais Mehmood, from the University of Veterinary and Animal Sciences, Lahore, focuses on integrating artificial intelligence in genomic surveillance, particularly in the context of the post-antibiotic era. Aim to develop innovative strategies to monitor and respond to antimicrobial resistance through advanced genomic analysis. By leveraging AI technologies, my work seeks to enhance our understanding of microbial behavior and resistance patterns.

E. mail: qmahmood.536@gmail.com ORCIC: 0009-0003-5750-2423

Prof. Dr. Mehmet OZASLAN received his PhD in 1995 Institute of Natural Sciences at Cukurova University, Turkiye. He is a Professor in Molecular Biology and Genetics. His research interests are included Cancer Genetics, Molecular Virology, Molecular Genetics, Microbiology, and Genetic mutations etc. He has published more than 200 research articles in national and international well reputed journals. He also has written many book chapters as well as edited books. He is an editor and editor in chief of many well reputed national and international journals.
E-mail: ozaslanmd@gantep.edu.tr, ORCID: 0000 0001 9380 4902

Similarity Index
The similarity index obtained from the plagiarism software for this book chapter is 7%.

To Cite This Chapter:

Rashid, Z. (2025). Decoding Resistance: AI-Powered Genomic Surveillance for a Post-Antibiotic Era. Current Studies in Life Sciences (pp.233-245). ISRES Book Series. ISRES Publishing.

Advances in Immunotherapy

Muhammad FAYYAZ
Khawar ALI
Hamna TARIQ
Mehmet OZASLAN

Introduction

Immunotherapy has perceived remarkable advancements in recent years, positioning itself as a pivotal strategy in the treatment of various cancers. One of the most compelling developments is the introduction of combination therapies that synergize traditional treatments with immunotherapeutic strategies to enhance efficacy while minimizing adverse effects. For instance, the combination of novel intravesical xenogeneic urothelial cell immunotherapy and chemotherapy has shown promise, particularly in preclinical models of bladder cancer, where it increases anti-tumor efficacy through a localized delivery system that mitigates systemic exposure and potential immune reactions (Huang et al., 2021). This localized approach is especially crucial in managing non-muscle-invasive bladder cancer (NMIBC), where ongoing trials are exploring novel therapeutic modalities to improve treatment outcomes and reduce toxicity. Furthermore, the integration of immune checkpoint inhibitors such as PD-1/PD-L1 and CTLA-4 inhibitors into treatment regimens has provided new avenues for cancer therapy. These agents have demonstrated efficacy across various malignancies, including lung cancer, where their application alongside chemotherapy has led to improved patient outcomes in some subsets of patients. The clinical success of immune checkpoint inhibitors has inspired further investigative efforts into identifying biomarkers that could predict treatment responses, thereby enhancing personalized therapy approaches in immuno-oncology. The emergence of bispecific antibodies represents another significant advancement in cancer immunotherapy. These agents, designed to simultaneously bind two distinct targets, are particularly beneficial in treating hematological malignancies such as multiple myeloma. The efficacy of bispecific T-cell engagers has been noted in the treatment of acute lymphoblastic leukemia, where they can redirect T cell activity towards cancer cells, highlighting their potential as robust therapeutic agents. Nevertheless, the challenge remains to manage resistance mechanisms that limit the effectiveness of these therapies, necessitating further research into combinatorial strategies and novel drug development. The use of patient-derived organoids and advanced preclinical models such as humanized mice has revolutionized the landscape of immunotherapy research. These models are instrumental in evaluating treatment responses and conducting translational studies that bridge the gap between laboratory discoveries and clinical applications (Chuprin et al., 2023). Moreover, ongoing investigations into the use of traditional agents from Chinese medicine have opened new potential pathways for enhancing immune responses against tumors, demonstrating that integrating traditional and modern medical practices can yield innovative therapeutics. Recent advances in immunotherapy are characterized by novel therapeutic modalities, the integration of combination strategies, and the development of refined models for preclinical research. As the field evolves, addressing challenges such as

treatment resistance and optimizing patient selection through the identification of predictive biomarkers will be essential in maximizing the benefits of immunotherapy for cancer patients.

Checkpoint Inhibitors Beyond PD-1/PD-L1

Immunotherapy, particularly with immune checkpoint inhibitors (ICIs), has revolutionized cancer treatments, especially with the development of PD-1 and PD-L1 inhibitors. However, resistance mechanisms limiting their efficacy and the quest for alternative targets have become focal points in ongoing research. Among the notable emerging checkpoint inhibitors beyond PD-1/PD-L1 is the cytotoxic T lymphocyte-associated protein 4 (CTLA-4), which has been successfully targeted by drugs such as ipilimumab. Studies have demonstrated that CTLA-4 blockade invigorates T cell activity, enhancing anti-tumor responses across various cancers. Studies indicated that additional checkpoints, such as LAG-3 (lymphocyte-activation gene 3) and TIM-3 (T cell immunoglobulin and mucin domain 3), are crucial targets in overcoming immune evasion in tumor microenvironments resistant to PD-1/PD-L1 inhibitors. Emerging findings suggest that targeting TIM-3 could restore T cell activation in tumors where PD-1 inhibition alone proves insufficient. Additionally, novel combination approaches utilizing these antagonists alongside standard therapies are being explored to enhance overall efficacy. Another crucial area of focus is understanding the mechanisms of acquired resistance to ICIs. Evidence suggests that tumors may adapt through various pathways, such as upregulating immunosuppressive ligands or enacting changes in T cell activity. For example, loss of PHF8 has been linked to a viral mimicry response, revealing a novel mechanism that cancer cells exploit for immune evasion (Liu et al., 2023). Furthermore, regulatory T cells (Tregs) can actively suppress anti-tumor responses, underscoring the need for strategies that target Treg populations or their inhibitory effects (Suzuki et al., 2024). Recent findings have identified distinctive immune profiles and modulators that influence treatment responses. The expression of VISTA, a member of the B7 family of immune checkpoints, has been observed to modulate T cell activity and is being investigated as a promising target in solid tumors. Moreover, the interplay between tumor-infiltrating lymphocytes (TILs) and the tumor microenvironment is critical for determining outcomes; research indicates that a higher presence of tumor-associated macrophages (TAMs) may correlate with reduced efficacy of immunotherapies. As the landscape of cancer immunotherapy evolves, ongoing studies focus on a variety of targets, including novel bispecific antibodies and combination therapies that aim to break through existing resistance mechanisms. Understanding the complex interactions within the immune microenvironment will prove essential to refining and optimizing immunotherapeutic strategies. PD-1 and PD-L1 inhibitors remain at the forefront of cancer immunotherapy, emerging targets such as CTLA-4, LAG-3, and TIM-3, alongside an improved understanding of resistance mechanisms, are pivotal in developing next-generation immunotherapies. The ongoing interplay between tumor biology, immune modulation, and clinical responses will shape the future of immuno-oncology.

Next-Generation CAR-T and CAR-NK Therapies

The field of cancer immunotherapy has evolved significantly with the introduction of Chimeric Antigen Receptor (CAR) therapies, notably CAR-T and CAR-NK (Natural Killer) cell therapies. These approaches harness the body's immune system to target and eliminate cancer

cells more effectively. This response outlines the engineering advancements and clinical impacts of next-generation CAR-T and CAR-NK therapies, highlighting their potential, challenges, and emerging targets. The engineering of CAR-T and CAR-NK cells has focused on enhancing their targeting efficacy and safety profiles. CAR-NK cells have the advantages of innate antitumor activity without the need for prior sensitization to tumor antigens, making them compelling candidates for cancer immunotherapy. Recent studies have indicated that combining CAR-T with CAR-NK cells can exploit the strengths of both cell types, such as the precise antigen targeting and robust proliferation of CAR-T cells and the innate immune response capabilities of CAR-NK cells. Moreover, innovations like the integration of CD38 CAR into NK cells have been shown to effectively target different cancer types, including T-cell Acute Lymphoblastic Leukemia (ALL), reflecting the adaptability of CAR design to specific malignancies. Further advancements include the development of CAR constructs that enhance the persistence and proliferation of NK cells. For example, the inclusion of the IL-15/IL-15Rα complex has been shown to significantly improve the anti-tumor efficacy of CAR-NK cells, expanding their potential application in solid tumors. In contrast, the use of genetic engineering in NK cells allows for the creation of "off-the-shelf" therapies, which can be pre-manufactured from healthy donor cells, thereby reducing the time and costs associated with individual patient therapies. The clinical impact of CAR therapies has been promising, particularly in hematological malignancies. CAR-T therapies targeting CD19, for instance, have demonstrated significant efficacy in treating B-cell malignancies, with real-world applications solidifying their role as a frontline treatment option (Testa et al., 2025). However, CAR-T therapies are associated with severe adverse effects such as cytokine release syndrome (CRS) and neurotoxicity, which limit their broader applicability in solid tumors. The development of CAR-NK cells seeks to mitigate these risks, as they have shown a favorable safety profile and reduced incidence of these severe toxicities while retaining strong anti-tumor activity. Moreover, CAR-NK cells are being explored for advanced solid tumors, including triple-negative breast cancer and glioblastoma. Studies reveal their ability to effectively engage and eliminate cancer cells that express specific tumor antigens, such as epidermal growth factor (EGFR) and tissue factor. As research progresses, dual targeting of tumors using CAR-NK cells has shown to overcome the heterogeneity of antigen expression often found in cancers, thereby increasing treatment efficacy. Despite their potential, both CAR-T and CAR-NK cell therapies face significant challenges. The complexity of tumor microenvironments often contributes to therapeutic resistance, necessitating innovative strategies to enhance in vivo efficacy. Emerging research is focused on the integration of additional immune modulators or checkpoint inhibitors with CAR therapies to enhance their overall anti-tumor responses. Another critical area of development is the fine-tuning of CARs, where the affinity and specificity of the engineered receptors are optimized to ensure targeted killing of tumor cells while sparing healthy tissues. The field of CAR-T and CAR-NK cell therapies is evolving rapidly, marked by significant engineering advancements and promising clinical outcomes. Ongoing research is expected to refine these therapies further, addressing their limitations and exploring new targets and combinations that could lead to broader applications in cancer treatment.

Bispecific Antibodies and T-cell Engagers (BiTEs)

Bispecific antibodies and T-cell engagers (BiTEs) represent a significant advancement in cancer immunotherapy, characterized by their unique ability to target two distinct antigens simultaneously. This innovative approach enables more precise targeting of tumor cells while engaging T cells or other immune effectors, facilitating effective anti-tumor immune responses. The emergence of bispecific antibodies, particularly in the treatment of malignancies, has attracted considerable interest in both clinical and research settings. Bispecific antibodies, including BiTEs, are designed to bind concurrently to a T-cell receptor, typically CD3, and a specific tumor-associated antigen, such as HER2 or CD19. This dual-binding capability allows T cells to recognize and directly attack cancer cells, thereby redirecting the immune response against tumors. Blinatumomab, a BiTE targeting CD19 and CD3, has demonstrated notable efficacy in treating relapsed or refractory acute lymphoblastic leukemia (ALL). Similarly, BiTEs targeting p95HER2 have shown promise in the treatment of HER2-positive breast cancer. Emerging designs of bispecific antibodies have focused on enhancing binding affinities and increasing tumor infiltration. Research indicates that the affinities of the targeting arms significantly influence therapeutic efficacy and safety, with specific bispecific formats exhibiting robust tumor-killing capabilities while minimizing off-target effects. This advancement is crucial for refining therapeutic indices and addressing resistance mechanisms associated with various cancers. Figure 1 indicates the bispecific antibodies and BiTE-based therapies enable simultaneous engagement of tumor-associated antigens and immune effector cells, offering improved specificity and reduced collateral damage compared to conventional monoclonal antibody treatments.

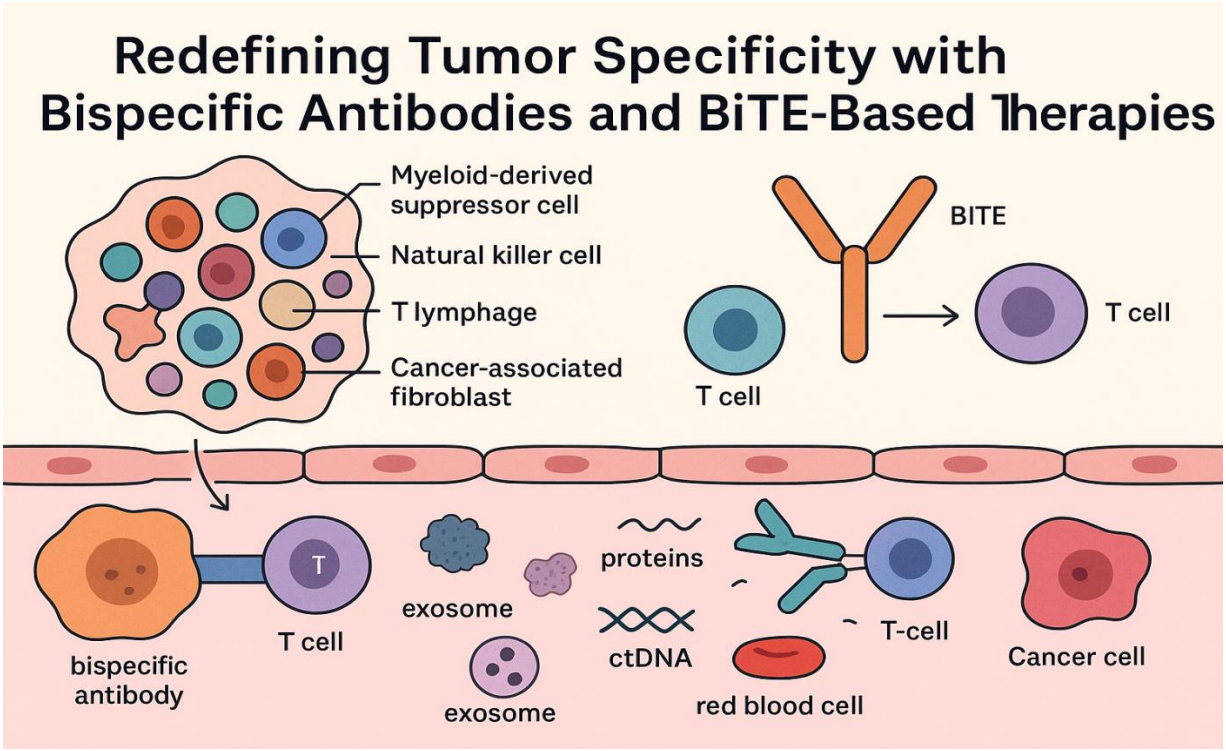


Figure 1. Redefining Tumor Specificity with Bispecific Antibodies and BiTE-Based Therapies

The therapeutic application of bispecific antibodies has expanded across various malignancies, including hematological cancers and solid tumors. Several bispecific antibodies are currently under clinical evaluation, demonstrating efficacy in conditions such as melanoma and various hematological malignancies. The use of bispecific antibodies in multiple myeloma has shown positive therapeutic outcomes, particularly due to their ability to redirect T cells toward myeloma cells expressing BCMA (B-cell maturation antigen). The recent progress of bispecific approaches has prompted further investigation into combination therapies that leverage the synergistic effects of bispecific antibodies alongside other immunomodulatory treatments, including checkpoint inhibitors. This strategy aims to enhance therapeutic efficacy while addressing immune evasion tactics employed by tumors, highlighting the versatility of bispecific antibodies in modern cancer therapy. Despite these advances, several challenges remain in the clinical implementation of bispecific antibodies. The potential for immune-related adverse effects is a significant concern, particularly regarding the non-specific activation of the immune system (Markouli et al., 2023). Additionally, the complexity inherent in the development and manufacturing of bispecific antibodies presents logistical hurdles that must be navigated to ensure consistency in production and delivery. Resistance mechanisms that may diminish the long-term efficacy of bispecific therapies are also a central concern. Ongoing research to understand these resistance pathways is essential for improving the therapeutic design of bispecific antibodies and for ensuring sustained clinical benefits. Identifying co-stimulatory pathways involved in T-cell activation could optimize bispecific antibody design to bypass these resistance mechanisms and enhance patient outcomes. The future of bispecific antibodies and T-cell engagers in oncology is promising, with ongoing research focused on innovative antibody formats, such as dual-variable domain antibodies, which offer enhanced specificity by targeting multiple tumor antigens simultaneously. Increased investment in preclinical and clinical trials is expected to reveal new therapeutic options and establish efficacy benchmarks across diverse cancer types. The bispecific antibodies and T-cell engagers signify a paradigm shift in targeted cancer immunotherapy. By more effectively harnessing the immune system through dual-targeting strategies, these therapies are redefining tumor specificity and providing new hope for patients facing malignancies. Continued innovation and research will be critical to overcoming current challenges and fully realizing the potential of these therapeutic modalities.

Tumor Microenvironment Modulation

The tumor microenvironment (TME) plays a critical role in shaping the immune response to cancer, often leading to immunosuppression and promoting tumor progression. Targeting immunosuppression and stromal barriers within the TME has emerged as a promising strategy to enhance anti-tumor immunity. This response synthesizes recent findings on the key components of the TME, their interactions, and therapeutic strategies aimed at reprogramming this environment to favor anti-tumor responses. Many malignancies, including pancreatic cancer and glioblastoma, are characterized by a highly immunosuppressive TME primarily driven by myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs). These cells inhibit normal immune function and promote tumor growth by secreting immunosuppressive cytokines such as IL-10 and TGF-β, which inhibit T cell activation and promote regulatory T cell (Treg) expansion (Kemp et al., 2021). For instance, in pancreatic cancer, MDSCs have been

shown to mediate immune suppression through adenosine production, significantly compromising the efficacy of immunotherapies. Furthermore, studies indicate that the presence of immunosuppressive layers, such as the extracellular matrix (ECM), can physically hinder the infiltration of T cells into tumors. Specifically, stiffened ECM conditions limit T cell migration and function, with strategies to modulate ECM components like hyaluronic acid proposed to enhance T cell access and promote TAM reprogramming to a more pro-inflammatory M1 phenotype.

Therapeutic Approaches to Modulate the Tumor Microenvironment

Transitional Reprogramming of Myeloid Cells: Targeting the signaling pathways involved in MDSC and TAM activation holds promise for altering the TME. For instance, CSF1/CSF1R blockade has shown the potential to reprogram tumor-infiltrating macrophages, enhancing therapeutic responses to T-cell checkpoint inhibitors. Similarly, agents targeting the IL-6 signaling pathway effectively disrupt the immunosuppressive functions of MDSCs and restore T cell activity.

Angiogenesis and Vascular Normalization: Vascular normalization via antiangiogenic therapies has been noted to reprogram the immunosuppressive TME, improving the efficacy of concurrent immunotherapies. Antiangiogenic treatments aim to remodel the abnormal tumor vasculature, which can reduce hypoxia and enhance the infiltration and activation of T cells within the tumor.

Nanoparticle-Based Approaches: Innovative nanomedicine strategies are being developed to target and modify the TME directly. For example, the use of mannose and hyaluronic acid dual-modified nanoparticles has enhanced T cell responses by polarizing macrophages and transforming "cold" tumors into "hot" tumors that are more responsive to immunotherapy. Nanoparticles can deliver specific drugs or nucleic acids directly to target myeloid cells, thereby modulating their immunosuppressive activity.

Exosomal RNA Delivery: The targeting of tumor-derived exosomal circRNAs has been identified as a crucial regulatory mechanism in mediating immunosuppression. By understanding and manipulating the molecular signals within the exosomes, researchers aim to develop therapeutics that restore immune function against tumors.

Targeting Immunosuppressive Molecules: The deployment of therapeutic agents that inhibit key immunosuppressive molecules, such as CD73 and TGF-β, is another promising approach. Inhibition of the CD73 enzyme, which generates adenosine, can disrupt the immunosuppressive "halo" surrounding tumors, thus promoting a more active immune response. Research into TGF-β siRNA delivery through nanoparticles exemplifies the development of strategies aimed at enhancing anti-tumor immunity. The modulation of the tumor microenvironment presents a multifaceted challenge and opportunity in cancer therapy. By targeting the immunosuppressive components of the TME and disrupting stromal barriers, novel therapeutic strategies can improve the overall efficacy of cancer treatments. Ongoing research is essential to identify effective interventions that not only enhance T cell immunogenicity but also create a supportive microenvironment conducive to sustained anti-tumor immunity.

Neoantigen-Based Personalized Cancer Vaccines

The advent of neoantigen-based personalized cancer vaccines represents a significant breakthrough in oncology, aiming to tailor therapeutic approaches to individual tumor genotypes and enhance patient outcomes through precise immune activation. Neoantigens, which are unique peptides derived from tumor-specific mutations, offer novel targets for immunotherapy, harnessing the body's immune system to recognize and destroy malignant cells. Despite considerable progress over recent years, significant challenges remain that must be addressed to optimize the efficacy and clinical translation of these personalized vaccines. Studies has demonstrated that tumor mutation burden (TMB) plays a critical role in the efficacy of neoantigen-based vaccines, as tumors with higher mutation rates are typically linked to a greater number of neoantigens, thereby enhancing the likelihood of triggering a robust immune response. However, there is emerging evidence suggesting that even tumors characterized by low mutation loads may respond favorably to neoantigen immunotherapy. This insight is crucial as many cancers, especially certain subtypes, exhibit relatively low TMB, raising questions about the universality of neoantigen therapy's effectiveness. Personalized neoantigen vaccines are primarily developed through the identification of tumor-specific mutations. Techniques such as whole-exome sequencing are utilized to pinpoint mutations, which are then screened for their potential to induce immune responses. Following identification, computational algorithms assess the predicted binding affinity of these neoantigens to patient-specific major histocompatibility complex (MHC) molecules, ensuring an effective triggering of T cell-mediated immunity (Huff et al., 2023). Strategies aimed at optimizing the selection of ideal neoantigens are fundamental, as the immunogenicity of these antigens varies significantly, influenced by factors such as mutation type, expression levels, and the tumor microenvironment. Clinical trials have underscored the promise of personalized neoantigen vaccines in various cancers including melanoma, non-small cell lung cancer (NSCLC), and colorectal cancer. Efficacy results have shown that personalized neoantigen vaccines can significantly augment T cell responses, improve progression-free survival, and reduce tumor burden when combined with checkpoint inhibitors like anti-PD-1 therapies. Moreover, neoantigen vaccines have been associated with reduced toxicity profiles compared to conventional therapies, thus presenting a safer therapeutic option. For instance, a study showed that patients receiving neoantigen-pulsed dendritic cell vaccines experienced favorable immune responses measured by an increase in neoantigen-specific T cells with minimal adverse effects. The efficacy of personalized cancer vaccines is encouraging; several hurdles complicate their clinical implementation. The integration of complex logistical processes from tumor biopsies to vaccine production and administration can result in delays that may adversely affect treatment outcomes, leading to the consideration of "off-the-shelf" therapies as a pragmatic alternative. Additionally, some trials have reported challenges related to immune evasion tactics employed by tumors, which can undermine the effectiveness of neoantigen-targeted therapies, calling for innovative strategies to counteract these adaptive mechanisms. The burgeoning scope of neoantigen vaccine development is also represented by advances in delivery systems and adjuvants that enhance their immunogenicity. The combination of neoantigens with novel adjuvants, such as TLR (Toll-like receptor) agonists, has demonstrated potential in boosting immune responses against cancer. For example, studies show that the use of combinatorial therapies, incorporating neoantigen vaccines with systemic treatments, leads to enhanced

immunogenicity and therapeutic efficacy, suggesting that multi-faceted approaches may be essential for overcoming current limitations in vaccine efficacy across diverse tumor types (Niemi et al., 2022). The durability of immune responses induced by personalized neoantigen vaccines is another critical consideration. Research indicates that while initial T cell responses can be robust, maintaining these responses over time poses a challenge, often requiring additional vaccine booster doses or combinatorial approaches with other immunotherapies to elicit sustained antitumor activity. Monitoring T cell dynamics post-vaccination remains essential for optimizing treatment strategies and addressing potential relapse or disease progression. While the field of neoantigen-based personalized cancer vaccines has made remarkable strides, continued research and collaborative efforts are necessary to tackle the myriads of challenges associated with their development and clinical application. The integration of advanced biotechnological methodologies, comprehensive biomarker analyses, and the strategic pairing of vaccines with established and novel immunotherapies will enhance the therapeutic landscape for patients with cancer. The ongoing refinement of personalized approaches holds the promise of not only improving the specificity and efficacy of cancer treatments but also fostering more adaptive and resilient therapeutic regimens that can evolve alongside the disease.

Microbiome-Driven Immunotherapy Response

The interplay between the gut microbiome and the response to cancer immunotherapy has emerged as a significant area of research, driven by accumulating evidence suggesting that the microbial composition within the gastrointestinal tract affects therapeutic outcomes in various malignancies. This influence is particularly notable in the context of immune checkpoint inhibitors (ICIs), where the microbiome may modulate the host's immune responses, ultimately impacting the effectiveness and safety of cancer treatments. Recent studies have highlighted how the gut microbiome confers individual variability in response to immunotherapy, particularly in melanoma and colorectal cancer. Peters et al. demonstrated that specific microbial profiles could be associated with favorable responses to immunotherapy in melanoma patients, indicating that a diverse and enriched gut microbiota correlates with enhanced therapeutic efficacy. In a study, the researchers connected the gut metagenome and meta-transcriptome to the patients' clinical responses, reinforcing the concept that the microbiome is a vital player in influencing immune responses during cancer treatment. There was association in analyzing the gut microbiome's role in patients with colorectal cancer and noting distinct variations in microbiome composition between responders and non-responders to immunotherapy. These findings support the hypothesis that dysbiosis an imbalance in microbial communities can lead to adverse outcomes and diminished efficacy during cancer treatment. Other analyses, like those conducted by Cheng et al., also reported on the clinical implications of gut microbiome composition, linking certain bacterial taxa with successful clinical responses. The mechanisms by which the gut microbiome influences immunotherapy effectiveness likely involve complex interactions between immune modulation and microbial metabolites. Studies have indicated that certain beneficial bacteria may enhance the host's immune response against tumors by promoting the activation of cytotoxic T cells and the production of specific cytokines, thereby potentiating the immunotherapeutic effect. Xu et al (2020) specifically pointed to metabolic pathways within the gut microbiome that could provide insights into how immunotherapy affects microsatellite-stable (MSS) colorectal cancer,

showcasing microbial interactions that potentially enhance the efficacy of PD-1 antibodies. This indicates a sophisticated biochemical terrain in which microbial constituents actively participate in influencing the immune landscape. Moreover, the relationship between gut microbiota diversity and ICI outcomes points toward significant potential for personalized medicine. Numerous studies have documented that higher microbial diversity is associated with better immunotherapy outcomes, suggesting that assessing the microbiome could serve as an adjunct strategy for predicting therapy responses. These observations pave the way for future clinical strategies that might include microbiome profiling as part of the standard approach to immunotherapy. The emerging therapeutic implications of manipulating the gut microbiome to improve immunotherapy efficacy cannot be overstated. Utilizing fecal microbiota transplantation (FMT) shows promise for restoring beneficial microbiota in patients who exhibit resistance to immunotherapy. As noted by Mao et al., FMT from donors with favorable clinical responses to immunotherapy in melanoma led to enhanced CD8+ T cell activation in recipients who previously showed resistance. Such findings underscore the potential for microbiome modulation as a therapeutic strategy, particularly in enhancing the effectiveness of existing immunotherapeutic approaches. In assessing biomarkers for predicting immunotherapy outcomes, the gut microbiome represents a compelling target. As highlighted by Gao et al., there is strong potential for integrating microbiome analysis including both profiling and metabolic assessments with established immune biomarker evaluations to create a multifaceted predictive model for immunotherapeutic efficacy (Gao et al., 2024). Furthermore, dietary interventions aimed at optimizing the gut microbiome may serve dual purposes in preventing disease and enhancing treatment response, as suggested by research linking plant-based diets with beneficial microbiome profiles that favor positive immunotherapeutic responses. Despite the promise that the gut microbiome holds as a predictive biomarker and therapeutic target, challenges remain in translating these findings into routine clinical practice. The heterogeneity of patient populations, variations in microbial resistance to antibiotics, and differing methodologies in microbiome analysis contribute to the complexity of establishing standardized protocols. Studies suggest that specific strains of gut bacteria correlate with enhanced response to therapy, though these associations vary widely across studies. Establishing clear, reproducible relationships between specific microbiota and clinical outcomes will be crucial for developing tailored interventions and comprehensive treatment schemas. The integration of gut microbiome analysis into cancer immunotherapy strategies represents a transformative approach that holds potential for enhancing patient outcomes. As clinical studies continue to explore the intricate relationships between microbiota and immunotherapy responses, the quest to identify potent microbial biomarkers and develop innovative microbiome-modulating therapies will likely shape the future landscape of cancer treatment. Ultimately, optimizing the gut microbiome could serve as an adjunct pathway to improving the immune response against diverse cancers and reducing the occurrence of treatment-related adverse effects, heralding a new era in personalized cancer care.

Liquid Biopsies and Circulating Tumor DNA

Liquid biopsies, particularly those involving circulating tumor DNA (ctDNA), have revolutionized the ability to monitor and predict responses to cancer immunotherapy. ctDNA offers a minimally invasive approach to assess dynamic changes in a patient's tumor burden and

genetic profile, making it a valuable tool in the management of various malignancies treated with immune-therapeutics. The predictive value of ctDNA as a biomarker in the context of immunotherapy has been effectively demonstrated in several studies. For instance, it was revealed that in melanoma patients receiving combined CTLA-4 and PD-1 antibody therapy, baseline ctDNA levels correlated with treatment response (Forschner et al., 2019). Patients without detectable ctDNA at baseline, or those whose ctDNA became undetectable within 12 weeks, were significantly more likely to respond favorably to immunotherapy. This study underscores the potential of ctDNA to serve as an early indicator of clinical benefit, complementing traditional metrics like performance status. Similarly, the role of ctDNA dynamics in predicting outcomes for patients with operable non-small cell lung cancer (NSCLC) undergoing neoadjuvant immunotherapy and the research demonstrated that changes in ctDNA levels could predict recurrence-free survival, suggesting that ctDNA dynamics closely correlate with pathological responses to treatment. This suggests that ctDNA could potentially be utilized to tailor therapy based on individual responses, paving the way for more personalized treatment strategies. As demonstrated in Figure 2, ctDNA analysis via liquid biopsies offers a minimally invasive approach to track tumor dynamics, evaluate immunotherapy response, and guide personalized treatment strategies (Souza et al., 2023).

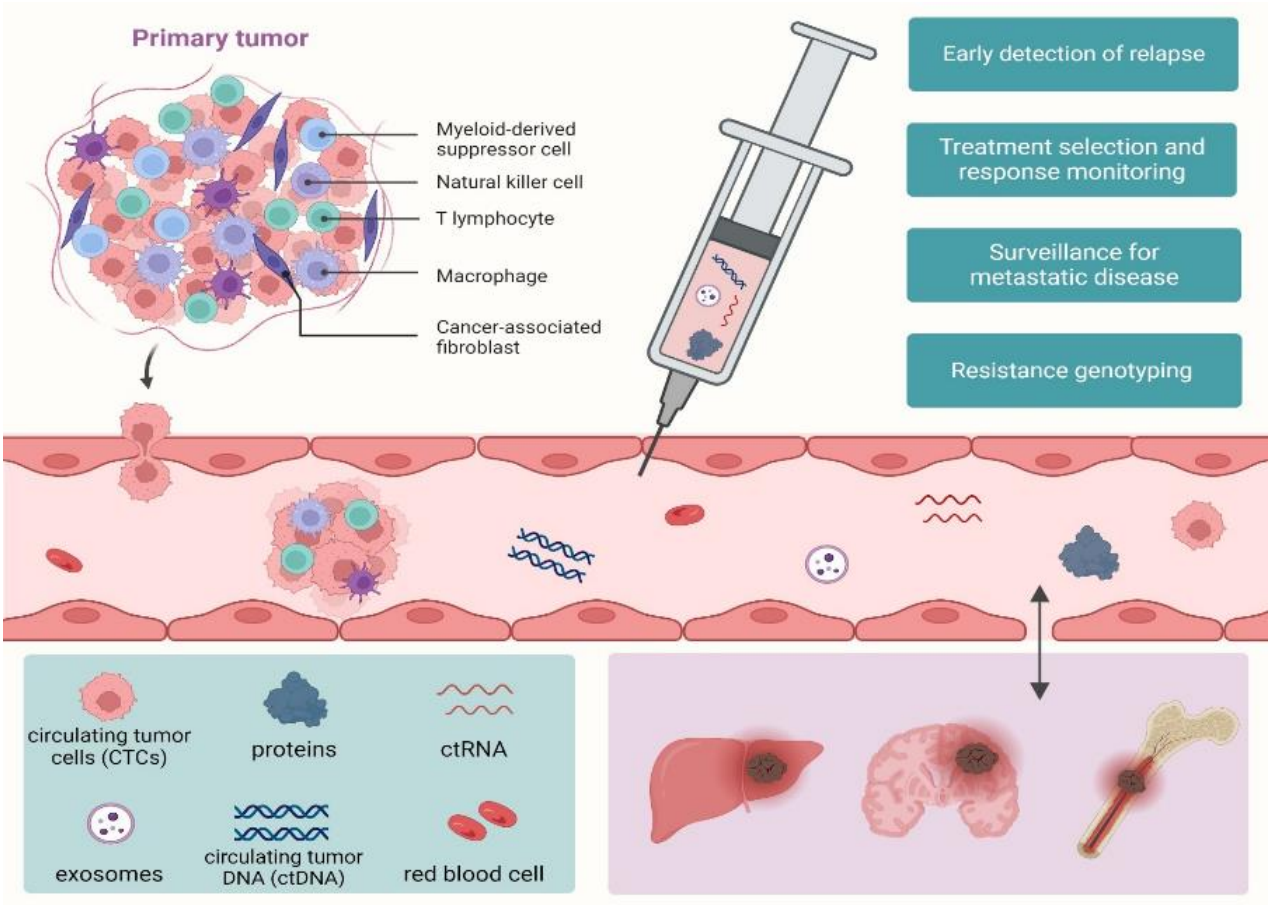


Figure 2. ctDNA and Liquid Biopsies: Precision Tools for Tracking Immunotherapy Efficacy (Souza et al., 2023)

In the realm of lung cancer, Goldberg et al. emphasized the utility of ctDNA analyses for assessing immunotherapy responses at an early stage, indicating its applicability as an effective biomarker for real-time monitoring. They further argued that tracking ctDNA levels could enable oncologists to adjust treatment approaches proactively based on the observed molecular shifts in ctDNA, achieving a more refined treatment course. Emerging data from others, including Cabel et al., reveal that ctDNA can be used to monitor changes during PD-1 inhibitor therapy, offering insights into minimal residual disease (MRD) and the potential for relapse. Continuous tracking of ctDNA could provide timely information on disease status that standard imaging assessments may not capture, indicating whether tumors are responding or developing resistance to treatment. A meta-analysis supports the increasing reliance on liquid biopsy techniques, noting that ctDNA is effective in monitoring treatment responses in advanced melanoma and could guide decisions on the continuation or modification of therapeutic regimens. This adaptation is critical, as cancer treatment responses can vary greatly among individuals. The clinical implications of utilizing ctDNA extend beyond just monitoring treatment efficacy; its integration into clinical practice could transform how treatment responses are evaluated in real time, enhancing the concept of personalized medicine. Notably, recent studies have shown consistent results indicating that patients with undetectable ctDNA levels often correlate with improved survival outcomes. However, despite these promising advances, challenges in standardization and validation of ctDNA assays persist, as highlighted by Kurtz, who pointed out that without properly validated methods, the clinical utility of ctDNA may be hampered. Furthermore, integrating ctDNA analysis into existing clinical practice requires overcoming obstacles such as regulatory approval, logistical considerations of sample handling, and ensuring that ctDNA assays remain cost-effective. As research advances and technologies refine the sensitivity and specificity of ctDNA detection, the role of liquid biopsies in predicting and monitoring responses to immunotherapy is likely to expand. The evidence suggests a pivotal shift toward integrating ctDNA analysis into routine clinical practice, providing valuable insights that could significantly enhance patient outcomes through timely and personalized interventions.

Combining Radiotherapy with Immune Checkpoint Blockade

Combining radiotherapy with immune checkpoint blockade (ICB) has emerged as a promising strategy to enhance cancer treatment efficacy, primarily through the activation of immune responses that can lead to systemic antitumor effects, including the elusive abscopal effect. This effect, characterized by tumor regression in non-irradiated areas, has garnered significant attention for its potential to improve overall patient outcomes when integrating these modalities. The abscopal effect has been documented in various cancer types following the administration of localized radiation therapy alongside ICB, such as anti-PD-1 or anti-CTLA-4 therapies. For instance, studies have shown that the administration sequence of PD-1 inhibitors relative to local tumor irradiation significantly influences the induction of abscopal responses. This point highlights the critical importance of treatment sequencing, where optimal timing can enhance the immunogenicity of the irradiated tumors and promote systemic immunity against distant metastatic lesions. Chiu et al. explored innovative approaches by combining boron neutron capture therapy with PD-1 blockade, illustrating a synergistic enhancement in immune responses. This combination strategy exemplifies how advanced therapeutic modalities can be tailored to

harness the immune system's power in conjunction with radiotherapy. Recent clinical findings support the rationale for combining radiotherapy with immune checkpoint inhibitors. For instance, Britschgi et al. documented an abscopal effect in a patient with metastatic non-small cell lung cancer (NSCLC) after stereotactic body radiotherapy (SBRT) in conjunction with nivolumab. These results indicate that the immunologic stimulation induced by radiation can overcome barriers posed by the tumor microenvironment, facilitating both local and systemic immune responses. As depicted in Figure 3, liquid biopsies combined with ctDNA profiling enable dynamic monitoring of tumor evolution during immunotherapy, facilitating timely treatment modifications and improved patient outcomes (Shannon et al., 2023).

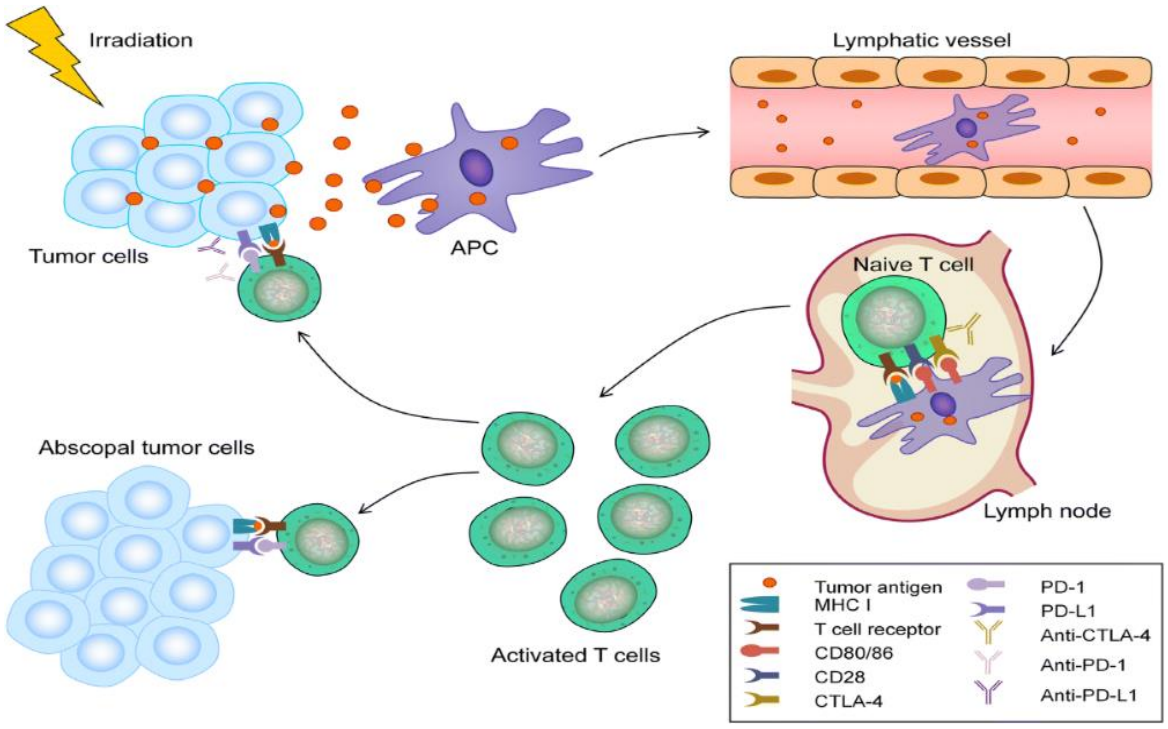


Figure 3. Monitoring Immunotherapy Response through Liquid Biopsies and Circulating Tumor DNA Analysis (Shannon et al., 2023)

Further, the timing of ICB introduction post-irradiation plays a pivotal role in determining treatment success. Precise sequencing could either enhance or diminish the potential for eliciting an abscopal effect, thus necessitating strategic planning in clinical settings. This complexity underscores the need for a comprehensive understanding of biological interactions between radiation and immune responses. The mechanisms underlying combined therapies involve various biological interactions. For example, radiotherapy can lead to the release of tumor antigens and the activation of dendritic cells, which in turn prime T cells to target not only the irradiated tumors but also metastatic lesions. This immunogenic cell death induced by radiation is crucial for triggering robust adaptive immune responses. Moreover, studies indicate that radiation can alter the tumor microenvironment by promoting the infiltration of immune effector cells and enhancing the expression of immune checkpoint molecules. These changes can improve tumor recognition and increase the effectiveness of immune checkpoint inhibitors, creating a constructive feedback loop between radiotherapy and immunotherapy. In addition to abscopal effects, the clinical

application of radiotherapy in combination with immunotherapy is also being explored in specific tumor settings, such as in hepatocellular carcinoma. Lee et al. noted that the integration of ICB with local irradiation strategies can shift treatment paradigms and yield improved patient prognoses. Such advancements advocate for clinical trials evaluating combination strategies to assess their effectiveness across various cancers. Nevertheless, while the combination of radiotherapy with immune checkpoint inhibition offers great promise, challenges remain. The optimization of radiation dose, fractionation schedules, and the management of potential adverse effects are critical for maximizing treatment efficacy and minimizing toxicity (Buchwald et al., 2018). Additionally, ongoing research is necessary to identify reliable biomarkers that could predict which patients are most likely to benefit from these combination therapies. The integration of radiotherapy with immune checkpoint blockade represents a significant leap forward in cancer treatment, providing a dual approach that leverages the immune system alongside conventional cancer therapies. The potential for achieving abscopal effects and enhancing systemic antitumor immunity through this combination holds the promise for more effective and durable responses in cancer patients. Continued research into the precise mechanisms and optimal dosing strategies will be essential for the successful translation of these findings into standardized clinical practice.

Adjuvant and Neoadjuvant Immunotherapy in Solid Tumors

The roles of neoadjuvant and adjuvant immunotherapy in treating solid tumors have garnered attention for their potential to enhance therapeutic outcomes and improve survival rates. Neoadjuvant immunotherapy refers to the administration of immune-modulating treatments prior to surgical resection, aimed at shrinking tumors and reducing the overall burden of cancer, while adjuvant therapy is applied post-surgery to eliminate residual disease and prevent recurrence. Both strategies leverage the immune system's ability to target and destroy cancerous cells, often leading to increased survival rates in various malignancies. Recent studies have highlighted the importance of timing and sequencing in the administration of neoadjuvant immunotherapy. Liu et al. emphasized that the timing of neoadjuvant treatment relative to surgical intervention is crucial for achieving optimal outcomes. Their investigation reported that 30% of patients achieved complete or near-complete pathological responses, demonstrating that pre-surgical immunotherapy can significantly impact treatment success. These results underscore the potential for neoadjuvant strategies to invigorate the immune response against tumors, setting the stage for improved surgical outcomes. In addition to timing, the mechanisms by which neoadjuvant immunotherapy exerts its effects have been elucidated through various studies. Zhang et al (2020) discussed adjuvant strategies that aim to modify the tumor microenvironment (TME) while inducing antitumor immune responses. Their approach involves delivering immunomodulating agents that sensitize tumors to immune attack, improving the effectiveness of subsequent therapies. This aligns with the overall goal of neoadjuvant therapy to enhance immunogenicity and help the immune system recognize and target tumor cells more effectively. A comparative analysis by Guo et al. contrasted neoadjuvant and adjuvant immunotherapy, revealing the distinct advantages of administering immunotherapy prior to surgery. Their findings suggest that neoadjuvant therapies can lead to a deeper systemic immune response, particularly beneficial in operable patients, while adjuvant treatment focuses on addressing micro-metastatic disease and preventing recurrence. This perspective is critical for informing clinical decision-making

regarding the optimal therapeutic approach for various cancers. The success of neoadjuvant immunotherapy is further supported by recent trials in early-stage non-small-cell lung cancer (NSCLC), such as NEOSTAR and NA_00092076. Szeto et al. reported improved rates of pathological complete response (pCR) with nivolumab, suggesting that these neoadjuvant protocols can establish a robust immune response that potentially leads to long-term remission (Szeto et al., 2021). Similarly, a review of outcomes in pancreatic cancer patients indicated improvements in overall survival associated with neoadjuvant immunotherapy, highlighting the utility of such approaches in traditionally difficult-to-treat malignancies. Despite the clear benefits, challenges persist in the practical application of neoadjuvant and adjuvant immunotherapy. The risk of immune-related adverse events, which can complicate the therapeutic landscape, requires careful monitoring and management. Additionally, the heterogeneity of tumor response to immunotherapy necessitates further studies to identify predictive biomarkers that can guide treatment decisions. Future research should focus on refining treatment protocols and optimizing patient selection to maximize the potential benefits of these innovative therapeutic strategies. Both neoadjuvant and adjuvant immunotherapy play crucial roles in the management of solid tumors, with the potential to improve clinical outcomes significantly. As the understanding of the mechanisms underlying these therapies expands, combined with advances in personalized medicine, we can expect to see enhanced strategies that leverage the immune system's power more effectively in fighting cancer.

Immunotherapy for Triple-Negative Breast Cancer (TNBC)

Immunotherapy has emerged as a transformative approach to treating triple-negative breast cancer (TNBC), a subtype characterized by its aggressive nature and the absence of estrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2 (HER2). The therapeutic landscape for TNBC has evolved considerably with the introduction of immune checkpoint inhibitors and novel combination strategies, enhancing both treatment efficacy and patient outcomes. One of the most critical advancements in immunotherapy for TNBC is the use of immune checkpoint inhibitors, such as pembrolizumab and atezolizumab. These agents have demonstrated significant efficacy when combined with chemotherapy. The pivotal IMpassion131 trial evaluated the combination of atezolizumab with nab-paclitaxel in patients with locally advanced or metastatic TNBC, leading to improved overall survival in patients with PD-L1-positive tumors. Similarly, the KEYNOTE-355 trial reinforced the benefits of pembrolizumab combined with standard chemotherapy, showing notable improvements in progression-free survival. The inclusion of immunotherapy in neoadjuvant settings has also proven beneficial. For example, recent meta-analyses indicate that neoadjuvant chemotherapy paired with immune checkpoint blockade can increase pathological complete response rates, ultimately translating to better long-term outcomes. This suggests that early intervention may stimulate a more robust immune response, aiding in the eradication of micro-metastatic disease. The complexity of TNBC necessitates innovative combination therapies to optimize treatment responses. Combining immune checkpoint inhibitors with targeted therapies, such as PARP inhibitors, shows promise in enhancing therapeutic efficacy. Olaparib and talazoparib have been approved for TNBC patients with BRCA1/2 mutations, demonstrating a progression-free survival benefit when used alongside immune checkpoint inhibitors. Additionally, studies investigating cabazitaxel's effects on

macrophage activation have indicated improved outcomes when paired with CD47-targeted immunotherapy, aiming to enhance phagocytosis of tumor-associated macrophages. This synergistic approach can potentially overcome tumor evasion mechanisms, further enhancing the immune-mediated destruction of cancer cells. The identification of predictive biomarkers is essential for personalizing treatment strategies in TNBC. For instance, PD-L1 expression levels have been validated as a crucial determinant of response to immunotherapy, influencing clinical decisions regarding the use of immune checkpoint inhibitors. Moreover, other biomarkers, such as tumor mutational burden and specific immune cell populations, can provide additional insights into potential responsiveness, allowing for more tailored therapeutic approaches. Emerging research into methods that characterize the tumor microenvironment, including the presence of tumor-infiltrating lymphocytes (TILs) and their associations with immunogenicity, can further enhance the predictability of responses to immunotherapy. Understanding these dynamics will be crucial in the design of upcoming clinical trials and treatment paradigms.

Despite promising developments, several challenges persist in optimizing immunotherapy for TNBC. The tumor's heterogeneous nature often leads to varied responses among patients, necessitating ongoing research to refine combination therapies and address issues related to treatment resistance (Yang et al., 2023). Novel strategies exploring gene expression modulation, such as targeting pathways like MYC and employing mRNA vaccines, might offer new avenues for enhancing the effectiveness of existing therapies. As we move forward, the need for robust clinical trials to validate the efficacy of these combination approaches will be pivotal. Future research should also focus on overcoming barriers related to tumor microenvironment interactions and developing strategies to mitigate immune resistance, thereby improving overall outcomes for patients with TNBC. The integration of immunotherapy into TNBC treatment regimens, especially when combined with other therapies, represents a significant advancement in the management of this challenging cancer subtype. Ongoing research and clinical trials will be essential for refining these approaches, ultimately enhancing survivability and quality of life for patients afflicted by TNBC.

Immunotherapy in Early-Stage vs. Metastatic Settings

The paradigm shifts in cancer treatment, particularly between early-stage and metastatic settings, emphasizes the role of immunotherapy as a transformative approach. In the early-stage setting, the integration of immunotherapy, particularly as neoadjuvant treatment, has been associated with enhanced therapeutic efficacy and patient outcomes. Studies have shown that neoadjuvant immunotherapy can induce a more robust immune response due to the presence of abundant tumor antigens at the time of treatment initiation, allowing for a greater chance of achieving complete pathological responses (Witt et al., 2022). Early-stage triple-negative breast cancer (TNBC) is one area where neoadjuvant immunotherapy has become standard practice, as it enhances response rates and survival outcomes compared to traditional approaches. The incorporation of immune checkpoint inhibitors has led to improved pathological complete response rates in these patients, demonstrating the capability of immunotherapy to shift the treatment paradigm from surgical interventions and chemotherapy alone to novel immunotherapeutic strategies.

Conversely, in the metastatic setting, the application of immunotherapy has also transformed patient management, highlighting its potential in offering durable responses where traditional therapies often fall short. Studies have outlined the effectiveness of checkpoint inhibitors in advanced non-small cell lung cancer (NSCLC) and other malignancies, establishing them as essential components of the treatment arsenal. Given the high mutational burden typical of these cancers, immunotherapy not only improves survival but can also lead to better quality of life by minimizing the toxicity associated with conventional chemotherapeutics. Furthermore, there is a growing consensus that specific biomarkers, such as PD-L1 expression levels, can guide treatment choices and predict responses to immunotherapy. This biomarker-driven approach is increasingly important as it allows clinicians to tailor patient management strategies based on individual tumor characteristics rather than a one-size-fits-all method. Nevertheless, certain challenges remain, such as the phenomenon of primary resistance to immunotherapy, underscoring the necessity for ongoing research to enhance therapeutic efficacy and broaden patient eligibility. The shifting paradigms between early-stage and metastatic cancer treatment illustrate the evolving landscape of immunotherapy. Comparatively, early-stage applications focus on maximizing immune engagement prior to surgical intervention, while the metastatic landscape relies more extensively on immunotherapy to achieve systemic control. This dual approach highlights the critical need for personalized medicine strategies to further optimize patient outcomes across the spectrum of cancer stages.

Immune-Related Adverse Events (irAEs)

The management of immune-related adverse events (irAEs) associated with immune checkpoint inhibitors (ICIs) is critical for enhancing patient outcomes in cancer immunotherapy. IrAEs, which can manifest in numerous organ systems, range from mild to severe and can significantly impact the course of cancer treatment. Understanding the risk factors, predictive biomarkers, and management strategies for irAEs is essential in optimizing therapeutic efficacy while minimizing adverse effects. Recent studies indicate that specific hematological markers can serve as predictive biomarkers for irAEs. For example, elevated absolute eosinophil counts have been identified as a strong predictor of irAEs in patients treated with PD-1/PD-L1 inhibitors. Furthermore, peripheral blood biomarkers such as lymphocyte counts and cytokine levels have shown promise in stratifying patients at risk for developing severe irAEs. The analysis of baseline cytokines like IL-13 has been correlated with severe irAE occurrences, supporting the development of predictive models for risk assessment. Additionally, the absolute lymphocyte count has been proposed as a useful predictor for irAEs in patients treated with Nivolumab, enabling personalized patient management. The class of immunotherapy and the sequence of treatment can influence the incidence of irAEs. Notably, patients progressing to subsequent lines of treatment tend to experience higher rates of severe irAEs compared to those on first-line therapy. In addition, combination therapies that integrate ICIs with chemotherapy or other targeted agents have been shown to heighten the risk of adverse events, making patient monitoring imperative during these treatment phases (Bai et al., 2021). Effective management of irAEs involves early recognition and intervention. Given that irAEs can mimic autoimmune disorders, a multidisciplinary approach is encouraged to navigate the complexities of treatment. Corticosteroids are often the first line of treatment for managing severe irAEs, while guidance

from specialists in the affected organ systems may be required for more complicated cases. Timely discontinuation of immune checkpoint inhibitors may be necessary, especially in cases of life-threatening adverse events, but must be balanced against the potential benefits of continued immunotherapy. The clinical repercussions of irAEs are substantial, as they can lead to therapy discontinuation and negatively influence overall treatment efficacy. Conversely, some studies have suggested that the presence of irAEs may correlate with improved treatment responses in certain patient cohorts, further complicating decision-making. Thus, stratifying patients based on identifiable risk factors and biomarkers not only aids in predicting the occurrence of irAEs but also informs the best management strategies to maintain therapeutic benefits. The prediction and management of immune-related adverse events are essential components of immunotherapy for cancer patients. By employing predictive markers and early intervention strategies, healthcare providers can mitigate the risks associated with ICIs, thereby enhancing patient outcomes in cancer treatment.

Targeting Epigenetic Silencing and PTMs

Targeting epigenetic silencing and post-translational modifications (PTMs) presents a critical opportunity to enhance immune responsiveness in cancer therapy. The interplay between epigenetics and the immune system is becoming increasingly relevant, as epigenetic alterations not only regulate tumor-associated antigen expression but also impact the efficacy of immune checkpoint inhibitors (ICIs) and overall therapeutic outcomes. Various studies highlight the role of epigenetic modifications in immune evasion by tumors. For instance, DNA methylation, particularly within tumor suppressor genes and immune checkpoint molecules such as PD-1, CTLA-4, and PD-L1, can significantly inhibit T cell responses and immunogenicity in the tumor microenvironment (TME). Specifically, hypermethylation of promoters for these immune checkpoints leads to their silencing, limiting the capacity of T cells to mount effective anti-tumor responses. Additionally, preclinical evidence supports that disrupting these epigenetic silences can enhance immune recognition of tumor cells, promoting cytotoxic T cell activity against cancer. The role of post-translational modifications, particularly histone modifications, on immune responsiveness has garnered attention. Histone acetylation and methylation can dictate the accessibility of chromatin, influencing the expression of genes involved in immune responses. For example, the Wdr5-H3K4me3 axis has been demonstrated to regulate pancreatic tumor immunogenicity, indicating a functional link between histone modifications and immune activity. Furthermore, inhibitors targeting histone deacetylases (HDAC) have shown promise in reversing tumor resistance by reactivating silenced immune genes and enhancing T cell infiltration in various malignancies. Combining epigenetic therapies with immunotherapies, such as ICIs, has emerged as a significant strategy to overcome immune resistance. The introduction of DNA methyltransferase inhibitors (DNMTi) alongside ICIs has demonstrated the potential to prime T cell responses by increasing the expression of tumor-associated antigens. For instance, preliminary studies suggest that DNMTi treatment can augment the immunogenicity of tumors, making them more susceptible to ICIs, and thus improving patient outcomes. Moreover, HDAC inhibitors (HDACi) not only enhance the efficacy of ICIs but also promote a favorable TME by reducing the presence of immunosuppressive cells, such as myeloid-derived suppressor cells. Increasing attention has focused on specific epigenetic regulators, such as EZH2 and KDM5B,

that play pivotal roles in governing immune responses. EZH2, for instance, is implicated in the negative regulation of PD-L1; inhibiting this protein has been associated with increased expression of this immune checkpoint, potentially sensitizing tumors to PD-1 blockade. Similarly, the depletion of KDM5B has been shown to activate T cell immunity through enhanced expression of retroelements that trigger cytosolic nucleic acid sensing pathways. The integration of epigenetic modulation alongside immunotherapy represents a promising avenue to enhance therapeutic efficacy in oncology. As our understanding of the epigenetic landscape and its influence on immune responses deepens, future strategies that harness these mechanisms are poised to significantly improve outcomes in cancer patients. By targeting the epigenetic silencing of critical immune pathways and enhancing immune cell function through PTMs, researchers can develop innovative approaches that not only activate antitumor immunity but also overcome existing therapeutic resistances.

Immunotherapy in Hepatocellular Carcinoma (HCC)

Immunotherapy has emerged as a promising treatment modality for hepatocellular carcinoma (HCC), particularly in the context of immune checkpoint inhibitors (ICIs) and combination therapies. The efficacy of these therapies is significantly influenced by various biomarkers that can predict patient outcomes and tailor treatment strategies. Clinical studies have identified several biomarkers that hold potential in predicting treatment response and overall survival in patients undergoing immunotherapy for HCC. For instance, a study by Zhu et al. demonstrated that combining alpha-fetoprotein (AFP) levels with the neutrophil-to-lymphocyte ratio (NLR) can effectively predict treatment responses and survival outcomes in patients treated with ICIs. This dual approach enhances predictive capabilities due to AFP‘s established role as a diagnostic marker for HCC, thereby providing better insight into the patient's immunological landscape and potential response to therapy. Additionally, the immune microenvironment‘s role in determining therapeutic responses. Their study demonstrated that β-catenin activation within HCC tumors promotes immune evasion, correlating with T-cell exclusion and resistance to PD-1 therapies. Understanding how tumor genetics, such as CTNNB1 mutations, influence immune responses offers new avenues for targeted therapy in HCC. The real-world effectiveness of immunotherapy in HCC has been corroborated by several clinical trials and observational studies. The Checkmate 040 trial indicated that the duration of response (DOR) among patients treated with nivolumab can reach up to 17 months, suggesting durable benefits, especially for patients not effectively treated by conventional therapies. Furthermore, the combination of atezolizumab and bevacizumab has shown promising results in real-world settings, with findings indicating significant improvements in overall survival (OS) and progression-free survival (PFS) rates compared to traditional therapies like sorafenib. A multicenter propensity score matching analysis reported in a study by Xu et al (2024). further underscores the effectiveness of adjuvant immunotherapy after curative resection in HCC. Their findings suggest that adjuvant therapies improve recurrence-free survival (RFS) and overall survival (OS) rates for patients with intermediate-advanced stages of the disease, emphasizing the potential benefits of integrating immunotherapy into standard treatment protocols. Despite these advancements, several challenges remain in optimizing immunotherapy for HCC. The tumor microenvironment in HCC is notoriously immunosuppressive, often driven by various cytokines and cell populations that

inhibit effective immune responses. The role of cellular factors such as FAM210B on immune modulation further complicates the therapeutic landscape by affecting immune cell infiltration and response. Addressing these challenges may require innovative therapy approaches that combine ICIs with other therapeutic modalities, such as locoregional therapies or novel immunotherapeutic agents targeting these immunosuppressive mechanisms. The incorporation of biomarkers and real-world evidence into treatment protocols represents a significant evolution in the management of HCC with immunotherapy. Ongoing research is pivotal in exploring novel combinations and refining patient selection to enhance treatment efficacy and address the challenges posed by the HCC tumor microenvironment. A proactive approach in understanding these dynamics will ultimately pave the way for improved outcomes in this challenging malignancy.

Evidence of Immunotherapy Use

The integration of immunotherapy into real-world clinical practice has significantly influenced treatment paradigms across various cancers, demonstrating an effect on patient outcomes and healthcare delivery. This discussion synthesizes findings on real-world evidence regarding the use of immunotherapy, particularly focusing on retrospective insights from community practice.

Research has shown an increase in the adoption of immunotherapy in community settings, particularly in diseases such as metastatic melanoma and non-small cell lung cancer (NSCLC). A cohort study by Moyers et al. indicated that earlier cancer diagnoses correlated with higher immunotherapy utilization, underscoring the importance of early intervention in improving survival rates in patients. Parallel findings in the study conducted by Torphy et al. highlight shifting treatment strategies reflecting a broader acceptance of immunotherapy beyond specialized centers into community practices. Additionally, Bonanno et al. emphasize the changes brought about by the introduction of chemo-immunotherapy in extended small cell lung cancer (SCLC), showcasing improved durability of response compared to older treatments. Despite the promise of immunotherapies, several barriers impact their implementation in community settings. Puri et al. discussed the financial constraints imposed by Medicare reimbursement models, which can create significant risks for oncology practices and lead to treatment disparities. Such financial environments have led to inconsistent availability of immunotherapy options, limiting the ability of community oncologists to provide these novel therapies promptly. Moreover, the study by Marin-Acevedo et al. (2018) sheds light on the need for improvements in education and training among healthcare professionals managing immunotherapy side effects, as insufficient knowledge in this area could lead to suboptimal patient care. This emphasizes that, while immunotherapy has transformed treatment landscapes, existing infrastructural and educational gaps require urgent attention to ensure comprehensive patient management. Understanding patient experiences with immunotherapy is crucial for improving communication and care quality. Boulanger et al. conducted interviews illustrating the uncertainty and hope patients experience when receiving immunotherapy, revealing the psychological dimensions that accompany such treatment modalities. Effective communication regarding potential outcomes and side effects is essential for aligning treatment goals with patient expectations. Additionally, studies highlight the importance of patient education regarding the immune system and expected side effects of immunotherapy to foster better relationships between healthcare providers and patients. These educational efforts can

empower patients to actively participate in treatment decisions, ultimately enhancing satisfaction and adherence to therapy. Outcomes from immunotherapy in real-world settings often reflect those seen in clinical trials, but they may also highlight disparities based on sociodemographic factors. For instance, Wu et al. report variations in immunotherapy effectiveness across diverse populations, necessitating a refined approach to treatment that accounts for these differences in patient background. Moreover, the analysis by Amin et al. of the National Cancer Database revealed that patients undergoing immunotherapy for pancreatic adenocarcinoma showed promising survival benefits compared to those receiving conventional therapies. Such findings support the effectiveness of immunotherapy in a broader patient population and encourage further research into optimizing treatment regimens across different cancer types. The real-world evidence on immunotherapy reflects a paradigm shift in oncological treatment, with substantial progress seen in various cancer types. However, challenges remain particularly concerning healthcare delivery models, training among providers, and addressing patient-specific needs. As more data emerge from community practices, there is an opportunity to refine these therapies further, creating a more inclusive and effective treatment landscape for cancer patients.

Cell-Free mRNA Therapies and Neoantigen Delivery Platforms

Cell-free mRNA therapies and neoantigen delivery platforms are emerging as innovative strategies for enhancing the efficacy of immunotherapeutic interventions. Utilization of mRNA enables precise targeting and can stimulate robust immune responses against tumors, particularly through the delivery of neoantigens, which are unique to cancer cells. Recent advancements have leveraged cell-free systems for mRNA production, such as using human primary peripheral blood mononuclear cells. This approach allows for the potential synthesis of personalized mRNA-based therapeutics without the complications of cell-based systems, thus streamlining the production process. The optimization of delivery mechanisms is vital, as mRNA must efficiently reach the cytoplasm to be translated into proteins that elicit an immune response. To improve mRNA stability and delivery, various strategies have been developed. For example, Yoshinaga et al. detailed the benefits of PEGylation of mRNA, which provides stabilization without relying on cationic materials that can introduce toxicity. This enhancement can significantly affect the efficiency of mRNA delivery systems, making them safer and more effective for therapeutic use. Moreover, lipid nanoparticles (LNPs) have become a preferred vehicle for mRNA delivery. Research indicates that these nanoparticles can protect mRNA from degradation and ensure effective uptake by target cells. LNPs have demonstrated success in delivering mRNA vaccines during the COVID-19 pandemic and are now being extensively applied in cancer immunotherapy scenarios. For instance, lipid-peptide-mRNA nanoparticles have been observed to augment radioiodine uptake in anaplastic thyroid cancer, showcasing the versatility of this delivery method in targeting specific oncological contexts. A significant focus in the application of mRNA therapies is the development of neoantigen-based vaccines capable of stimulating specific T-cell responses. In a study led by Rojas et al., personalized RNA neoantigen vaccines were shown to successfully activate T-cells in patients with pancreatic cancer, proving their potential in eliciting targeted immune responses against tumors. This achievement highlights the clinical relevance of neoantigen delivery platforms, as they offer tailored therapeutic interventions based on individual tumor mutational profiles. Furthermore, biomimetic nanoparticles designed to deliver mRNAs

encoding costimulatory receptors have enhanced CD8+ T cell responses, thus reinforcing the immune system's ability to recognize and eliminate cancer cells. Such strategies emphasize the importance of not only delivering neoantigen mRNA but also ensuring that these antigens effectively mount an immune response through adequate co-stimulation. Despite the advances, challenges remain in optimizing mRNA delivery systems and ensuring sustained immune responses. Issues such as mRNA instability and variability in patient responses necessitate ongoing research. Enhanced understanding of the tumor microenvironment and immune response dynamics is essential for further developing effective therapies. Overall, the future of mRNA therapies in oncology appears promising, particularly as technologies advance to create safer and more effective delivery methods. As more data surface regarding the efficacy of combined approaches utilizing mRNA alongside traditional therapies, they hold immense potential to transform cancer treatment paradigms.

Artificial Intelligence and Machine Learning

Artificial Intelligence (AI) and Machine Learning (ML) are increasingly playing crucial roles in the design and prediction of outcomes for immunotherapy trials. The utilization of these advanced computational techniques has the potential to optimize patient selection, enhance treatment stratification, and improve predictive accuracy regarding treatment responses across various cancer types. Recent studies have demonstrated the effectiveness of machine learning models in predicting the response to immunotherapy. For example, Sun et al. utilized a random forest algorithm integrated with immune cell scores from the tumor microenvironment to identify subtypes of nasopharyngeal carcinoma that showed differential responses to treatment, achieving an area under the curve (AUC) of 0.75 for IFNG expression as a predictive marker. This illustrates how ML can parse complex datasets to derive actionable insights for clinical use. In the context of breast cancer, Lu et al. developed a gene panel aimed at predicting the benefit from neoadjuvant chemoimmunotherapy specifically for HER2-negative subtypes. Their study indicates that applications of existing biomarkers may be controversial or insufficient in providing predictive power. This highlights the urgent need for refined models using advanced ML techniques. Such predictive capabilities can significantly aid in clinical decision-making by identifying patients most likely to benefit from specific therapies. The integration of multi-omic data using machine learning presents another frontier in enhancing predictive capabilities for immunotherapy outcomes. The combining transcriptomic data with ML algorithms allowed for a comprehensive assessment of colorectal cancer patients undergoing immunotherapy, significantly improving the accuracy of survival predictions. This approach underscores the significance of leveraging extensive biological data to inform and direct treatment strategies. Furthermore, Anagnostou et al. propose the development of multimodal genomic features to predict outcomes following immune checkpoint blockade in non-small-cell lung cancer (NSCLC). Their approach emphasizes the need for more complex, integrated predictive models that account for both static and dynamic biomarkers. These innovations represent a fundamental shift in understanding the multifaceted nature of cancer immune responses and could drastically improve patient stratification in clinical trials. Despite the promising advances, several challenges persist in the application of AI and ML in immunotherapy trial design. One significant concern is ensuring the interpretability of machine learning models, which may operate in black-box settings, thereby

limiting trust and usability in clinical environments. As acknowledged by Liu et al., developing explainable models that provide insight into the decision-making process will be critical for achieving clinical adoption and optimizing treatment protocols. Moreover, data quality and variety pose additional challenges. The efficacy of ML models depends on the availability of robust and diverse datasets encompassing various demographic, clinical, and biological factors. Improving data interoperability and alignment among different studies can enhance the robustness of machine learning outcomes. AI and ML are reshaping the landscape of immunotherapy trial design and prediction through innovative modeling approaches that enhance treatment stratification and patient outcomes. As research progresses toward integrating diverse omics data and improving model interpretability, these technologies hold significant promise for advancing personalized medicine in oncology. Continued collaboration among computational biologists, clinicians, and data scientists is essential for translating these advancements from conceptual frameworks into practical, real-world applications.

The Role of Beta Blockers and Metabolic Modulators

The integration of beta blockers and metabolic modulators represents a significant frontier in enhancing the efficacy of immunotherapy for cancer treatment. This approach aims to manipulate the tumor microenvironment (TME) and metabolic pathways to bolster anti-tumor immune responses. Beta blockers, commonly known for their cardiovascular applications, have garnered attention for their immunomodulatory effects in cancer patients. Research has indicated that adrenergic stress can impair anti-tumor immunity; therefore, using beta blockers may help mitigate the immunosuppressive effects linked to chronic stress in cancer patients. Chen et al. demonstrated that adrenergic stress constrains the immune response, suggesting that beta-adrenergic antagonists could be utilized alongside immunotherapies to enhance their effects by improving the infiltration and function of CD8-positive T lymphocytes. This represents an innovative application of beta blockers, shifting their traditional role toward a supportive therapy in cancer immunology. Moreover, Guerra et al. highlighted the importance of understanding nutrient competition within the TME, showing that beta blockers could indirectly influence metabolic processes that affect immune cell function and tumor growth, thereby improving patient outcomes when combined with immune-therapeutics (Guerra et al., 2020). Metabolic modulators play a pivotal role in the context of cancer treatment by reprogramming tumor-associated metabolic pathways and enhancing immune responses. Different strategies targeting metabolic processes have shown promise in improving the efficacy of immunotherapy. For instance, Liu et al. explored how glutamine metabolism alters the immune landscape and immune responsiveness in lung adenocarcinoma, demonstrating that inhibiting aspects of glutamine metabolism can activate CD8+ T cells and augment the efficacy of anti-PD-1 therapies. Optimizing T cell metabolism through 4-1BB co-stimulation was shown to enhance mitochondrial function, leading to more effective anti-tumor immune responses. This underscores the potential for metabolic modulation as a complementary strategy to conventional immunotherapy. Additionally, reprogramming lipid metabolism in T cells, as described by Liu et al., prevents senescence and dysfunction, thereby improving the overall efficacy of immunotherapeutic interventions. Combining beta blockers and metabolic modulators could create a synergistic effect, enhancing both anti-tumor immunity and the efficacy of immunotherapeutic agents. As Kundu et al. noted,

the TME is complex and requires multifaceted approaches to modulate its various components effectively. Metabolic manipulations, such as targeting glucose or fatty acid metabolism, combined with the immunomodulatory effects of beta blockers, could lead to improved infiltration of immune cells and a more favorable TME. The interaction between tumor metabolism and immune response cannot be understated, as highlighted by the evidence suggesting that modifications in metabolic pathways can influence immune checkpoints in the TME, affecting the overall treatment outcomes of immunotherapy. Future research should focus on elucidating the mechanisms by which these metabolic and pharmacological interventions can be integrated into clinical protocols, thereby maximizing therapeutic benefits for cancer patients undergoing immunotherapy. The dual approach of utilizing beta blockers and metabolic modulators offers a promising avenue to enhance the efficacy of immunotherapy in cancer treatment. By reshaping the TME and improving immune cell functionality through metabolic reprogramming, these strategies present a holistic treatment modality that may lead to improved long-term outcomes for patients. As ongoing research elucidates these interactions further, the potential for combinatorial strategies based on metabolic and adrenergic modulation in cancer care is indeed compelling.

Immunotherapy in Non-Oncologic Diseases

Immunotherapy is emerging as a pivotal treatment modality not only in oncology but also in addressing autoimmune diseases and infectious diseases. The adaptability of immunotherapeutic approaches in these diverse settings stems from the innovative mechanisms by which they modulate the immune response. Recent developments in Chimeric Antigen Receptor T-cell (CAR-T) technology demonstrate promising applications in autoimmune diseases. Traditionally a staple of cancer treatment, CAR-T cells have shown potential beyond oncology, notably in autoimmune conditions where dysregulated immune responses are prevalent. Research indicates that CAR-T cell technology can effectively target specific antigens associated with certain autoimmune diseases, thereby resetting dysfunctional immune pathways. This suggests a significant paradigm shift in how these conditions may be managed. Additionally, immune checkpoint inhibitors, widely used in oncology, have been implicated in facilitating immune modulation in autoimmune diseases. They function by unleashing T-cells from inhibition, potentially reversing immune tolerance that characterizes many autoimmune diseases. While these applications show promise, they come with risks for immune-related adverse events, where the activated immune system may damage healthy tissues, highlighting the need for careful patient selection and management. Infectious diseases also present an avenue for immunotherapy's innovative applications. CAR-T cell technology is being explored for its efficacy against viral infections such as HIV, demonstrating a capability to redirect the immune response toward viral antigens. This re-engineering not only promotes viral clearance but also potentially contributes to long-term immunity, akin to the model for cancer immunotherapy. Moreover, advancements in vaccine development, particularly those leveraging nanotechnology, have been proposed as novel strategies to enhance immune responses against pathogens (Jyotsna et al., 2023). The relationship between immunotherapy and infectious disease opens the door for synergistic approaches. For example, immunotherapies that successfully manage cancer-related immune suppression could potentially enhance host responses in relation to concurrent infections, thereby improving overall patient outcomes. Research indicates that simultaneous treatment strategies, such as the use of

immune checkpoint inhibitors in tandem with antiviral therapies, might enhance clinical efficacy in specific populations, offering a dual benefit (Jyotsna et al., 2023). Despite the exciting prospects of using immunotherapy in both autoimmune and infectious diseases, there are significant challenges. The specificity of immune responses can lead to variability in treatment efficacy, necessitating a personalized approach. Further research is crucial to elucidate the safe and effective deployment of these therapies across non-oncologic diseases while minimizing adverse effects. Moreover, the development of better predictive biomarkers is essential to select appropriate candidates for immunotherapy, especially given the diverse responses between patients. As the field progresses, combining insights from oncology with those from infectious disease management might yield novel therapeutic strategies that incorporate the best of both worlds, potentially improving the overall efficacy of treatments across a range of diseases. Immunotherapy serves as a versatile and promising avenue for treating both autoimmune diseases and infectious diseases. The principles and technologies developed in oncology have the capacity to be repurposed effectively, but they require meticulous research and clinical validation to ensure broad applicability and safety.

Ethnic Disparities and Access to Novel Immunotherapies

Ethnic disparities in access to novel immunotherapies represent a critical area of concern in contemporary medical science. A meta-analytical perspective elucidates how various demographic factors contribute to inequities in treatment access, potentially hindering effective healthcare delivery, particularly among racial and ethnic minority populations. Recent studies have consistently highlighted the racial and ethnic disparities in access to immunotherapy treatments across various cancers. For instance, Ramkumar et al. elucidated how racial minorities, specifically Black patients, face substantial barriers in receiving immunotherapy for head and neck cancers compared to their White counterparts. This reflects a broader trend, as Freeman et al. observed disparities in the likelihood of initiating immunotherapy among patients with stage IV non–small cell lung cancer, where Black patients were 12% less likely than White patients to begin treatment, particularly in non-high-volume healthcare facilities. Such patterns underscore systemic inequalities tied to both race and the structural aspects of healthcare delivery. In addition to racial disparities, socioeconomic factors significantly exacerbate inequities in immunotherapy access. A study conducted by Ermer et al. revealed that Black and Hispanic patients were less likely to receive immunotherapy treatments even before the FDA approval era for several agents, indicating a broader systemic issue that reflects historical and ongoing inequities in healthcare access. These disparities have persisted even after regulatory changes, with access to advanced therapies like immunotherapy still disproportionately skewed against minorities. Furthermore, the landscape of access disparities is multifaceted, influenced by geographical, financial, and institutional factors. Wong et al. provided compelling evidence that mortality rates among various racial/ethnic groups within the Veterans Health Administration exhibited significant disparities, linked to differences in service delivery and accessibility, which can be extrapolated to understand access to advanced therapies, including immunotherapy. Their findings suggest that institutions aiming to equalize access must account for such geographic and institutional variances. The implications of these disparities are profound, necessitating targeted policy interventions. The Affordable Care Act has shown promise in narrowing some disparities in health insurance

coverage; however, significant gaps remain in actual healthcare service delivery and access to innovative treatments like immunotherapies among racial and ethnic minorities. Paradoxically, while such policies aim to address inequities, disparities in treatment uptake persist, indicating that mere policy implementation is insufficient without addressing the underlying social determinants of health. Studies suggest a need for enhanced outreach efforts aimed at minority communities to foster trust, improve health literacy, and build better channels for communication between healthcare providers and patients (Ashrafzadeh et al., 2021). Additionally, researchers like Nazha et al. highlight the critical need for inclusive clinical trials to ensure better representation of minority groups in studies that inform treatment strategies, which is essential for tailoring therapies effectively. The ethnic disparities in access to novel immunotherapies are driven by a complex interplay of historical, social, and institutional factors. Addressing these disparities requires a concerted effort from policymakers, healthcare providers, and researchers to implement comprehensive strategies that not only enhance access but also ensure equitable treatment outcomes for all racial and ethnic groups.

References

Ashrafzadeh, S., Asgari, M., & Geller, A. (2021). The need for critical examination of disparities in immunotherapy and targeted therapy use among patients with cancer. Jama Oncology, 7(8), 1115. https://doi.org/10.1001/jamaoncol.2021.1322

Bai, R., Chen, N., Chen, X., Li, L., Song, W., Li, W., … & Cui, J. (2021). Analysis of characteristics and predictive factors of immune checkpoint inhibitor-related adverse events. Cancer Biology and Medicine, 18(4), 1118-1133. https://doi.org/10.20892/j.issn.2095-3941.2021.0052

Buchwald, Z., Wynne, J., Nasti, T., Zhu, S., Mourad, W., Yan, W., … & Khan, M. (2018). Radiation, immune checkpoint blockade and the abscopal effect: a critical review on timing, dose and fractionation. Frontiers in Oncology, 8. https://doi.org/10.3389/fonc.2018.00612

Chuprin, J., Buettner, H., Seedhom, M., Greiner, D., Keck, J., Ishikawa, F., … & Brehm, M. (2023). Humanized mouse models for immuno-oncology research. Nature Reviews Clinical Oncology, 20(3), 192-206. https://doi.org/10.1038/s41571-022-00721-2

Forschner, A., Battke, F., Hadaschik, D., Schulze, M., Weißgraeber, S., Han, C., … & Biskup, S. (2019). Tumor mutation burden and circulating tumor dna in combined ctla-4 and pd-1 antibody therapy in metastatic melanoma – results of a prospective biomarker study. Journal for Immunotherapy of Cancer, 7(1). https://doi.org/10.1186/s40425-019-0659-0

Gao, H., Acharya, D., Haddad, E., Comstock, S., Jorgensen, R., Hamdan, M., … & Srkalović, G. (2024). Analysis of blood immune biomarkers and fecal microbiome in subjects with and without malignant solid tumors: report from a feasibility study. Gastroenterol Funct Med, 2(1). https://doi.org/10.54844/gfm.2024.529

Guerra, L., Bonetti, L., & Brenner, D. (2020). Metabolic modulation of immunity: a new concept in cancer immunotherapy. Cell Reports, 32(1),107848. https://doi.org/10.1016/j.celrep.2020.107848

Huang, C., Yang, C., & Shyr, C. (2021). Utilizing xenogeneic cells as a therapeutic agent for treating diseases. Cell Transplantation, 30. https://doi.org/10.1177/09636897211011995

Huff, A., Longway, G., Mitchell, J., Andaloori, L., Davis-Marcisak, E., Chen, F., … & Zaidi, N. (2023). Cd4 t cell–activating neoantigens enhance personalized cancer vaccine efficacy. Jci Insight, 8(23). https://doi.org/10.1172/jci.insight.174027

Jyotsna, F., Ikram, J., Nageeta, F., Komal, F., Anjlee, F., Patel, H., … & Patel, T. (2023). Unlocking the potential of immunotherapy in cardiovascular disease: a comprehensive review of applications and future directions. Cureus. https://doi.org/10.7759/cureus.42790

Kemp, S., Magliano, M., & Crawford, H. (2021). Myeloid cell mediated immune suppression in pancreatic cancer. Cellular and Molecular Gastroenterology and Hepatology, 12(5), 1531-1542. https://doi.org/10.1016/j.jcmgh.2021.07.006

Liu, Y., Hu, L., Wu, Z., Yuan, K., Hong, G., Lian, Z., … & Pang, X. (2023). Loss of phf8 induces a viral mimicry response by activating endogenous retrotransposons. Nature Communications, 14(1). https://doi.org/10.1038/s41467-023-39943-y

Markouli, M., Ullah, F., Ünlü, S., Omar, N., Lopetegui-Lia, N., Duco, M., … & Dima, D. (2023). Toxicity profile of chimeric antigen receptor t-cell and bispecific antibody therapies in multiple myeloma: pathogenesis, prevention and management. Current Oncology, 30(7), 6330-6352. https://doi.org/10.3390/curroncol30070467

Niemi, J., Sokolov, A., & Schiöth, H. (2022). Neoantigen vaccines; clinical trials, classes, indications, adjuvants and combinatorial treatments. Cancers, 14(20), 5163. https://doi.org/10.3390/cancers14205163

Shannon AH, Manne A, Diaz Pardo DA and Pawlik TM (2023) Combined radiotherapy and immune checkpoint inhibition for the treatment of advanced hepatocellular carcinoma. *Front. Oncol.* 13:1193762. doi: 10.3389/fonc.2023.1193762

Souza, V. G. P., Forder, A., Brockley, L. J., Pewarchuk, M. E., Telkar, N., de Araújo, R. P., Trejo, J., Benard, K., Seneda, A. L., Minutentag, I. W., Erkan, M., Stewart, G. L., Hasimoto, E. N., Garnis, C., Lam, W. L., Martinez, V. D., & Reis, P. P. (2023). Liquid Biopsy in Lung Cancer: Biomarkers for the Management of Recurrence and Metastasis. *International Journal of Molecular Sciences*, *24*(10), 8894. https://doi.org/10.3390/ijms24108894

Szeto, C., Shalata, W., Yakobson, A., & Agbarya, A. (2021). Neoadjuvant and adjuvant immunotherapy in early-stage non-small-cell lung cancer, past, present, and future. Journal of Clinical Medicine, 10(23), 5614. https://doi.org/10.3390/jcm10235614

Testa, U., Castelli, G., & Pelosi, E. (2025). Emerging role of chimeric antigen receptor-natural killer cells for the treatment of hematologic malignancies. Cancers, 17(9), 1454. https://doi.org/10.3390/cancers17091454

Witt, R., Erstad, D., & Wargo, J. (2022). Neoadjuvant therapy for melanoma: rationale for neoadjuvant therapy and pivotal clinical trials. Therapeutic Advances in Medical Oncology, 14. https://doi.org/10.1177/17588359221083052

Yang, K., Zhao, Y., Sun, G., Zhang, X., Cao, J., Shao, M., … & Wang, L. (2023). Clinical application and prospect of immune checkpoint inhibitors for car-nk cell in tumor immunotherapy. Frontiers in Immunology, 13. https://doi.org/10.3389/fimmu.2022.1081546

About The Authors

Muhammad Fayyaz is currently serving as a Lecturer in Allied Health Sciences at the University of Sargodha, Pakistan. He is also pursuing his Ph.D. at the University of Gujrat, Pakistan. His research interests encompass several key disciplines, including Pathology, Immunology, Molecular Biology, Oncology and Biotechnology. With a strong academic and professional background, he has made meaningful contributions to the advancement of Health Sciences and Medical Education.
Email : fayyazahmad198@gmail.com **ORCID:** 0009-0006-6368-8799

Khawar Ali is currently pursuing his Ph.D. at North University of China, following his M.S. in Molecular Biology. He has previously served as a Research Associate at the Virtual University of Pakistan and Cholistan University of Veterinary and Animal Sciences, Bahawalpur, where he gained extensive experience in Molecular Biology research and laboratory techniques. Additionally, he worked as a Molecular Biologist at IBN-E-SINA Clinical Laboratory & Research Center in Lahore, contributing to clinical diagnostics and research operations. Khawar Ali has published several research articles in reputable peer-reviewed scientific journals. His research interests include Oncology, Molecular Biology, Genomics, and Biotechnology, with a particular focus on Molecular Diagnostics and Cancer Biology. Through his academic and professional endeavors,

Email: khawarali1202@gmail.com **ORCID:** 0000-0002-0565-4535.

Dr. Hamna Tariq (PhD) currently working as Assistant Professor and Head of Department of Department of Molecular Biology, University of Okara Pakistan. She has expertise in Whole genome sequencing for identifying clues and culprits responsible for non-syndromic human hearing impairment. Her research interests include Genetics, Biotechnology, Precision Medicine, Molecular Biology and Non-Syndromic Human Disorders. During her doctoral research she worked on the understanding how human genetic blue print carries a legacy of ailments.

E-mail: hamna.tariq@uo.edu.pk **ORCID:** 0009-0002-1332-8761.
Prof. Dr. Mehmet OZASLAN received his PhD in 1995 Institute of Natural Sciences at Cukurova University, Turkiye. He is a Professor in Molecular Biology and Genetics. His research interests are included Cancer Genetics, Molecular Virology, Molecular Genetics, Microbiology, and Genetic mutations etc. He has published more than 200 research articles in national and international well reputed journals. He also has written many book chapters as well as edited books. He is an editor and editor in chief of many well reputed national and international journals.
E-mail: ozaslanmd@gantep.edu.tr, ORCID: 0000 0001 9380 4902

The similarity index obtained from the plagiarism software for this book chapter is 14%.
To Cite This Chapter:
Fayyaz M et al., (2025). Advances in Immunotherapy. In Current Studies in Life Sciences 2025 (pp.246-273---). ISRES Publishing.

Digital Methods for Refining Nanoparticles Based Drug Delivery in Lung Cancer

Nehal ALI
Saba SANA
Samina TAHIR
Shahid SHERZADA

1. Introduction

The disease of lung cancer continues to cause extensive death worldwide despite its status as a severe cancer form and scarce treatment choices. The medical approaches of chemotherapy and radiation display poor treatment precision because lung cancer manifests complex features and substantial variation that limits treatment impact and produces severe adverse effects among patients. The applications of nanotechnology delivered efficient drug delivery systems (DDS) which enhance precise tumor targeting while minimizing systemic toxicity to deliver better therapeutic results. Unique physical features of nanoparticles demonstrate strong potential to deliver anticancer agents specifically to cancerous cells through a mechanism that safeguards healthy tissues.

Drug delivery systems which use nanoparticles demand complex research and expensive development work. Current experimental methods require extensive time and expense while also producing incomplete explanations of biological and physiological drug delivery elements. Digital methods comprised of artificial intelligence (AI) along with machine learning (ML) and computational modeling together with digital twin technology serve this purpose. The advanced tools give researchers the capability to forecast and enhance nanoparticle performance within human body which leads to faster development of customized optimal lung cancer treatments.

2. Overview on Cancer

Cancer a complicated health condition is due to abnormal cell multiplication. Such cells transition between natural selection patterns. The natural course of cancer development is directed by gene mutations along with environmental factors while producing multiple cancer forms with distinct mortality patterns. Recent worldwide statistics present a substantial global cancer impact where new cases reached 19.3 million while excess deaths exceeded 10 million counts in 2020 (Mundel, Dhadwal, Bharti, & Chatterjee, 2023).

The development of mutations in cancer cells produces survival benefits that foster competition in the tumor region. Evolutionary processes in cancer commonly select genes which regulate fundamental cellular operations because these genes determine survival capabilities (Ostrow, Barshir, DeGregori, Yeger-Lotem, & Hershberg, 2014). Treatment efforts become complex due to the adaptability of cancer cells as outlined by an evolutionary perspective. Worldwide cancer stands as the second leading cause of fatalities since it cause 15% of recorded deaths (Tanday, 2015).

Breast cancer together with prostate cancer and lung cancer represent the highest occurrence types of cancer. Such types are causing notable death rates (Mundel et al., 2023).

2.1. Types of Cancer

Common cancer types include breast cancer, lung cancer, bladder cancer, colorectal cancer, uterine cancer, skin cancer and kidney cancer. Although breast cancer is most common type of cancer to occur among women but the death rate of such type of cancer is still less. Lung cancer proved to be the most frequent form of cancer-related death as 10 million fatalities were documented in 2020 (Hjartåker, Weiderpass, & Bray, 2014).According to the Organization for Economic Co-operation and Development (OECD) cancer presented at 21 percent of all deaths in 2021 while lung cancer stood first at 20 percent (Siegel, Miller, Wagle, & Jemal, 2023).The most lethal type of cancer that has high death rates is Lung Cancer. According to National Cancer Institute, 611,720 number of deaths have been reported in US in 2024, among these 125,070 deaths are caused due to Lung and Bronchus cancer. **Table 1** shows that lung cancer remains the leading cause of cancer-related deaths globally and among OECD countries, followed by colorectal, liver, breast, and prostate cancers.

Table 1. Global and OECD mortality rates of major cancer types

Cancer Type	**Global Mortality Rate %**	**OECD Mortality Rate %**	**References**
Lung cancer	18.7	21	(Bray et al., 2024),(Hjartåker et al., 2014)
Colorectal cancer	9.3	11	(Bray et al., 2024)
Liver cancer	7.8	NA	(Bray et al., 2024)
Breast cancer	6.9	15	(Bray et al., 2024)
Prostate cancer	7.3	10	(Bray et al., 2024)

2.2. Oncogenes

Two main classes of cancer genes are

- **Proto-Oncogenes** promote cancer. The main purpose of this gene is to help cells to grow and divide. These can convert normal cells to become cancerous because of mutation. These are more prone to mutation and their muted version is known as **oncogenes**. It includes **rat sarcoma virus** (RAS family) (KRAS, NRAS, HRAS), mutation of such genes cause lung, colon and pancreatic cancer (Moore, Rosenberg, McCormick, & Malek, 2020). **Myelocytomatosis oncogene** (MYC), activates cell proliferation, often deregulated in breast and blood cancers (Dhanasekaran et al., 2022).
- **Tumor Suppressor Genes** are the genes that prevent cancer. These typically function to stop excessive cell proliferation by regulating the cell cycle and fixing DNA. Cancer,

however, arises when mutations in tumor suppressor genes counteract these safeguards and unleash unregulated cell growth. It includes TP53 (p53), the "guardian of the genome," acts to suppress mutations. More than half of human cancers have this altered. **Retinoblastoma Protein** (RB1) (L. H. Wang, Wu, Rajasekaran, & Shin, 2018) regulates cell cycle progression; mutated in retinoblastoma and other cancers. **BRCA1 and BRCA2**, aids in DNA repair; mutation increases risk of breast and ovarian cancers (Budak & Segmen, 2022).

Table 2 summarizes key cancer therapies including chemotherapy, targeted therapy, immunotherapy, hormonal therapy, and radiopharmaceuticals along with representative drugs and major drawbacks.

Table 2. Overview of cancer treatment modalities, associated drugs, and limitations

Treatment	Available Drugs	Drawbacks	References
Chemotherapy	Cisplatin,,Doxorubicin, Paclitaxel	Non-selectivity, Drug resistance, Severe side effects	(S. U. Khan, Fatima, Aisha, & Malik, 2024)
Targeted Therapy	Erlotinib (Tarceva), Imatinib (Gleevec), Trastuzumab (Herceptin)	Limited applicability, Development of resistance, High cost	(Guirgis, 2012; Ou, Gao, Habaz, & Wang, 2024)
Immunotherapy	Pembrolizumab (Keytruda), Nivolumab (Opdivo), Ipilimumab (Yervoy)	Autoimmune reactions, Not effective for all cancers, Delayed response	(Garg et al., 2024)
Hormonal Therapy	Tamoxifen, Anastrozole (Arimidex), Leuprolide (Lupron)	Limited to hormone-sensitive cancers, Hormonal side effects, Resistance development	(B. Liu, Zhou, Tan, Siu, & Guan, 2024)
Radiopharmaceuticals	Radium-223 (Xofigo), Lutetium-177 (Lutathera)	Radiation exposure risks, Limited targeting ability, Short half-life	(B. Liu et al., 2024)

3. Lung Cancer

Lung cancer is the most related cause of cancer related deaths, 15% of an average 5 year survival rate in US. Forty nine percent rate of new cases and 32.4% of death cases among 100,000 men and women have been reported of lung cancer (Schabath & Cote, 2019). Smoking is the most

common cause of lung cancer. It is characterized as small cell carcinoma and non-small cell carcinoma (Collins, Haines, Perkel, & Enck, 2007).

Small cell carcinoma is aggressive, more sensitive to chemotherapy and radiation, having high remission rates. SCLC comprises of two conditions

1. Limited-disease SCLC: In such condition the cancer is only present at the affected area and has not spread yet but long term survival rate is not possible. Studies shows only 7% of the patients survive more than 5 years.
2. Extensive-disease SCLC: In this state the cancer has spread and has affected all other parts of body and the survival rate is not much possible beyond 5 years. Studies shows only 1% survive in such conditions (Wittes, 1993).

Non-small cell carcinoma (NSCLC) is the most common lung cancer caused by smoking, cases are of about 75% and the survival rate is also very poor. Studies shows less than 10% patient survives of about 5 years (Dancey & Le Chevalier, 1997).

Table 3 outlines major gene mutations implicated in lung cancer, detailing their biological functions, prevalence, and literature references. High-frequency mutations in ***TP53, KRAS***, and ***EGFR*** highlight their significance in tumor progression and therapeutic targeting

Table 3. Key mutated genes in lung cancer, their roles, and mutation frequencies

Gene Symbol	Gene Name	Role	Mutation Frequency%	References
TP53	Tumor Protein p53	Tumor suppressor that monitors DNA damage and regulates cell growth.	40-51	(Qin, Hou, Liang, & Zhang, 2020)
KRAS	Kirsten Rat Sarcoma Viral Oncogene Homolog	Regulates cell division via the RAS/MAPK pathway; mutations lead to uncontrolled growth.	30	(Li et al., 2020)
EGFR	Epidermal Growth Factor Receptor	Produces a receptor protein that regulates signaling pathways of cell growth and proliferation.	10-15	(Li et al., 2020)
ALK	Anaplastic Lymphoma Kinase	Involved in cell growth; mutations can lead to uncontrolled proliferation.	5	(Li et al., 2020)
BRAF	B-Raf Proto-Oncogene	Produces a protein that regulates cell growth; mutations can lead to uncontrolled growth.	3	(Li et al., 2020)

HER2	Human Epidermal Growth Factor Receptor 2	Involved in cell growth and differentiation; mutations can lead to uncontrolled growth.	1-4	(Li et al., 2020)
MET	MET Proto-Oncogene	Encodes a protein involved in cell growth and differentiation; mutations can lead to uncontrolled growth.	8-10	(Li et al., 2020)
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	Produces a protein that regulates cell growth; mutations can lead to uncontrolled growth.	Up to 6	(Li et al., 2020)
RET	RET Proto-Oncogene	Involved in cell growth; mutations can lead to uncontrolled proliferation.	Less than 2	(Li et al., 2020)
ROS1	ROS Proto-Oncogene 1	Encodes a receptor tyrosine kinase involved in cell growth; mutations can lead to uncontrolled growth.	Less than 2	(Li et al., 2020)

4. Importance of Drug Delivery System

The power of drug delivery systems (DDS) emerges from their enhanced therapeutic results together with better patient treatment devotion and reduced side effects. Swing-based drug delivery systems have received major advancements through modern technological capabilities that enable controlled medication release systems essential for disease treatment.

4.1. Efficacy and Targeting

Through DDS healthcare providers can send drugs to precise tissues or cells leading to enhanced patient outcomes and decreased drug levels in the body (Vikal et al., 2024). Liposomes along with nanoparticles serve as technological tools which enable disease-focused delivery that permits site-specific medication treatment (Venkateswara Reddy, 2024).

4.2. Controlled Release Mechanisms

The Controlled Drug Delivery Systems (CDDS) sustain therapeutic levels of medications for sustained periods which increases both drug performance and reduces unwanted reactions (Sune, Jumde, Hatwar, Bakal, & Korde, 2024). Different mechanisms use diffusion responses together with environmental detection methods to achieve precise drug release control ("Advances in Drug Delivery Systems: Review Article," 2024).

4.3. Innovations in Drug Delivery

The latest developments in drug delivery technology include intelligent delivery systems which adapt to body conditions to optimize drug availability as well as patient medication compliance ("Advances in Drug Delivery Systems: Review Article," 2024). The development of 3D printing technology along with gene delivery methods serves as fundamental elements in building personalized medicine strategies which meet individual patient treatment requirements (Vikal et al., 2024).

5. Nanoparticles Based Drug Delivery Systems

Research and applications in nanoscience and nanotechnology have progressed over the last few decades at an unparalleled rate. A great expectation now is that medicine will reap nanotechnology and hence make large breakthroughs in detecting and curing many diseases. Such medical applications would include the delivery of medications, *in-vitro* and *in-vivo* diagnostics, nutraceuticals, and improved development of biocompatible materials. Engineered nanoparticles are an essential tool in bringing many of these applications to practice. Note that not all medicinal particles meet the newly defined and widely accepted criterion of ≤100 nm (De Jong & Borm, 2008).

Nanoparticles (NPs) find applications in most fields of life today because of their small size and rich surface area. NPs have been applied as pharmaceutical medicine vehicles for diagnostics and therapeutics. Their therapeutic relevance is dependent on various characteristics such as their physical and chemical properties, drug loading efficiency, drug release, and, most critically, the carrier's low or no toxicity. Higher surface to mass ratios, quantum properties, and the ability to adsorb and transport other chemicals make them more appealing for medical applications. NPs have relatively large functional surfaces that can bind, adsorb, and transport other molecules including medicines, probes, and proteins (De Jong & Borm, 2008; Najahi-Missaoui, Arnold, & Cummings, 2020).

The major role of nanoparticle based drug delivery comprises of target site delivery of drug, maintenance of maximum therapeutic effect, reduced toxicity, safety and biocompatibility. (Najahi-Missaoui et al., 2020).

5.1.1. Types of Nanoparticles

5.1.2. Polymer based Nanoparticles

Polymeric nanoparticles (PNP) are frequently employed as drug carriers that provide regulated and sustained release. The enclosed object can be affixed to the surface of a nanosphere or nanocapsule, as well as embedded into the polymer's matrix or shell. Polylactic glycolic acid (PLGA) and chitosan are two commonly used polymers that have been approved via Food and Drug Administration (FDA) for clinical usage, partly because these are biocompatible and biodegradable (Najahi-Missaoui et al., 2020; Y. Wang, Li, Truong-Dinh Tran, Zhang, & Kong, 2016).

5.1.3. Gold Nanoparticles

Gold nanoparticles have been widely used in cancer treatments, as these are easy to synthesis, have surface modification, excellently biocompatible properties also these are very well enhanced and can change their optical characteristics. It provide good penetration ability of substances for therapy and diagnosis as compared to conventional drugs in cancer therapy. It also shows minimum toxic effect. Due to their size shape and surface properties, these are more efficient in cancer diagnosis, therapy and radiotherapy. It is a type of solid nanoparticle (Chithrani et al., 2010; Huang & El-Sayed, 2010; Mesbahi, 2010; Najahi-Missaoui et al., 2020).

5.1.4. Silver Nanoparticle

Silver nanoparticles also got great attention as it serve as antimicrobial agents, biomedical device coatings, drug-delivery carriers, imaging probes, and diagnostic and optoelectronic platforms. It can absorb and scatter light and their function can be tailored by diverse size and shapes (Lee & Jun, 2019; Najahi-Missaoui et al., 2020).

5.1.5. Carbon Based nanoparticles

Carbon based nanoparticles have been a topic of consideration in many biomedical fields like drug delivery and gene therapy. By interaction with various biomarkers, it can help in indication and localization of targeted cells. A major class of carbon based nanoparticles that have a major role in biomedical fields is carbon nanotubes, as these are highly stable, specific surface chemistry and have unique physiochemical properties but still it show toxic effects against healthy tissue (Diez-Pascual, 2021; Najahi-Missaoui et al., 2020).

5.1.6. Lipid Based nanoparticles

Lipids based nanoparticles play a major role in cancer immunotherapy. Advances have made lipid based nanoparticles to deliver small drug particles as well as for the achievement of anticancer immunity system. This can be done via cytotoxic immune cell activation, checkpoint blockade, and chimeric antigen receptor cell therapies. Liposome is a major example of lipid based nanoparticles. These are vesicular systems, spherical shape, having a lipophilic bilayer present between two hydrophilic layers. It have a major role in treatment of cardiovascular disease, neurodegenerative disease, diabetes, cancer and inflammation. Their major properties include high encapsulation efficacy and extended circulation time. It have shown least toxic effects for *in-vivo* applications (Hald Albertsen et al., 2022; Najahi-Missaoui et al., 2020; Zhang, Yao, Hu, Zhao, & Lee, 2022).

5.2. Mode of Action of Novel Drug Delivery System

Novel Drug Delivery Systems (NDDS) function through innovative delivery approaches that boost drug potency and microorganism accessibility and specific drug location and help eliminate adverse effects. The transport mechanisms of NDDS make use of niosomes alongside nanoparticles and emulsions to encase drugs which release them in regulated amounts. Research demonstrates that nano drug delivery systems within NDDS show excellent potential to defeat drug resistance

and simultaneously enhance the stability properties and improved bioavailability of anticancer drugs (J. Liu et al., 2022).The ability of NDDS helped design nanovaccine platforms alongside drug reutilization strategies for COVID-19 treatment which proves the wide range of uses NDDS provides to handle critical health emergencies (Prabhu, 2022).

- ❖ The ability of NDDS to manage drug release at controlled rates leads to more effective therapies together with improved adherence from patients (S. Khan & Ali, 2022).
- ❖ Drugs administered through niosomes and nanoparticles can receive specific tissue or cellular targeting capabilities which enhances drug concentration at the target site while lowering the distribution to systemic tissues (J. Liu et al., 2022; Tarun Parashar, 2012).
- ❖ NDDS systems enhance drug bioavailability by improving soluble drug properties which results in better drug effectiveness rates (J Med Discov, Kumar, Devraj, & S.S.Apte, 2017).

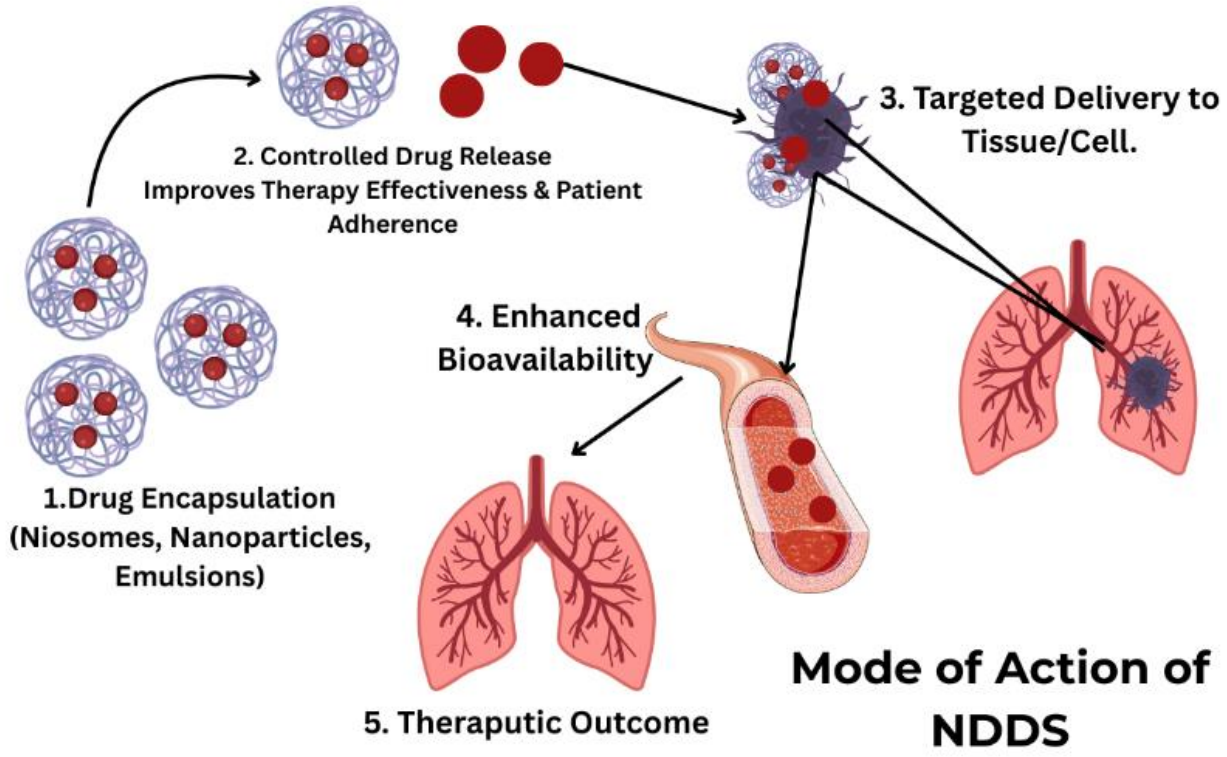


Figure 1. This figure outlines the sequential stages of NDDS in lung cancer treatment, (1) Beginning with drug encapsulation in nanocarriers such as niosomes or nanoparticles. (2) Slow release maintains therapeutically effective drug levels and minimizes undesirable exposure elsewhere in the body. (3) Digital optimization helps guide the drug to specific tissues and maximizes the treatment‘s efficiency. (4) Improved bioavailability promotes effective absorption and cellular uptake, (5) and results in enhanced treatment response with lower adverse effects.

5.3. Challenges and limitations

The successful delivery of NDDS drugs towards their target areas is prevented by critical barriers when operating inside complex biological systems. NDDS that release drugs without control give rise to suboptimal therapeutic results. The *in-vivo* performance evaluation of NDDS

systems faces limitations because there is a lack of suitable monitoring methods for these systems. NDDS remain unavailable on the market because of their high production and manufacturing expenses. People fail to follow their medicine plan because the drug administration processes are challenging to manage and they have difficulty understanding their dosage requirements. Drugs find it extremely difficult to pass through the blood-brain barrier because of its limited permeability during nervous system medication delivery (Varsha, Bagade, Kuldeep, Bindiya, & Riddhi, 2014). NDDS technology progress has generated an aggressive market that hinders innovation because of monetary barriers (Rao Khadam et al., 2024).

To overcome such limitations, digital methods have adopted for refining of nanoparticles in drug delivery systems.

6. Digital Methods for Refining Nanoparticle-Based Drug Delivery

Recent advancements of technologies have been proved efficient enough to overcome many of the limitations related to nanoparticle-based drug delivery. Many of the techniques have been adopted in accordance with the desire need of changes in drug delivery systems.

6.1. Artificial Intelligence and Machine Learning

The advent of Artificial Intelligence (AI) technology has brought transformative changes to numerous sectors such as medicine and drug discovery. The incorporation of artificial intelligence technologies into cancer research has led to marked enhancements in operational efficiency and result accuracy. It helps in producing better patient outcomes. The algorithm include machine learning, deep learning and neural networks which provide insights for nanoparticle formulation, targeted site delivery and reduced toxicity.

6.1.1. Role of AI and ML in Drug discovery and cancerous target identification

AI and ML helps in identification of anticancer points and drug aspirant by griping them with different algorithms like network based algorithms and machine learning methods. Additionally it is also efficient in understanding carcinogenesis and precision medicine by analyzing complex biological links via integration of multiomics data.

6.1.1.1. Screening and Molecular Docking

Artificial intelligence programs perform accelerated examination of extensive chemical collections to detect compounds that demonstrate effective binding to lung cancer targets. The use of machine learning models enables the prediction of molecular interactions which enhances the selection process for nanoparticle-loaded drugs (A. P. Das, Mathur, & Agarwal, 2024).

6.1.1.2. De-novo Drug Design

Generative AI models including generative adversarial networks (GANs) facilitate the creation of new drug molecules that possess ideal pharmacokinetic and pharmacodynamic characteristics through de novo drug design processes. Artificial intelligence systems examine current pharmaceutical compounds to discover potential new uses for lung cancer treatment which accelerates drug development processes while lowering associated expenses (L. Wang et al., 2023).

6.1.1.3. Multiomics

The integration of multiomics data through artificial intelligence techniques enables the combination of genomic, transcriptomic, and proteomic information to discover new lung cancer biomarkers which support the creation of targeted therapeutic approaches (D. Huang, Z. Li, T. Jiang, C. Yang, & N. Li, 2024).

6.1.1.4. Targeted Site Delivery

Artificial intelligence systems perform the mapping of biological pathways that drive lung cancer progression to identify essential genes and proteins which enable targeted nanoparticle-based drug delivery (You et al., 2022).

6.1.1.5. Personalized Treatment Strategies

AI algorithms perform intricate analyses of patient data to identify biomarkers linked to lung cancer progression which then allow for the development of personalized treatment strategies (Lococo et al., 2024).

6.1.2. Factors affecting nanoparticle-based drug delivery

Nanoparticle-based drug delivery systems encounter numerous obstacles including inadequate bioavailability combined with unintended systemic effects and inconsistent tumor penetration. The application of artificial intelligence in nanoparticle design processes leads to improved therapeutic results.

6.1.2.1. Physiochemical properties

The key barrier in nanomedicine practice remains the poor efficiency at which nanoparticles reach cancerous tumor locations. Physicochemical properties in combination with tumor models and cancer types allow AI and ML models to predict the delivery efficiency of nanoparticles. Deep neural networks deliver accurate predictions concerning maximum delivery efficiency (DEmax) and delivery efficiency at specific times (DE24 and DE168) (Lin et al., 2022).

6.1.2.2. Preparation of Smart Nanoparticles

The application of artificial intelligence techniques facilitates the creation of nanoparticles that respond to specific stimuli by releasing drugs when it detect signals from the tumor microenvironment such as pH levels, temperature changes, or enzyme activity.

6.1.2.3. Machine Learning Models for Nanoparticle Biodistribution

The biodistribution prediction for nanoparticles in tumor, liver, spleen and kidney organs utilizes Kernel Ridge Regression (KRR) as a ML model. The analysis of nanoparticle properties through these models evaluates zeta potential, core material and targeting approaches to enhance drug delivery optimization (Lin et al., 2022; Mahdi, Alhowyan, & Obaidullah, 2025).

6.1.2.4. AI-Driven Design of Nanoparticles

Artificial intelligence uses extensive analysis of biological system responses to nanoparticle properties to accelerate the process of designing new nanoparticles. The use of AI algorithms leads to exact and efficient computation of nanoparticle solvent-accessible surface area measurements (SASA) which represents a vital parameter for therapeutic applications (Jahandoost, Dashti, Houshmand, & Hosseini, 2024).

6.1.2.5. Ligand Optimization

The process of ligand optimization for targeting involves artificial intelligence systems predicting optimal ligands such as antibodies, proteins, and peptides to functionalize nanoparticles. It enhances their selective binding capacity to lung cancer cells. Through the application of artificial intelligence scientists develop nanoparticles capable of avoiding immune detection. It results in prolonged circulation periods and improved drug bioavailability (K. P. Das & J, 2022; Jena, Patra, Jammula, Rana, & Chand, 2024).

6.1.3. Integrating Artificial Intelligence with Nanomedicine for Advanced Lung Cancer Care

Medical researchers have developed AI-driven nanorobots supposed to work for accurate tumor targeting and drug delivery systems. The nanorobots employ integrated AI algorithms to move autonomously through the human body where they can selectively pick cancerous cells without harming healthy tissue (Siva, R, P, & P, 2024). Lung cancer treatment received significant improvement through the nanoparticle-based delivery of polo-like kinase 1 (PLK1) inhibitors together with programmed death-ligand 1 (PD-L1) antibodies. AI optimization of these nanoparticles seeks to create more effective drug release mechanisms which bring about better treatment results (Reda et al., 2021).

Scientists have created lipid-polymer hybrid nanoparticles (LPHNPs) which serve as path for pulmonary delivery and provide fast initiation after deposition in the lungs. By utilizing AI multiple factors about these nanoparticles can be improved including stability functions and release profiles alongside biocompatibility properties (Akhtar et al., 2024; Kassaee, Richard, Ayoko, & Islam, 2024). Nanoparticles act simultaneously as diagnostic tools to find cancer early and theranostic agents to perform diagnosis along with treatment. AI algorithms evaluate diagnostic data through accurate imaging and precise targeted treatment decisions (S. Das, Mazumdar, Khondakar, & Kaushik, 2024).

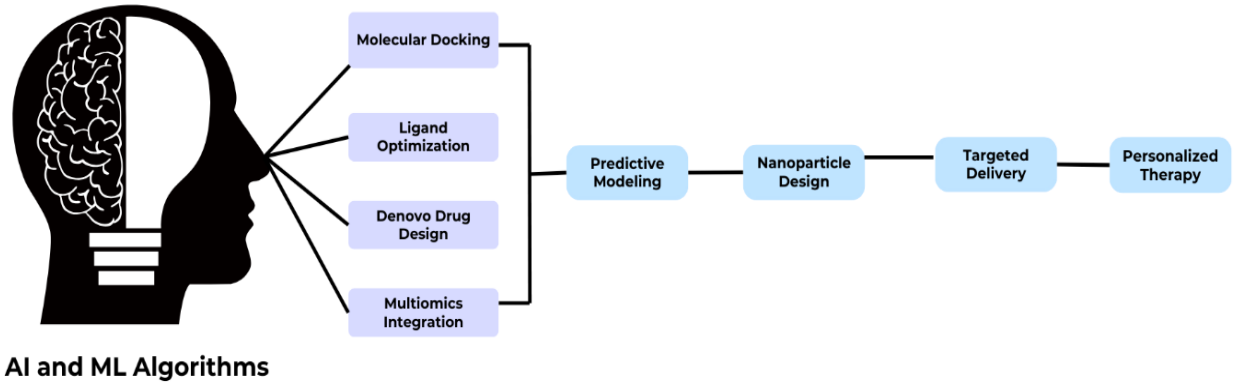


Figure 2. AI and ML optimize nanoparticle drug delivery for lung cancer through computational methods such as molecular docking, ligand optimization, de novo drug design, and multiomics analysis. This data-driven approach enables targeted, personalized therapy, improving treatment effectiveness and patient outcomes.

6.2. Computational Modeling and Simulations

The development of computational models serves as a vital mechanism to study and enhance the process of nanoparticle delivery to lung cancer cells. Research models supply scientists with predictive capabilities regarding the movement of NPs together with their deposition behavior and diffusion patterns and cellular absorption.

6.2.1. Role in Nanoparticle Based Drug Delivery System

6.2.1.1. Mechanistic Modeling and Simulations

The development of mathematical frameworks to explain nanoparticle pharmacokinetics and pharmacodynamics makes up mechanistic modeling. The application of mathematical models in anti-microRNA-155 therapy studies with immunotherapy demonstrated optimal therapeutic pairing through simulation (Cave et al., 2023). Additionally researchers developed an agent-based model for multicellular analysis. It examines nanoparticle uptake and drug release effect on tumor system dynamics and nanoparticle cellular division impact on treatment success rates (Y. Wang et al., 2024).

6.2.1.2. Multi-Scale Modeling

Multi-scale simulation combines data from molecules and organs to provide complete knowledge about the behavior of nanoparticles in biological structures. A study on solid tumors employed a multi-scale model to evaluate the impact of nanoparticle size, binding affinity, and drug release rate on treatment efficacy. It concludes that smaller nanoparticles with low binding affinity and controlled release rates are more effective. Another study utilized multi-scale simulations to investigate nanoparticle transport barriers, including circulation, extravasation, and cellular internalization. It provides insights into nanoparticle design for improved delivery (Farshad Moradi Kashkooli, Abazari, Soltani, Akbarpour Ghazani, & Rahmim, 2022; F. Moradi Kashkooli, Hornsby, Kolios, & Tavakkoli, 2024).

6.2.1.3. Physiologically Based Pharmacokinetic (PBPK) Modeling

PBPK modeling serves as an effective mathematical approach to estimate how nanoparticles spread across the body and their ability to reach intended targets. Multiple body physiological parameters together with tissue composition details and nanomaterial properties allow the method to construct body drug delivery simulations (Xie et al., 2024).

6.2.1.4. *In Silico* Modeling

In silico modeling has brought a revolutionary change to nanomedicine through the ability to replicate complicated biological processes in computer simulations. Researchers used *in silico* models for nanomedicine to develop a better understanding of how nanoparticles deliver their content to cells, how cells accept these nanoparticles and how medications release inside them. The researchers applied *in silico* modeling to determine individualized cancer therapies for NSCLC patients which helped identify resistance-breaking treatment combinations (Yilun, Yaojing, & Hongcan, 2024).

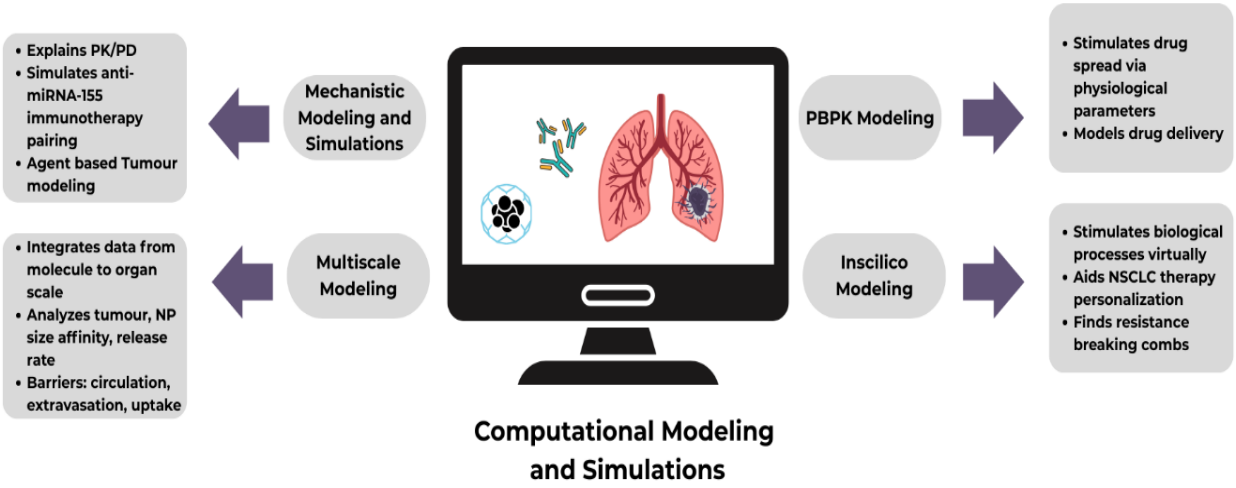


Figure 3. Computational modeling approaches in nanoparticle-based lung cancer therapy. Mechanistic, multiscale, PBPK, and in silico models simulate drug delivery, tumor interaction, and therapy personalization to optimize treatment outcomes.

6.2.2. Predictive Modeling and Simulation of Nanoparticle Behavior in Lung Cancer Treatment

Inhalable nanoparticles represent an effective strategy which can achieve localized treatment of lung cancer. The application of computational models allowed scientists to develop nanoparticles with optimal aerodynamic features to achieve efficient lung deposition without exposing the body system. Scientists have established models which achieve better drug access into solid tumors through changes to the extracellular matrix that functions as a barrier to medication penetration (A et al., 2024; Abdel-Hafez, Gallei, Wagner, & Schneider, 2024; Tarawneh et al., 2022).

Computational analyses which use images have been utilized to model drug delivery to solid tumors. These predictive models use authentic data on tumor and capillary networks to determine therapeutic responses. It assess how both drug release rate and nanoparticle dimensions affect treatment success. These frameworks become essential tools for planning optimized delivery approaches and they help estimate individual patient responses (Farshad Moradi Kashkooli et al., 2022; F. Moradi Kashkooli et al., 2024).

Scientific models exist to investigate how nanoparticles affect both pharmacological drug absorption and pharmacological drug effects. The predictive models help to monitor the way anti-microRNA-155 nanoparticles work alongside traditional immunotherapies by uncovering drug interaction potential and developing suitable drug release schedules. Future treatment opportunities show promise because they offer better clinical performance alongside reduced medication toxicities and decreased exposure to drugs (Cave et al., 2023).

6.3. Digital Twin Technology

A Digital Twin serves as an exact digital copy of physical objects which runs through real-time information together with AI algorithms and simulations. The healthcare field uses virtual representations of patients' whole bodies and tumors alongside their biological systems to enable research teams and medical staff to optimize treatment methods including nanoparticle drugs before they reach the clinical phase.(Machado & Berssaneti, 2023) Digital twins enable modeling of essential elements which concentrate on nanoparticle-based drug delivery within the human body (Bruynseels, Santoni de Sio, & van den Hoven, 2018).

- Lung anatomy and airflow patterns
- Tumor location and physiology
- Nanoparticle behavior within lung tissues
- Drug absorption, distribution, and clearance

The technique integrates patient-oriented data from medical scans and biomarkers as well as lung testing functions into algorithmic code to produce a computational simulation of patient lung tissue. (Corral-Acero et al., 2020).The twin automatically updates through real-time clinical data which allows its development to track disease progression and treatment effects. Multi-physics simulation: Integrates different modeling techniques, such as Computational Fluid Dynamics (CFD) for airflow and Molecular Dynamics (MD) for nanoparticle-cell interactions (Tao, Qi, Wang, & Nee, 2019).

Every patient experiences unique behavior within their lung tumors because of the high heterogeneity of the disease. Drug resistance together with evolving tumors prevents standard treatments from succeeding (Stahlberg et al., 2022). Patient-specific digital treatment models help enhance therapy outcomes when built by digital twin technology.

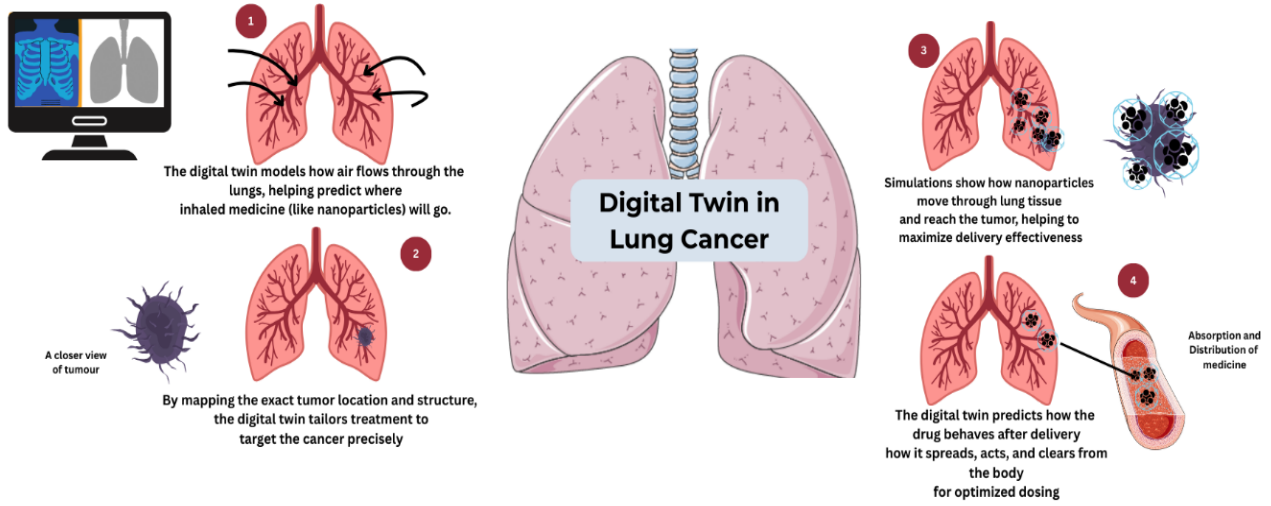


Figure 4. Digital twin models simulate airflow, tumor localization, nanoparticle transport, and drug behavior to enable precise targeting and optimized dosing.

6.3.1. Real-Time Digital Twin Simulations for Monitoring and Adjusting Cancer Nanotherapy

Digital representation of lung cancer patients‘ anatomical and physiological features requires construction. A model should demonstrate how nanoparticles moving through the lungs and tumors after inhalation or intravenous delivery. The model helps scientists predict how drugs distribute in the lungs and tumors while determining both the absorption rates and targeting Differences in genetic variations together with variations in tumor microenvironments make every patient with lung cancer react uniquely to treatment methods (J. E. Holder et al., 2023).

The digital twin requires testing with various nanoparticle sizes as well as shapes and surface coating combinations. Select the nanoparticle formulation which maximizes its ability to stay in the lungs while reaching tumors while keeping patients safe (Carrasco-Esteban et al., 2021).

A simulation must be conducted to assess the performance of nanoparticle-based chemotherapy through extended treatment sessions. Medical staff should make dosage schedule adaptations following simulations of tumor reduction alongside patient immune response and adverse reactions (J. Wang, Zhou, Liu, Chen, & Yu, 2022).

The digital twin runs ongoing updates with recently collected clinical data points which may include tumor growth information and immune system metrics. The model enables proactive treatment changes through which the nanoparticle delivery approach or dosage plan can be adjusted when the prediction forecasts suboptimal drug distribution (Katsoulakis et al., 2024).

6.4. Digital Imaging and Analysis

The characterization of nanoparticles and drug monitoring and therapeutic outcome assessment in lung cancer therapy heavily depend on digital imaging and analysis. *In-vivo* tracking along with advanced image processing joined with high-resolution imaging techniques gives important data regarding nanoparticle actions and efficiency.

6.4.1. High Resolution Imaging Technology

Nanoparticle study requires detailed imaging techniques to investigate their shape features alongside their dimension spread and external facial parameters. Popular techniques used for this purpose include the following:

6.4.1.1. Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy functions as a strong method to observe nanoparticles through atomic-level resolution. This method enables researchers to inspect nanoparticle structures in combination with examining uniformity of sizes and determining drug encapsulation levels. A study with doxorubicin-loaded liposomes used TEM to monitor their spherical details and ensure suitable drug loading for lung cancer treatment (Cheng et al., 2014).

6.4.1.2. Scanning Electron Microscopy (SEM)

Surface morphology pictures of nanoparticles can be observed in detail through SEM. The technique shows its best value for inspecting drug delivery systems constructed with polymeric or metallic nanoparticles. Scientists applied SEM to analyze gold nanoparticles' spherical structure along with their surface modifications which increased the drug's ability to target lung cancer cells (Tang, Wang, Wei, Mu, & Han, 2021).

6.4.1.3. Atomic Force Microscopy (AFM)

AFM offers surface topography analysis in all dimensions and nanoparticles can be studied for their mechanical properties. This technique functions extensively to analyze interferactions among nanoparticles and drugs on the nanometer scale. The stability evaluation of polymeric nanoparticles for lung cancer *in-vivo* treatment under physiological conditions was done through AFM analysis (Fulton & Najahi-Missaoui, 2023).

6.4.2. Image Processing for Nanoparticle Characterization

The technology of artificial intelligence (AI) improves image segmentation methods which allow the characterization of nanoparticles present in drug delivery systems. A system identifies biological sample nanoparticle dimensions together with morphology and dispersion characteristics automatically. The accuracy of tumor tissue-nanoparticle analysis has increased. AI-controlled segmentation processes show that the technology manages to eliminate human mistakes for accurate nanoparticle analysis in lung cancer treatment applications. For instance ligand conjugation on gold nanoparticles for targeted lung cancer therapy was done via AI assisted image processing (Adir et al., 2020).

6.4.3. *In-vivo* Imaging for Real Time Monitoring

Research-grade monitoring of nanoparticles becomes necessary to measure their global travel patterns as well as their tumor sequestration and treatment performance:

6.4.3.1. Fluorescence Imaging

The fluorescent properties of labeled nanoparticles provide direct time-based imaging of drug delivery routes. The selectivity of polymeric nanoparticles to accumulate in lung tumors became

visible using fluorescence detection methods which demonstrated decreased impact on unintended targets (Sun et al., 2023).

6.4.3.2. Magnetic Resonance Imaging (MRI)

Through MRI technology researchers can visualize detailed tissue images of nanoparticles without harming the patient. Superparamagnetic Iron Oxide Nanoparticles (SpIONs) serve as superb MRI contrast enhancers for lung cancer detection purposes. The application of SPIONs with MRI guidance resulted in better delivery precision within lung tumors (Sun et al., 2023).

6.4.3.3. Positron Emission Tomography (PET) and Computed Tomography (CT)

PET and CT scanners allow researchers to evaluate real-time distribution patterns of radiolabeled nanoparticles by providing precise quantitative analysis. PET scanners using labeled liposomes indicated longer systemic presence and better lung malignancy targeting capabilities (Sun et al., 2023).

7. Challenges and Limitations

The widespread application and effectiveness of nanoparticle-based lung cancer therapy drug delivery systems through digital methods faces multiple technological and practical barriers. The barriers to implementation divide into technical barriers that combine with ethical, regulatory and practical limitations.

7.1. Technical Challenges

Digital methods depend completely on accurate and extensive data datasets. Researchers face difficulties when uniting information from diverse origin points (such as genomic and proteomic together with clinical information) because of conflicting data, missing entries and various document formats (Dongdong Huang, Zifang Li, Tao Jiang, Chaojuan Yang, & Ning Li, 2024). Accurate *in-vivo* modeling of nanoparticle behavior presents an ongoing major challenge to the complex workings of human beings. Tumor heterogeneity and immune responses along with the blood-brain barrier obstacle predictions through various stages of research (Lin et al., 2022). Reliable execution of complex simulations coupled with AI algorithms demands both substantial power and financing resources and significant periods of time. Real-time applications demand substantial resources for implementing digital twin technology and other programs (Jahandoost et al., 2024). Prediction capabilities of AI and ML models for nanoparticles remain restricted because both teaching data quality and biological interaction intricacy affect their output precision. The regular occurrence includes both over fitting problems alongside generalization concerns (Mahdi et al., 2025).

7.2. Ethical and Privacy Concerns

There exists substantial worry about patient data privacy together with data protection issues when AI and digital twin models are utilized. Following General Data Protection Regulation (GDPR) and Health Insurance Portability and Accountability Act (HIPAA) regulations demands attention while the enforcement requirements remain complex to satisfy (Bruynseels et al., 2018). The training data used in AI systems transfers biases that lead to different treatment outcomes

between demographic groups. It is vital to establish fairness in addition to inclusiveness when building AI-driven drug delivery frameworks (Topol, 2019).

7.3. Regulatory and Standardization issues

Advancements in digital technology methods have progressed faster than the development of governing regulations. Researchers along with companies developing nanoparticle-based therapies face an uncertain environment because of this condition (De Jong & Borm, 2008). The field lacks established set protocols for the use of AI alongside ML and computational modeling in drug delivery systems. It becomes impossible to verify research consistency due to this difficulty (Najahi-Missaoui et al., 2020).

7.4. Practical and Economic Barrier

Digital methods demand substantial budget allocations towards building automated systems together with software development and knowledge-based human resources. The limitation poses difficulties for small academic and industrial organizations (Rao Khadam et al., 2024). Patients tend to show lesser adherence to therapeutic programs using nanoparticle-based drugs because of their intricate dosing needs and challenging administration techniques. The advanced treatment methods often remain outside patient reach because of high costs together with limited accessibility (Vikal et al., 2024). The translation of preclinical digital methods fails to succeed in clinical applications due to incompatible differences between experimental laboratory conditions and real in-clinic environments (He, Liu, Morin, Liu, & Schwendeman, 2019).

7.5. Scientific and Biological Limitation

Since nanoparticles trigger immune system responses which leads to their removal from the body they become less effective. Manufacturers of digital nanoparticles must build immune system tolerance into their design processes (Wilhelm et al., 2016). Different areas within lung tumors show varying levels of response toward treatment according to their heterogeneity. The complexity of designing standard nanoparticle therapies increases because of this factor (Jessica E. Holder et al., 2023). Solid tumors resist nanoparticle penetration because they possess thick extracellular matrices along with high interstitial pressure. The delivery efficiency requires digital methods to overcome these barriers that prevent effective delivery (F. Moradi Kashkooli et al., 2024).

8. Summary

Total lung cancer treatment strategies benefit from digital methods when working with nanoparticle-based drug delivery platforms. Digital technology tools allow researchers to create precise nanoparticles for targeted drug delivery systems and individualized treatment customs. It helps to boost efficiency and minimize toxic effects. Several obstacles including technical barriers and ethical concerns also together with high costs and regulations create barriers to overcome. Using nanotechnology and digital intelligence enables significant progress in lung cancer therapy while improving treatment results toward eliminating lung cancer from being the leading cause of death. The achievement of advanced treatment maximum potential depends on both continuous innovation along with interdisciplinary collaboration.

References

A, R., Han, Z., Wang, T., Zhu, M., Zhou, M., & Sun, X. (2024). Pulmonary delivery of nano-particles for lung cancer diagnosis and therapy: Recent advances and future prospects. *Wiley Interdiscip Rev Nanomed Nanobiotechnol, 16*(1), e1933. doi:10.1002/wnan.1933

Abdel-Hafez, S. M., Gallei, M., Wagner, S., & Schneider, M. (2024). Inhalable nano-structured microparticles for extracellular matrix modulation as a potential delivery system for lung cancer. *Eur J Pharm Biopharm, 204*, 114512. doi:10.1016/j.ejpb.2024.114512

Adir, O., Poley, M., Chen, G., Froim, S., Krinsky, N., Shklover, J., ... & Schroeder, A. (2020). Integrating artificial intelligence and nanotechnology for precision cancer medicine. *Advanced materials*, *32*(13), 1901989.

Advances in Drug Delivery Systems: Review Article. (2024). *Journal of Pharma Insights and Research, 2*(3), 088–095. doi:10.69613/xakkge51

Akhtar, Y., Ahmad, S., Khalid, F., Junjua, M. U. D., Aryanfar, Y., Hendy, A. S., ... & Soliman, A. T. (2024). Machine learning-a new paradigm in nanoparticle-mediated drug delivery to cancerous tissues through the human cardiovascular system enhanced by magnetic field. *Scientific Reports*, *14*(1), 22048.

Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., & Jemal, A. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin, 74*(3), 229-263. doi:10.3322/caac.21834

Bruynseels, K., Santoni de Sio, F., & van den Hoven, J. (2018). Digital Twins in Health Care: Ethical Implications of an Emerging Engineering Paradigm. *Front Genet, 9*, 31. doi:10.3389/fgene.2018.00031

Budak, M., & Segmen, H. (2022). Cancer Genes and Breast Cancers. In M. Budak & R. Rajkumar (Eds.), *Molecular Mechanisms in Cancer*. Rijeka: IntechOpen.

Carrasco-Esteban, E., Domínguez-Rullán, J. A., Barrionuevo-Castillo, P., Pelari-Mici, L., Leaman, O., Sastre-Gallego, S., & López-Campos, F. (2021). Current role of nanoparticles in the treatment of lung cancer. *J Clin Transl Res, 7*(2), 140-155.

Cave, J., Shinglot, V., Butner, J. D., Cristini, V., Ozpolat, B., Calin, G. A., ... & Wang, Z. (2023, July). Mechanistic modeling of anti-microRNA-155 therapy combinations in lung cancer. In *2023 45th Annual International Conference of the IEEE Engineering in Medicine & Biology Society (EMBC)* (pp. 1-4). IEEE.

Cheng, L., Huang, F. Z., Cheng, L. F., Zhu, Y. Q., Hu, Q., Li, L., ... & Chen, D. W. (2014). GE11-modified liposomes for non-small cell lung cancer targeting: preparation, ex vitro and in vivo evaluation. *International journal of nanomedicine*, 921-935.

Chithrani, D. B., Jelveh, S., Jalali, F., Van Prooijen, M., Allen, C., Bristow, R. G., ... & Jaffray, D. A. (2010). Gold nanoparticles as radiation sensitizers in cancer therapy. *Radiation research*, *173*(6), 719-728.

Collins, L. G., Haines, C., Perkel, R., & Enck, R. E. (2007). Lung cancer: diagnosis and management. *Am Fam Physician, 75*(1), 56-63.

Corral-Acero, J., Margara, F., Marciniak, M., Rodero, C., Loncaric, F., Feng, Y., ... & Lamata, P. (2020). The ‗Digital Twin'to enable the vision of precision cardiology. *European heart journal*, *41*(48), 4556-4564.

Dancey, J., & Le Chevalier, T. (1997). Non-small cell lung cancer: an overview of current management. *Eur J Cancer, 33 Suppl 1*, S2-7. doi:10.1016/s0959-8049(96)00325-5

Das, A. P., Mathur, P., & Agarwal, S. M. (2024). Machine Learning, Molecular Docking, and Dynamics-Based Computational Identification of Potential Inhibitors against Lung Cancer. *ACS Omega, 9*(4), 4528-4539. doi:10.1021/acsomega.3c07338

Das, K. P., & J, C. (2022). Nanoparticles and convergence of artificial intelligence for targeted drug delivery for cancer therapy: Current progress and challenges. *Front Med Technol, 4*, 1067144. doi:10.3389/fmedt.2022.1067144

Das, S., Mazumdar, H., Khondakar, K. R., & Kaushik, A. (2024). Machine learning integrated graphene oxide-based diagnostics, drug delivery, analytical approaches to empower cancer diagnosis. *BMEMat, n/a*(n/a), e12117. doi:https://doi.org/10.1002/bmm2.12117

De Jong, W. H., & Borm, P. J. (2008). Drug delivery and nanoparticles:applications and hazards. *Int J Nanomedicine, 3*(2), 133-149. doi:10.2147/ijn.s596

Dhanasekaran, R., Deutzmann, A., Mahauad-Fernandez, W. D., Hansen, A. S., Gouw, A. M., & Felsher, D. W. (2022). The MYC oncogene - the grand orchestrator of cancer growth and immune evasion. *Nat Rev Clin Oncol, 19*(1), 23-36. doi:10.1038/s41571-021-00549-2

Diez-Pascual, A. M. (2021). Carbon-based nanomaterials. *International Journal of Molecular Sciences*, *22*(14), 7726.

Fulton, M. D., & Najahi-Missaoui, W. (2023). Liposomes in Cancer Therapy: How Did We Start and Where Are We Now. *International Journal of Molecular Sciences, 24*(7), 6615. Retrieved from https://www.mdpi.com/1422-0067/24/7/6615

Garg, P., Malhotra, J., Kulkarni, P., Horne, D., Salgia, R., & Singhal, S. S. (2024). Emerging Therapeutic Strategies to Overcome Drug Resistance in Cancer Cells. *Cancers (Basel), 16*(13). doi:10.3390/cancers16132478

Guirgis, H. M. (2012). Proposal for a novel methodology to screen and score cost versus survival for anticancer drugs in metastatic disease: could cost weigh in evaluation? *J Oncol Pract, 8*(4), 224-230. doi:10.1200/jop.2011.000390

Hald Albertsen, C., Kulkarni, J. A., Witzigmann, D., Lind, M., Petersson, K., & Simonsen, J. B. (2022). The role of lipid components in lipid nanoparticles for vaccines and gene therapy. *Adv Drug Deliv Rev, 188*, 114416. doi:10.1016/j.addr.2022.114416

Han, Z., Wang, T., Zhu, M., Zhou, M., & Sun, X. (2023). Pulmonary delivery of nano-particles for lung cancer diagnosis and therapy: Recent advances and future prospects. *Wiley Interdisciplinary reviews. Nanomedicine and Nanobiotechnology*, e1933-e1933.

Hjartåker, A., Weiderpass, E., & Bray, F. (2014). Cancer Mortality. In.

Holder, J. E., Ferguson, C., Oliveira, E., Lodeiro, C., Trim, C. M., Byrne, L. J., ... & Wilson, C. M. (2023). The use of nanoparticles for targeted drug delivery in non-small cell lung cancer. *Frontiers in oncology*, *13*, 1154318.

Huang, D., Li, Z., Jiang, T., Yang, C., & Li, N. (2024). Artificial intelligence in lung cancer: current applications, future perspectives, and challenges. *Front Oncol, 14*, 1486310. doi:10.3389/fonc.2024.1486310

Huang, X., & El-Sayed, M. A. (2010). Gold nanoparticles: Optical properties and implementations in cancer diagnosis and photothermal therapy. *Journal of Advanced Research, 1*(1), 13-28. doi:https://doi.org/10.1016/j.jare.2010.02.002

Kumar, G. P., Rambhau, D., & Apte, S. S. (2017). Development of NDDS of carbamazepine in epilepsy for medical discovery of formulation significance. *Journal of Medical Discovery*, *2*(2), 1-13.

Jahandoost, A., Dashti, R., Houshmand, M., & Hosseini, S. A. (2024). Utilizing machine learning and molecular dynamics for enhanced drug delivery in nanoparticle systems. *Sci Rep, 14*(1), 26677. doi:10.1038/s41598-024-73268-0

Jena, G. K., Patra, C. N., Jammula, S., Rana, R., & Chand, S. (2024). Artificial Intelligence and Machine Learning Implemented Drug Delivery Systems: A Paradigm Shift in the Pharmaceutical Industry. *Journal of Bio-X Research, 7*, 0016. doi:doi:10.34133/jbioxresearch.0016

Kassaee, S. N., Richard, D., Ayoko, G. A., & Islam, N. (2024). Lipid polymer hybrid nanoparticles against lung cancer and their application as inhalable formulation. *Nanomedicine (Lond), 19*(25), 2113-2133. doi:10.1080/17435889.2024.2387530

Katsoulakis, E., Wang, Q., Wu, H., Shahriyari, L., Fletcher, R., Liu, J., ... & Deng, J. (2024). Digital twins for health: a scoping review. *NPJ digital medicine*, *7*(1), 77.

Khadam, V. K. R., Singh, R. P., Prajapati, D., Yunus, M., Prajapat, B., Rai, S. K., ... & Gogoi, D. (2024). An Updated Comprehensive Review on Novel Drug Delivery Systems (NDDS) In the Pharmaceuticals. *Asian Journal of Pharmaceutical Research and Development*, *12*(1), 55-64.

Khan, S. U., Fatima, K., Aisha, S., & Malik, F. (2024). Unveiling the mechanisms and challenges of cancer drug resistance. *Cell Communication and Signaling, 22*(1), 109. doi:10.1186/s12964-023-01302-1

Khan, S., & Ali, H. (2022). Novel Drug Delivery Systems. In (pp. 235-250).

Lee, S. H., & Jun, B. H. (2019). Silver Nanoparticles: Synthesis and Application for Nanomedicine. *Int J Mol Sci, 20*(4). doi:10.3390/ijms20040865

Li, D., Ding, L., Ran, W., Huang, Y., Li, G., Wang, C., ... & Xing, X. (2020). Status of 10 targeted genes of non-small cell lung cancer in eastern China: A study of 884 patients based on NGS in a single institution. *Thoracic cancer*, *11*(9), 2580-2589.

Lin, Z., Chou, W. C., Cheng, Y. H., He, C., Monteiro-Riviere, N. A., & Riviere, J. E. (2022). Predicting Nanoparticle Delivery to Tumors Using Machine Learning and Artificial Intelligence Approaches. *Int J Nanomedicine, 17*, 1365-1379. doi:10.2147/ijn.S344208

Liu, B., Zhou, H., Tan, L., Siu, K. T. H., & Guan, X.-Y. (2024). Exploring treatment options in cancer: tumor treatment strategies. *Signal Transduction and Targeted Therapy, 9*(1), 175. doi:10.1038/s41392-024-01856-7

Liu, J., Li, S., Wang, J., Li, N., Zhou, J., & Chen, H. (2022). Application of Nano Drug Delivery System (NDDS) in Cancer Therapy: A Perspective. *Recent Pat Anticancer Drug Discov, 18*(2), 125-132. doi:10.2174/1574892817666220713150521

Lococo, F., Ghaly, G., Chiappetta, M., Flamini, S., Evangelista, J., Bria, E., ... & Mohammed, A. (2024). Implementation of artificial intelligence in personalized prognostic assessment of lung cancer: a narrative review. *Cancers*, *16*(10), 1832.

Machado, T. M., & Berssaneti, F. T. (2023). Literature review of digital twin in healthcare. *Heliyon, 9*(9), e19390. doi:10.1016/j.heliyon.2023.e19390

Mahdi, W. A., Alhowyan, A., & Obaidullah, A. J. (2025). Intelligence analysis of drug nanoparticles delivery efficiency to cancer tumor sites using machine learning models. *Sci Rep, 15*(1), 1017. doi:10.1038/s41598-024-84450-9

Mesbahi, A. (2010). A review on gold nanoparticles radiosensitization effect in radiation therapy of cancer. *Rep Pract Oncol Radiother, 15*(6), 176-180. doi:10.1016/j.rpor.2010.09.001

Moore, A. R., Rosenberg, S. C., McCormick, F., & Malek, S. (2020). RAS-targeted therapies: is the undruggable drugged? *Nat Rev Drug Discov, 19*(8), 533-552. doi:10.1038/s41573-020-0068-6

Moradi Kashkooli, F., Abazari, M. A., Soltani, M., Akbarpour Ghazani, M., & Rahmim, A. (2022). A spatiotemporal multi-scale computational model for FDG PET imaging at different stages of tumor growth and angiogenesis. *Scientific Reports, 12*. doi:10.1038/s41598-022-13345-4

Moradi Kashkooli, F., Hornsby, T. K., Kolios, M. C., & Tavakkoli, J. J. (2024). Ultrasound-mediated nano-sized drug delivery systems for cancer treatment: Multi-scale and multi-physics computational modeling. *Wiley Interdiscip Rev Nanomed Nanobiotechnol, 16*(1), e1913. doi:10.1002/wnan.1913

Mundel, R., Dhadwal, S., Bharti, S., & Chatterjee, M. (2023). A Comprehensive Overview of Various Cancer Types and Their Progression. In (pp. 1-17).

Najahi-Missaoui, W., Arnold, R. D., & Cummings, B. S. (2020). Safe Nanoparticles: Are We There Yet? *Int J Mol Sci, 22*(1). doi:10.3390/ijms22010385

Ostrow, S. L., Barshir, R., DeGregori, J., Yeger-Lotem, E., & Hershberg, R. (2014). Cancer evolution is associated with pervasive positive selection on globally expressed genes. *PLoS Genet, 10*(3), e1004239. doi:10.1371/journal.pgen.1004239

Ou, X., Gao, G., Habaz, I. A., & Wang, Y. (2024). Mechanisms of resistance to tyrosine kinase inhibitor-targeted therapy and overcoming strategies. *MedComm, 5*(9), e694. doi:https://doi.org/10.1002/mco2.694

Prabhu, R. (2022). Advances in NDDS: An expansive menu of choices in the management of pandemic. *International Journal of Frontiers in Biology and Pharmacy Research, 2*, 001-006. doi:10.53294/ijfbpr.2022.2.2.0031

Qin, K., Hou, H., Liang, Y., & Zhang, X. (2020). Prognostic value of TP53 concurrent mutations for EGFR- TKIs and ALK-TKIs based targeted therapy in advanced non-small cell lung cancer: a meta-analysis. *BMC Cancer, 20*(1), 328. doi:10.1186/s12885-020-06805-5

Reda, M., Ngamcherdtrakul, W., Nelson, M., Siriwon, N., Wang, R., Zaidan, H., ... & Yantasee, W. (2022). Development of novel immunotherapy based on nanoparticle co-delivering PLK1 and PD-L1 inhibitors for lung cancer treatment.

Schabath, M. B., & Cote, M. L. (2019). Cancer Progress and Priorities: Lung Cancer. *Cancer Epidemiol Biomarkers Prev, 28*(10), 1563-1579. doi:10.1158/1055-9965.Epi-19-0221

Siegel, R. L., Miller, K. D., Wagle, N. S., & Jemal, A. (2023). Cancer statistics, 2023. *CA: A Cancer Journal for Clinicians, 73*(1), 17-48. doi:https://doi.org/10.3322/caac.21763

Siva, S., R, D., P, S., & P, N. (2024). *Revolutionizing Cancer Therapy: Artificial Intelligence-Controlled Nanorobots for Precise and Non-Invasive Tumor Eradication*.

Stahlberg, E. A., Abdel-Rahman, M., Aguilar, B., Asadpoure, A., Beckman, R. A., Borkon, L. L., ... & Zervantonakis, I. (2022). Exploring approaches for predictive cancer patient digital twins: Opportunities for collaboration and innovation. *Frontiers in Digital Health*, *4*, 1007784.

Sun, L., Liu, H., Ye, Y., Lei, Y., Islam, R., Tan, S., ... & Cai, L. (2023). Smart nanoparticles for cancer therapy. *Signal transduction and targeted therapy*, *8*(1), 418.

Sune, P., Jumde, K., Hatwar, P., Bakal, & Korde, A. (2024). Advances in oral controlled release drug delivery systems. *GSC Biological and Pharmaceutical Sciences, 29*, 286-297. doi:10.30574/gscbps.2024.29.3.0475

Tanday, S. (2015). Global cancer cases on the rise. *Lancet Oncol, 16*(7), e317. doi:10.1016/s1470-2045(15)00022-4

Tang, X., Wang, Z., Wei, F., Mu, W., & Han, X. (2021). Recent Progress of Lung Cancer Diagnosis Using Nanomaterials. *Crystals, 11*(1), 24. Retrieved from https://www.mdpi.com/2073-4352/11/1/24

Tao, F., Qi, Q., Wang, L., & Nee, A. (2019). Digital Twins and Cyber–Physical Systems toward Smart Manufacturing and Industry 4.0: Correlation and Comparison. *Engineering, 5*, 653-661. doi:10.1016/j.eng.2019.01.014

Tarawneh, S., Dahmash, E., Alyami, H., Abu-Doleh, S., Hussein-Al-Ali, S., Iyire, A., & Abuthawabeh, R. (2022). Mechanistic Modelling of Targeted Pulmonary Delivery of Dactinomycin Iron Oxide Loaded Nanoparticles for Lung Cancer Therapy. *Pharmaceutical Development and Technology, 27*, 1-35. doi:10.1080/10837450.2022.2152047

Topol, E. J. (2019). High-performance medicine: the convergence of human and artificial intelligence. *Nat Med, 25*(1), 44-56. doi:10.1038/s41591-018-0300-7

Varsha, A., Bagade, O., Kuldeep, R., Bindiya, P., & Riddhi, P. (2014). Poles apart Inimitability of Brain Targeted Drug Delivery system in Middle of NDDS. *International Journal of Drug Development and Research, 6*, 15-27.

Venkateswara Reddy, B. (2024). Novel Drug Delivery Systems. In (pp. 713-775).

Vikal, A., Maurya, R., Bhowmik, S., Patel, P., Gupta, G. D., & Kurmi, B. (2024). From Conventional to Cutting-Edge: A Comprehensive Review on Drug Delivery Systems. *Drug Delivery Letters, 14*. doi:10.2174/0122103031304556240430161553

Wang, J., Zhou, T., Liu, Y., Chen, S., & Yu, Z. (2022). Application of Nanoparticles in the Treatment of Lung Cancer With Emphasis on Receptors. *Frontiers in Pharmacology, 12*. doi:10.3389/fphar.2021.781425

Wang, L. H., Wu, C. F., Rajasekaran, N., & Shin, Y. K. (2018). Loss of Tumor Suppressor Gene Function in Human Cancer: An Overview. *Cell Physiol Biochem, 51*(6), 2647-2693. doi:10.1159/000495956

Wang, L., Song, Y., Wang, H., Zhang, X., Wang, M., He, J., ... & Cao, L. (2023). Advances of artificial intelligence in anti-cancer drug design: A review of the past decade. *Pharmaceuticals*, *16*(2), 253.

Wang, Y., Bucher, E., Rocha, H., Jadhao, V., Metzcar, J., Heiland, R., ... & Macklin, P. (2024). Drug-loaded nanoparticles for cancer therapy: a high-throughput multicellular agent-based modeling study. *bioRxiv*.

Wang, Y., Li, P., Truong-Dinh Tran, T., Zhang, J., & Kong, L. (2016). Manufacturing Techniques and Surface Engineering of Polymer Based Nanoparticles for Targeted Drug Delivery to Cancer. *Nanomaterials (Basel), 6*(2). doi:10.3390/nano6020026

Wilhelm, S., Tavares, A. J., Dai, Q., Ohta, S., Audet, J., Dvorak, H. F., & Chan, W. C. (2016). Analysis of nanoparticle delivery to tumours. *Nature reviews materials*, *1*(5), 1-12.

Wittes, R. E. (1993). Small-cell lung cancer: an overview of issues in therapy. *Semin Surg Oncol, 9*(2), 127-134. doi:10.1002/ssu.2980090211

Xie, L., Xie, D., Du, Z., Xue, S., Wang, K., Yu, X., ... & Fang, C. (2024). A novel therapeutic outlook: Classification, applications and challenges of inhalable micron/nanoparticle drug delivery systems in lung cancer. *International Journal of Oncology*, *64*(4), 38.

Yilun, W., Yaojing, Z., & Hongcan, S. (2024). Nanoparticle trends and hotspots in lung cancer diagnosis from 2006-2023: a bibliometric analysis. *Frontiers in Oncology, 14*. doi:10.3389/fonc.2024.1453021

You, Y., Lai, X., Pan, Y., Zheng, H., Vera, J., Liu, S., ... & Zhang, L. (2022). Artificial intelligence in cancer target identification and drug discovery. *Signal Transduction and Targeted Therapy*, *7*(1), 156.

Zhang, Z., Yao, S., Hu, Y., Zhao, X., & Lee, R. J. (2022). Application of lipid-based nanoparticles in cancer immunotherapy. *Front Immunol, 13*, 967505. doi:10.3389/fimmu.2022.967505

About The Authors

Ms. Nehal Ali is currently pursuing a Bachelor's degree in Biotechnology (2021–2025) at Government College University, Lahore, Pakistan. She is passionate about the application of biotechnology in modern medicine and is particularly interested in nanoparticle-based drug delivery systems. Her academic focus is on integrating digital tools and artificial intelligence in biomedical research, with the aim of developing innovative solutions in cancer therapy.

Email: nehalali325@gmail.com ORCID: 0009-0001-2997-1017

Dr. Saba Sana is currently working as an Assistant Professor in Department of Microbiology Dr. Ikram ul Haq Institute of Industrial Biotechnology, Government College University Lahore. Previously she served as Lecture in UVAS Lahore Pakistan. She has earned her PhD degree in Microbiology from UVAS Lahore Pakistan. Her areas of interests are Mycology, mycotoxins, antimicrobial resistance, biosafety and biosecurity.

Email: saba.sana@gcu.edu.pk ORCID: 0009-0003-6837-3209

Ms. Samina Tahir is a dedicated visiting faculty member in Department of Biotechnology- Dr. Ikram ul Haq Institute of Industrial Biotechnology, Government College University Lahore, where she has been inspiring future biotechnologists for the past two years. She holds an M.Phil in Biotechnology from the KAM School of Life Sciences at Forman Christian College University, and completed her bachelor's degree at Kinnaird College for Women. Samina's research journey began with work on *Toxoplasma gondii* detection in pet cats and their owners, sparking her interest in public health. Her M.Phil research was on collaborative research project with PARB to develop herbicide-resistant transgenic cotton, contributing to sustainable agricultural practices.

Email: saminakinnaird@gmail.com ORCID: 0009-0008-2687-778X

Dr. Shahid Sherzada is currently working as Assistant Professor, Department of Zoology Government College University Lahore. Previously he served as Lecture in two well-known institutions, UVAS and Virtual University of Pakistan. He has earned is PhD degree in Zoology from University of the Punjab Lahore Pakistan. His field of interest is Aquatic Ecology and Biotechnology.

Email: shahid.sherzada@gcu.edu.pk ORCID: 0000-0003-0431-7294

Mobile And Wearable Sensor Technologies For Early Warning In Chemical, Biological, Radiological And Nuclear (CBRN) Risks

Ali SERT

Introduction

Chemical, Biological, Radiological, and Nuclear (CBRN) threats pose significant risks to both military and civilian populations. These threats may arise from deliberate terrorist attacks, industrial accidents, or natural disasters, and they can lead to large-scale human casualties, long-term health problems, and environmental conditions with adverse long-term impacts (Qzih & Ahmad, 2024). In particular, incidents occurring in densely populated urban areas often create emergencies that require immediate response, making it essential to prevent such disasters where possible, or at the very least, to implement precautionary measures (Shafiei-Moghaddam et al., 2024). At the core of these precautionary strategies lie early warning systems. Accurate, region-specific disaster risk analyses for the early detection of such events directly influence the timeliness and effectiveness of emergency interventions. However, a critical shortcoming arises from the limited effectiveness of conventional fixed detection systems, especially under dynamic field conditions, where they often fail to adapt, provide rapid data transmission, or meet user-centered requirements (Kegyes et al., 2024). At this juncture, mobile and wearable sensor systems offer an innovative and rapid solution for the real-time, precise, and swift detection of CBRN agents in the field. These systems typically integrate user-embedded sensors, wireless communication technologies, and AI-supported analytical software (Zhuang, 2024).

Wearable sensor systems

Stand out due to their simplicity, lightweight structure, small size, and particularly their low energy consumption, which makes them suitable for integration into the bodies of actively working personnel. These features enable individuals who operate or monitor the sensors to detect potential exposure to CBRN agents directly in the field where they are deployed. The systems typically function through sensor modules that can be integrated into clothing, simple belts, headgear, or directly onto skin-contact areas (Hooshmand, 2023). In particular, exposures resulting from chemical and radiological risks—which exert their effects very rapidly must be detected through the combined analysis of multiple sensor data, such as respiratory activity, skin contact, or body temperature. Such analyses allow for the periodic monitoring of any dose of hazardous agents to which an individual has been exposed over time, and, when necessary, the initiation of timely alarms. Advantages of wearable systems One of the key advantages of wearable systems is their ability to collect real-time data and transmit this information to command centers or mobile decision-support systems via wireless communication technologies. In this way, not only can the health status of field personnel be remotely monitored, but valuable insights can also be obtained regarding the intensity of environmental threats (Li et al., 2024). This capability is critical not only for personal protection but also for making operational decisions. Furthermore, some advanced wearable systems employ biosensor technologies to monitor vital signs such as heart rate, blood oxygen saturation, and respiratory rate, thereby detecting physiological changes that may occur as

a result of exposure to chemical or biological agents. Such detection provides an opportunity for the early recognition of systemic effects caused by CBRN agents and enables timely medical intervention. Looking ahead, the integration of wearable systems with artificial intelligence-based decision-support software is expected to enhance detection accuracy, reduce false positive and negative rates, and provide improved protection against exposure risks. In addition, ergonomic design considerations such as lightness, user comfort, durability, and suitability for long-term use must also be taken into account during the development process (Ali et al., 2025; Mukherjee et al., 2025).

Mobile Sensor Platforms

Mobile sensor systems consist of sensor components integrated into mobile carrier platforms such as unmanned aerial and ground vehicles, various robotic systems, or portable handheld devices. These systems enable the safe collection of environmental data in high-risk areas without requiring direct intervention by personnel. Among their advantages are the ability to perform large-scale area scanning and to allow field operators to collect data from hazardous zones. The applications of mobile sensors vary depending on the level of risk and specific geographical conditions. For instance, in the case of large-scale industrial accidents or chemical leaks, unmanned aerial vehicles can be employed to conduct rapid and comprehensive airborne monitoring, while in indoor or confined environments where biological agents are suspected, small-scale unmanned ground vehicles are deployed. These vehicles, particularly in scenarios where human intervention is extremely difficult, can leverage their mobility to collect samples, measure temperature and gas concentrations, and, in some cases, even perform surface decontamination tasks (Nerger et al., 2024; Schwaiger et al., 2024).

Energy Consumption and Portability Criteria

For mobile and wearable systems to function effectively and efficiently in the field, criteria such as energy efficiency and long battery life must be met. Consequently, sensor components with low energy consumption are generally preferred. Energy management is also critical both for ensuring the comfort of field personnel during operations and for preventing devices from overheating under certain conditions. To address the energy requirements of portable mobile systems, several strategies have been developed, including the use of energy-efficient processors, low-power wireless communication protocols, task-based operating modes, and the integration of energy-harvesting techniques (Neseem et al., 2020; Brito-Brito, 2024).

Types of Sensors Used in the Detection of CBRN Agents

The rapid and accurate detection of CBRN agents depends on the sensitivity, selectivity, and adaptability of the developed sensors to environmental conditions. Since these agents are highly potent even at very low doses, the sensor systems designed to detect them must demonstrate high sensitivity, fast response time, real-time data generation, and response accuracy. Recent advancements in sensor technologies have enabled the development of systems tailored to specific CBRN risks with highly sensitive detection parameters. The adoption of such systems is determined not only by their detection capabilities but also by their performance under varying environmental and field conditions. Nevertheless, the precise selectivity of sensors toward target

agents often constitutes a critical factor for reliability. Chemical and biological agents can share similar characteristics with naturally occurring substances in the field, thereby increasing the likelihood of false results. To minimize this risk, sensor systems are frequently designed based on multi-detection principles, employing hybrid systems that integrate different types of sensors simultaneously to enhance accuracy. Looking forward, advances in nanotechnology and biosensor research are expected to enable the development of sensor systems capable of detecting CBRN agents at lower doses, with faster response times and greater reliability. However, for these technologies to be widely implemented, factors such as cost-effectiveness, user training, maintenance and repair processes, and overall system durability must also be taken into account (Popa, 2025; Disley, 2023).

Detection of Chemical Agents

Among CBRN agents, chemical warfare agents and toxic industrial chemicals are most commonly encountered in gas or vapor form. This is primarily due to their ability to penetrate more rapidly compared to other physical forms of chemical agents, which makes inhalation the predominant route of exposure. The main sensor technologies employed for detecting these agents include Metal Oxide Semiconductor (MOX) Sensors, Electrochemical Sensors, Quantitative Infrared (IR) and Raman Sensors, and Mass Spectrometry-Based Sensors. In order to be effective, sensor technologies designed for chemical agent detection must exhibit high sensitivity, fast response times, and low false alarm rates.

Currently, the leading sensor technologies in this field can be summarized as follows:

- **Metal Oxide Semiconductor (MOX) Sensors**: These low-cost sensors detect gases through changes in surface resistance in the presence of specific agents. Their advantages include long operational lifetimes and good sensitivity. However, they are also highly susceptible to temperature and humidity fluctuations, which may lead to false results.
- **Electrochemical Sensors**: These sensors operate based on the occurrence of a chemical reaction on the electrode surface when exposed to the target chemical gas. They offer the capability of detection even at very low concentrations, making them highly suitable for early warning applications.
- **Quantitative Infrared (IR) and Raman Sensors**: By measuring the unique vibrational spectra of chemical gases, these sensors identify the presence of specific agents. A key advantage of this approach is its non-contact nature and high selectivity, which allow for remote detection.
- **Mass Spectrometry-Based Sensors**: These sensors separate various chemicals according to their molecular weights, enabling the precise identification of individual substances even within complex mixtures. Their major strength lies in their high accuracy in distinguishing suspected or targeted chemicals.

Although each of these sensor types can be used independently, combining them within hybrid systems can substantially reduce the probability of false detections when supported by appropriate programming and operational protocols. In the future, the integration of artificial intelligence-

supported data analysis is expected to become more prevalent in chemical agent detection. Such integration would enable the real-time processing of multi-sensor data, thereby reducing decision-making times on-site and enhancing the effectiveness of emergency response teams (Liu et al., 2025; Kim et al., 2024; Xu et al., 2025).

Detection of Biological Agents

Among CBRN agents, biological agents (bacteria, viruses, and toxins) can lead to large-scale outbreaks if not detected at an early stage. Rapid detection of these agents in the field is particularly challenging, as the identification of living organisms is time-consuming. The primary sensor technologies employed for their detection include biosensors, fluorescence- and luminescence-based sensors, and mini-laboratory systems such as LAMP and PCR platforms.

Compared to other CBRN agents, the detection of biological agents is more difficult. The main reasons for this include: the ability of living organisms or toxins to alter their behavior under different environmental conditions; their effectiveness at very low doses; and the necessity of laboratory-based identification processes to accurately characterize them.

The major technologies currently used are as follows:

- **Biosensors**: Detect target agents by using biological recognition elements such as enzymes, antibodies, or nucleic acid-based probes. In particular, immunosensors can rapidly identify surface antigens of biological agents.
- **Fluorescence- and Luminescence-Based Sensors**: Measure changes in light emission resulting from reactions with target organisms.
- **LAMP (Loop-Mediated Isothermal Amplification) Systems**: Amplify DNA/RNA at relatively low temperatures and within short time frames, enabling rapid identification of target microorganisms. Their portability and speed make them advantageous for field applications.
- **PCR (Polymerase Chain Reaction)-Based Mini-Laboratory Systems**: Amplify target genetic material with high sensitivity. However, their limitations include energy requirements and sample preparation processes.

Recent advances in nanobiotechnology and microfluidic systems have enabled the miniaturization of detection devices while significantly reducing energy consumption. In the future, wearable biosensors and autonomous mobile laboratory platforms are expected to play a leading role in the detection of biological agents (Zhang, 2025; Vo et al., 2024).

Detection of Radiological and Nuclear Agents

Radiological agents and radioactive materials released as a result of nuclear material decay pose serious risks, whether they originate from deliberate actions or accidental industrial leaks. The sensors used for their detection are generally classified according to the type of radiation they detect and the principles of measurement they employ. The most widely used field-deployable sensor technologies today include:

- **Geiger-Müller (GM) Counters**: Compact and portable devices capable of detecting alpha, beta, and gamma radiation.
- **Scintillation Detectors**: Operate by producing light (scintillation) when high-energy radiation interacts with crystal materials. The emitted light is then converted into an electrical signal by photodetectors. These systems offer high energy resolution, thereby providing valuable information on the type of radionuclides present.
- **Semiconductor Detectors**: Capable of performing high-resolution energy determination, these detectors are particularly used in laboratory settings and mobile analytical platforms for precise radiation analysis (El Amri, 2025; Patel & Mazumdar, 2023).

Challenges and Field Application Issues

Although mobile and wearable sensors offer significant advantages for countering CBRN risks in the field, they also present certain disadvantages. These disadvantages can be categorized as technical and operational challenges, which may arise either during active operation or even beforehand. Such challenges stem both from the hardware limitations of the sensors and from the demanding conditions encountered in real-world environments. These conditions may include external factors such as temperature, humidity, dust, vibration, and exposure to chemical vapors. Due to these environmental stressors, sensors may fail to deliver the expected level of performance under harsh conditions, and in some cases, may completely lose functionality.

This issue is particularly critical for sensors designed to detect biological agents such as viruses and bacteria, since their sample collection and laboratory analysis processes are highly sensitive to environmental conditions, which increases the risk of producing false results. Similarly, the sensitivity of sensors to CBRN agents can sometimes result in misleading information. For example, metal oxide semiconductor sensors may respond not only to specific chemical agents but also to substances with similar chemical structures. Such limitations may lead to errors in operational decision-making and reduce overall confidence in the systems. Therefore, it is of great importance that newly developed sensors undergo rigorous testing, including the application of multiple validation methods, to ensure their suitability for use under challenging field conditions.

Conclusion

Mobile and wearable sensor systems play a vital role in the early detection of CBRN threats and the mitigation of their impacts. Through these advanced systems, both individual exposure levels and environmental risk factors can be monitored in real time, while the acquired data can be rapidly transmitted to decision-support mechanisms. Parameters such as energy efficiency, portability, and artificial intelligence integration are among the key factors enhancing the operational effectiveness of these technologies.

Future advancements in nanotechnology, microfluidic systems, and hybrid sensor fusion are expected to enable the development of more reliable, cost-effective, and widely applicable systems. By strengthening rapid response capacity against CBRN threats, mobile and wearable sensors stand out as strategic components in safeguarding human life and minimizing environmental consequences.

References

Ali, S. M., Noghanian, S., Khan, Z. U., Alzahrani, S., Alharbi, S., Alhartomi, M., & Alsulami, R. (2025). Wearable and flexible sensor devices: Recent advances in designs, fabrication methods, and applications. *Sensors*, *25*(5), 1377.

Brito-Brito, Z., Velázquez-González, J. S., Mira, F., Román-Villarroel, A., Artiga, X., Mishra, S. K., ... & Llamas-Garro, I. (2024). Wireless Sensor Node for Chemical Agent Detection. *Chemosensors*, *12*(9), 185.

Disley, J., Gil-Ramírez, G., & Gonzalez-Rodriguez, J. (2023). A review of sensing technologies for nerve agents, through the use of agent mimics in the gas phase: Future needs. *TrAC Trends in Analytical Chemistry*, *168*, 117282.

El Amri, L., El Bounagui, O., Amsil, H., Elmokhtari, B., Didi, A., & Bounouira, H. (2025). Software Developed to Simulate and Optimize Geiger-Müller Detectors and Study Gamma Ray Attenuation. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment*, 170773.

Hooshmand, S., Kassanos, P., Keshavarz, M., Duru, P., Kayalan, C. I., Kale, İ., & Bayazit, M. K. (2023). Wearable nano-based gas sensors for environmental monitoring and encountered challenges in optimization. *Sensors*, *23*(20), 8648., https://civil-protection-knowledge-network.europa.eu/system/files/2025-03/role-of-uncrewed-vehicles-in-cbrn-defence.pdf 01.08.2025.

Kegyes, T., Süle, Z., & Abonyi, J. (2024). Machine learning-based decision support framework for CBRN protection. *Heliyon*, *10*(4).

Kim, Y., Jeon, Y., Na, M., Hwang, S. J., & Yoon, Y. (2024). Recent trends in chemical sensors for detecting toxic materials. *Sensors*, *24*(2), 431.

Li, Y., Guo, S., Wang, B., Sun, J., Zhao, L., Wang, T., ... & Lu, G. (2024). Machine learning-assisted wearable sensor array for comprehensive ammonia and nitrogen dioxide detection in wide relative humidity range. *InfoMat*, *6*(6), e12544.

Liu, Y., Zhao, S., You, L., Xu, Y., Si, R., & Zhang, S. (2025). Improving the Selectivity of Metal Oxide Semiconductor Sensors for Mustard Gas Simulant 2-Chloroethyl Ethyl Sulfide by Combining the Laminated Structure and Temperature Dynamic Modulation. *Sensors*, *25*(2), 525.

Mukherjee, M. D., Gupta, P., Kumari, V., Rana, I., Jindal, D., Sagar, N., ... & Dhand, C. (2025). Wearable Biosensors in Modern Healthcare: Emerging Trends and Practical Applications. Talanta Open, 100486.

Nerger, T., Neumann, P. P., & Weller, M. G. (2024). Drone-Based Localization of Hazardous Chemicals by Passive Smart Dust. *Sensors*, *24*(19), 6195.
Neseem, M., Nelson, J., & Reda, S. (2020, July). AdaSense: Adaptive low-power sensing and activity recognition for wearable devices. In *2020 57th ACM/IEEE Design Automation Conference (DAC)* (pp. 1-6). IEEE.

Patel, A., & Mazumdar, H. (2023). A review on Radiation Detectors for Various Radiation Detection Applications. *Journal of Radiation and Nuclear Applications*, *8*(2), 93-111.

Popa, M. (2025). Literature Review on Recent Technological Methodologies in Detection And Identifying Cbrn Agents. *Land Forces Academy Review*, *30*(2), 202-210.

Qzih, E. S., & Ahmad, M. M. (2024). Hospital-based preparedness measures for CBRNE disasters: A systematic review. *Environmental Health Insights, 18*. https://doi.org/10.1177/11786302241288859

Schwaiger, S., Muster, L., Novotny, G., Schebek, M., Wöber, W., Thalhammer, S., & Böhm, C. (2024). UGV-CBRN: An Unmanned Ground Vehicle for Chemical, Biological, Radiological, and Nuclear Disaster Response. *arXiv preprint arXiv:2406.14385*.

Shafiei-Moghaddam, P., Jahangiri, K., & Hassani, N. (2024). Chemical release risk assessment in earthquake: Natech event scenario. *Heliyon*, *10*(8).

Vo, D. K., & Trinh, K. T. L. (2024). Advances in wearable biosensors for healthcare: current trends, applications, and future perspectives. *Biosensors*, *14*(11), 560.

Xu, M., Hu, X., Zhang, H., Miao, T., Ma, L., Liang, J., ... & Sun, X. (2025). Detection and Pattern Recognition of Chemical Warfare Agents by MOS-Based MEMS Gas Sensor Array. *Sensors*, *25*(8), 2633.

Zhuang, Y., Wang, X., Lai, P., Li, J., Chen, L., Lin, Y., & Wang, F. (2024). Wireless flexible system for highly sensitive ammonia detection based on polyaniline/carbon nanotubes. *Biosensors*, *14*(4), 191.

About the Author

Ali SERT: Lecturer at Burdur Mehmet Akif Ersoy University. Currently a doctoral candidate in the CBRN Department, specializing in the organic chemistry laboratory. He has been working in this field for four years.

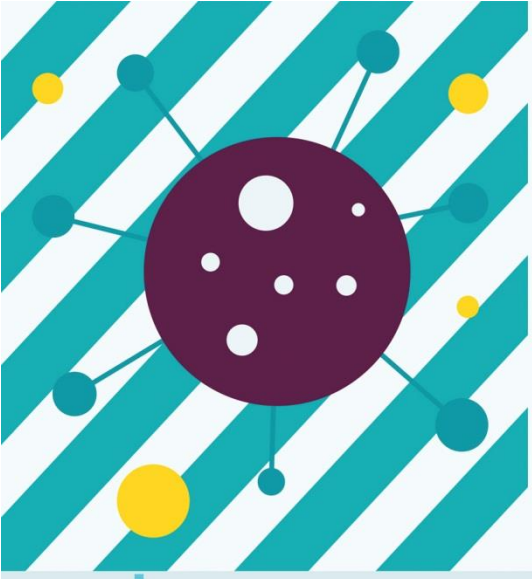
E-mail:alisert@mehmetakif.edu.tr

Similarity Index

The similarity index obtained from plagiarism detection software for this book chapter is 5%.

To Cite This Chapter

Sert, A. (2025). *Mobile and wearable sensor technologıes for early warning in chemical, biologıcal, radiological, and nuclear (CBRN) Risks*. Current Studies in Life Sciences, 2025 (pp. 300-307 ISRES Publishing.



ISRES

2025

CURRENT STUDIES IN LIFE SCIENCES

EDITOR
PROF.DR. MEHMET ÖZASLAN