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Current Research in Medical Microbiology and Human Infectious Diseases

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First Edition

Dr. R. B. Tripathi
Dr. Sowmya. N
Dr. Waseem S. M. H
Dr. Ashok Kumar







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Tamil Nadu, India

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Preface

Microorganisms are integral to life, yet their capacity to cause disease continues to challenge clinical practice, public health and global health security. Emerging and reemerging infections, antimicrobial resistance, environmental change and increasing interactions among humans, animals and ecosystems demand a broader understanding of infectious diseases. At the same time, advances in diagnostic technologies, molecular microbiology, vaccines and therapeutic strategies are creating new opportunities for prevention and treatment. Bringing these interconnected developments into a coherent scholarly discussion is the central purpose of ***Current Research in Medical Microbiology and Human Infectious Diseases***, published by **Thanuj International Publishers, Tamil Nadu, India**.

This first volume brings together twenty-two chapters addressing the biological foundations, clinical significance and public health dimensions of microbial diseases. The contributions connect environmental microbiology, pathogen biology, epidemiology and clinical practice, encouraging readers to examine infectious diseases within the wider settings in which they emerge, circulate and evolve.

The environmental foundations of the volume are introduced by **Dr. R. B. Tripathi** in *Microbial Diversity, Ecosystem Functions and Sustainable Environmental Management*. **Dr.Sowmya Nasimuddin** presents *One Health Approach for Infectious Diseases*, highlighting the interconnected nature of human, animal and environmental health. Complementing these perspectives, **Prof. Ashok Kumar** examines *Microbial Pathogens and the Dynamics of Emerging and Reemerging Disease Threats*, while **Prof.Susmita Srivastav** addresses *Microbiology and Epidemiology in Understanding and Controlling Infectious Diseases*. Together, these chapters establish a framework for understanding microbial threats through ecological, biological and population-level perspectives.

The importance of translating microbiological knowledge into effective clinical decisions is explored by **Dr.Waseem SMH** in *Diagnostic Stewardship and Antimicrobial Stewardship: Synergistic Approaches to Optimizing Patient Care*. Antimicrobial resistance receives further attention in **Dr.MohdAkmal's** chapter, *Antimicrobial Resistance in the Twenty-First Century: Emerging Challenges, Novel Therapeutics and One Health Perspectives*, and in **Dr. Monika S.**contribution, *Antimicrobial Resistance: Molecular Mechanisms and Global Surveillance*. **Dr. V. Pavithra** addresses a major clinical manifestation of this challenge in *Multidrug Resistant Tuberculosis (MDR TB) & Newer TB Regimens*. These contributions connect resistance mechanisms with surveillance, responsible antimicrobial use and therapeutic development.

The human–animal–environment interface forms another substantial theme. **Dr.AkankshaTripathi**, in *Microbiology and Zoonotic Diseases with Emphasis on Transmission, Prevention and the One Health Approach*, considers the pathways and prevention of zoonotic infections. **Mansi Patel** examines *Zoonotic Diseases and Emerging Pathogens: Challenges for Global Health*, while **Dr.SangeetaSarkhel** contributes *Emerging Zoonoses at the Human–Animal–Environment Interface*. Extending this ecological perspective, **Dr.PriyaSantharam** discusses *Climate Change and the Spread of Vector-Borne Diseases*. Collectively, these chapters underscore the need for coordinated approaches that account for changing environments and interactions across species.

Developments in diagnosis and pathogen-specific research are represented across several contributions. **Dr.Kayalvili K. K.** presents *Advances in Diagnostic Microbiology*, and **Dr. Fahad Affan T.** examines *Current Advances in Medical Virology: Emerging Viral Infections, Vaccines, and Antiviral Therapies*. **Dr.PriyaadarshiniUrjan Mohan** contributes *Current Research in Human Parasitic Diseases: Innovations in Diagnosis, Treatment, and Control*. **Dr. K. Hemanth Kumar** addresses *Emerging Trends in Medical Mycology: Diagnosis, Management, and Antifungal Resistance*, while **Dr.Sathishkumar G. M.** examines *Emerging and Re-emerging Bacterial Pathogens: Current Research and Clinical Challenges*. These chapters broaden the discussion across major pathogen groups and their associated diagnostic and therapeutic challenges.

The possibilities of preventive and therapeutic innovation are explored by **Dr. Sandhya Varshini S.** in *Next Generation Vaccines and Immunotherapy* and by **Dr.Afraa** in *Novel Therapeutic Approaches in Infectious Diseases: Phage Therapy, CRISPR and Nanomedicine*. These areas invite careful consideration of how scientific advances may translate into practical interventions through rigorous investigation and clinical validation. **J. Arockia John Paul's** chapter, *Earthworm Microbiota as a Source of Regenerative Medicine*, extends the volume towards exploratory biomedical applications. **Ms. Swetha V.**, through *Cardio Vascular Diseases*, adds a broader dimension of human health, while **Dr.Kaunesh Singh** connects prevention and public health in *Microbial Threats, Disease Prevention and Population Health*.

This volume is intended for undergraduate and postgraduate students, research scholars, teachers, microbiologists, clinicians and public health professionals. Its breadth offers opportunities for interdisciplinary learning and encourages readers to connect microbial mechanisms with epidemiological patterns, clinical decisions and population health outcomes. Across these diverse subjects, critical appraisal remains essential, particularly when distinguishing established applications from emerging research possibilities.

As editors, we express our appreciation to all contributing authors for sharing their knowledge and scholarly perspectives. Their collective participation brings together complementary areas of expertise and demonstrates the value of dialogue across environmental, biomedical and clinical disciplines. We also acknowledge **Thanuj International Publishers, Tamil Nadu, India**, for publishing this volume and providing a platform for the dissemination of scholarly work in medical microbiology and human infectious diseases.

Medical microbiology is a continually evolving field, and no single volume can encompass all its developments. This collection is offered as a contribution to an ongoing scientific conversation. We hope that it will support teaching, encourage thoughtful engagement with research and inspire further investigations directed towards better diagnosis, effective treatment, responsible antimicrobial use and sustainable disease prevention.

Editors

Dr. R. B. Tripathi
Dr. Sowmya Nasimuddin
Dr. Waseem SMH
Prof. Ashok Kumar

About the Editors



Dr.R.B.Tripathi is currently working as Assistant Professor in P.G.Department of Zoology, M.L.K.P.G.College, Balrampur-271201, Uttar Pradesh, India. He has been completed his Ph.D.in Zoology from Dr. R.M.L. Avadh University, Ayodhya, Uttar Pradesh, India. He has 25 years teaching experience in U.G and 21 years teaching experience in P.G classes, published 27 book chapters, 49 research papers in international and national reputed journals, participated and presented papers in many international and national seminars, conferences and workshops. He is Indian Zoologist, published by Surya Scientist Unique Researchers Yare Association, 2015. He is Associate Editor in International Journal of Advanced Research in Biological Sciences (ISSN:2348-8069), Editorial board member in International Journal of Advanced Multidisciplinary Research (ISSN:2393-8870), Published 16 Edited book served as Editor for Publication such as Recent Trends in Life Sciences Research (ISBN:978-81-947071-3-4), published by Darshan Publishers, Tamil Nadu, India, Recent Advancements and Research in Biological Sciences (ISBN:978-81-952529-1-6), Current Trends in Biological Sciences (ISBN:978-93-94638-00-6), Current Research in Life Sciences (ISBN: 978-93-94638-22-8), Recent Research in Biosciences (ISBN:978-93-94638-25-9), Current Advances in Biosciences (ISBN:978-93-94638-64-8),Advances in Pharmaceutical and Biosciences Research (ISBN:978-93-94638-87-7), Advance Research Trends in Biology (ISBN:978-93-94638-75-4), Biological Resources for Sustainable Research (ISBN:978-93-94638-90-7), Emerging Trends in Human Cardiology and Physiology (ISBN:978-93-94638-42-6), Concepts and Approach for Biosciences Research (ISBN:978-93-94638-53-2), Emerging Research Concept in Life Sciences (ISBN:978-93-94638-91-4), Insights to the Key Components of Biology-An Introduction (ISBN:978-93-94638-55-6), Biosciences and its Application for Plants and Animal Research (ISBN:978-93-94638-60-0), Emerging Trends of Bioresearch (978-93-49618-42-8) and Advances in Human Health Deficiencies and Biosciences Research (ISBN:978-93-49618-19-0) published by Thanuj International Publishers Tamil Nadu,India.



Dr. Sowmya N

MBBS, MD (Microbiology)

Dr. Sowmya N, MBBS, MD (Microbiology), is a Professor in the Department of Microbiology at Sri Muthukumaran Medical College Hospital & Research Institute, Chennai, with over 14 years of academic and diagnostic experience.

She currently serves as the IQAC Director, NABL-accredited Quality Manager for the BSL-2 Molecular Laboratory, and Coordinator of the Institutional Scientific Advisory and Editorial Boards. Her administrative responsibilities also include membership in the Hospital Infection Control Committee, Institutional Research Board, Pharmacovigilance Committee, and Anti-Ragging Committee. In addition, she serves as the In-charge of the CRMI and ECFMG Curriculum.

Her clinical and research expertise encompasses bacteriology, mycobacteriology, mycology (including antifungal susceptibility testing), serology (including immunofluorescence), parasitology, and molecular diagnostic techniques such as PCR and RT-PCR.

A Gold Medallist from The Tamil Nadu Dr. M.G.R. Medical University, Dr. Sowmya was awarded the JSB Gold Medal for Best Thesis. She has also received multiple Best Researcher Awards at both national and international levels, including the ISSN Best Researcher Award (2024) and the Best Researcher Award from The Tamil Nadu Dr. M.G.R. Medical University (2025).

Dr. Sowmya has authored more than 23 research papers published in indexed national and international journals and has contributed chapters to reputed medical textbooks. She currently serves as an Editorial Board Member of the International Journal of Current Research in Medical Sciences for the term 2025–2027.



Dr. Waseem S. M. H. is a Medical Microbiologist with an MD in Microbiology from Sri Ramachandra Medical College and Research Institute, Chennai, and a Diploma in Infection Prevention and Control (DIPC) from the Infection Control Academy of India, University of Hyderabad. He currently serves as an Assistant Professor and Hospital Infection Control Coordinator at Sri Muthukumaran Medical College Hospital and Research Institute, Chennai, and as a Consultant Microbiologist at Dr. Kamakshi Memorial Hospitals, Chennai.

His areas of interest include clinical microbiology, antimicrobial resistance, molecular diagnostics, hospital infection prevention and control, and antimicrobial stewardship. His research work encompasses carbapenemase-producing bacteria, molecular characterization of antimicrobial resistance, and the synergistic activity of antibiotics against multidrug-resistant pathogens. He has published research articles and clinical case reports in peer-reviewed journals, including *Access Microbiology* and the *Journal of Clinical and Diagnostic Research*. He has also presented research at national scientific conferences, including MICROCON 2025.

His academic and professional interests focus on strengthening diagnostic and antimicrobial stewardship through evidence-based laboratory practices, interdisciplinary collaboration, and effective infection prevention strategies to improve patient outcomes.



Prof. Ashok Kumar is Professor and Head of the Department of Zoology at **M.L.K. P.G. College, Balrampur, Uttar Pradesh, India.** With more than nineteen years of undergraduate and postgraduate teaching experience and thirteen years of research experience, he combines academic leadership with a sustained commitment to zoological research and scientific education.His research interests encompass fish physiology, aquatic toxicology, immunology, parasitology, entomology, biodiversity conservation and environmental sustainability. His studies examine the effects of pollutants and environmental stress on aquatic organisms, as well as the potential of natural dietary supplements and immunostimulants to improve fish health.His scholarly contributions include **47 research papers, 10 books, five edited volumes, 30 book chapters, and 04 Patent.** His books cover immunology, toxicology, microbiology, animal behaviour, entomology, sericulture, dairy and poultry science, and applied pisciculture. He also supervises doctoral research and contributes to scientific exchange through invited lectures, conferences, seminars and workshops.Prof. Kumar serves as **Convenor of the Boards of Studies in Zoology, Home Science and Microbiology at Maa Pateswari University, Balrampur,** Uttar Pradesh and as a member of its **Academic Council and Executive Council.** His responsibilities include curriculum development, coordination of pre-Ph.D. coursework and institutional quality assurance. As Convenor of the **Tiger Nature Club,** he promotes environmental awareness and biodiversity conservation.He holds fellowships from five scientific and professional organisations, including the **Asian Biological Research Foundation,** the **International Association of Zoology** and the **Nepal Aquaculture Society.** Through his teaching, research and writing, he seeks to make biological knowledge accessible, strengthen research skills and connect scientific understanding with environmental and societal needs.

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Microbial Diversity, Ecosystem Functions and Sustainable Environmental Management

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Abstract

Environmental microbiology examines microorganisms in natural and modified environments and their influence on ecosystems, environmental quality and health. This chapter describes microbial diversity, distribution, ecological interactions and contributions to decomposition, nutrient cycling, primary production and pollutant transformation. It discusses applications in agriculture, wastewater treatment, resource recovery and bioremediation, alongside risks involving pathogens, harmful algal blooms and antimicrobial resistance.Microbial monitoring approaches include cultivation, microscopy, molecular assays, sequencing and activity measurements. Emerging themes encompass climate change, plastic-associated biofilms and sustainable microbial technologies.The chapter emphasises integrating biological,chemical and ecological evidence to distinguish microbial presence, functional potential and demonstrated environmental processes.

Keywords: Microbial ecology; biogeochemical cycles; bioremediation; wastewater treatment; metagenomics; antimicrobial resistance; One Health.

Introduction

Microorganisms are central to the functioning of the biosphere. They transform matter, obtain energy through diverse metabolic pathways and interact with plants, animals and other microorganisms. Many processes that sustain ecosystems occur at scales invisible to the unaided eye, including decomposition within soil aggregates, nitrogen transformations in sediments and microbial growth on submerged surfaces. Environmental microbiology is the study of microorganisms in relation to their surroundings. Its scope includes their identity, abundance, distribution, metabolism, interactions and responses to environmental change. It also investigates how microbial processes can be used to improve water quality, restore contaminated sites and

recover useful resources from waste. Microbial ecology provides the conceptual foundation, while environmental engineering, molecular biology, chemistry and public-health science extend its applications. Microorganisms should not be viewed exclusively as disease-causing agents. Most environmental microbial activities sustain ecosystem processes, although particular organisms or communities may produce harmful effects under suitable conditions. Understanding both beneficial functions and environmental risks is essential for interpreting changes in microbial communities (Falkowski et al., 2008).

Diversity of Environmental Microorganisms

Bacteria exhibit considerable metabolic diversity.Depending on the organism and environmental conditions, they obtain energy from light, organic compounds or inorganic chemical reactions. They participate in decomposition, nitrogen fixation, nitrification, denitrification and the transformation of contaminants. Genera such as *Pseudomonas*, *Bacillus* and *Streptomyces* contain species of environmental and biotechnological importance, but ecological functions must be established at an appropriate taxonomic level rather than assigned indiscriminately to an entire genus. Archaea occur in ordinary soils, oceans, freshwater systems and animal-associated habitats as well as extreme environments. Methanogenic archaea generate methane in oxygen-free settings, including waterlogged soils, anaerobic sediments and digesters. Ammonia-oxidising archaea contribute to nitrogen transformations. Their recognition has broadened understanding of both global microbial diversity and ecosystem metabolism.Environmental fungi include yeasts and filamentous forms. Fungi secrete extracellular enzymes that break down complex organic materials, and their hyphae explore soil and decomposing substrates. Mycorrhizal fungi associate with plant roots and help plants acquire nutrients. Some fungi can transform pollutants, although laboratory degradation does not automatically demonstrate effective remediation in the field. Microscopic algae and cyano- bacteria support aquatic primary production and food webs.Cyanobacteria are photosynthetic bacteria, whereas algae include diverse eukaryotic lineages. Some cyanobacteria fix atmospheric nitrogen. Excess nutrient loading can favour blooms, and certain bloom-forming organisms produce toxins. Bloom toxicity depends on the organisms and strains present; a visible bloom alone does not establish toxin production.Protozoa graze on bacteria and other small organisms, contributing to food-web dynamics and nutrient regeneration. Some protozoa also include environmentally transmitted parasites. Viruses infect microbial and other hosts,

influencing population dynamics and releasing cellular material through lysis. Viruses are acellular infectious entities rather than independently metabolisingcells, but they are an important component of environmental microbiology.

Microorganisms in Major Environmental Habitats:

Soil contains heterogeneous microbial habitats shaped by mineral particles, organic matter, pores, moisture and oxygen availability. Different conditions can occur within a single aggregate. The rhizosphere—the soil influenced by roots—receives root-derived compounds that support microbial growth. Root-associated microorganisms can influence nutrient availability and plant performance, but their effects depend on soil properties, plant species and community interactions. Aquatic microorganisms occur in the water column, on suspended particles and within biofilms and sediments. Rivers and streams experience flowing-water conditions, whereas ponds and lakes often retain materials for longer periods.These differences influence microbial dispersal and the development of oxygen gradients. Organic pollution may increase microbial oxygen consumption and contribute to hypoxia, affecting fish and other aquatic organisms.Aquatic microbial studies should therefore combine biological observations with measurements such as temperature, pH, dissolved oxygen, nutrients and organic loading. Microbial abundance alone cannot establish the cause or severity of ecological stress.

Sediments receive organic matter and contaminants and frequently exhibit strong chemical gradients. As available electron acceptors change with depth, different respiratory processes become favourable. Oxygen respiration, nitrate reduction, metal reduction, sulfate reduction and methanogenesis may occur in different zones, although the actual distribution depends on local conditions. Disturbance or resuspension can alter these processes and release sediment-associated materials.Air transports bacterial cells, fungal spores, viruses and biological fragments in bioaerosols. Sources include soil, vegetation, water droplets, animals and waste-handling operations. Detection in air does not necessarily establish viability or infection risk. Engineered habitats include activated-sludge tanks, compostpiles, anaerobic digesters and drinking-water distribution systems.These environments demonstrate how microbial activity can be managed for beneficial purposes while unwanted growth and contamination are controlled.

Environmental Factors and Microbial Interactions:

Temperature affects growth and enzyme activity;pH influences physiology and chemical availability;moisture determines diffusion and water availability;and oxygen conditions regulate the metabolic pathways that can operate. Nutrient supply, salinity, light, pollutants and predation further shape communities.Microbial responses are context-dependentand no single environmental variable explains all observed patterns.Communities include competition, mutualism, predation and parasitism. In syntrophy, cooperating organisms exchange metabolites in ways that permit reactions that would otherwise be energetically unfavourable under the prevailing conditions. Such interactions are important in anaerobic organic-matter degradation. Biofilms are surfaceassociat-ed microbial communities embedded in a matrix containing extracellular polymers. The matrix provides structural organisation and retains materials near cells. Biofilms contribute to nutrient transformation and wastewater treatment, but can also complicate maintenance of water infrastructure (Flemming & Wingender, 2010). Their properties vary with species composition, surface characteristics and environmental conditions.

Microbial Roles in Biogeochemical Cycles:

Biogeochemical cycles connect biological activity with transformations of chemical elements. Microorganisms link these cycles through coupled metabolic reactions (Falkowski et al., 2008).

Cycle	Important microbial processes	Environmental significance
Carbon	Carbon fixation, decomposition, respiration, methanogenesis and methane oxidation	Primary production, organic-matter turnover and greenhouse-gas balance
Nitrogen	Fixation, mineralisation, nitrification, denitrification and anammox	Nutrient availability, nitrogen removal and nitrous oxide production
Sulfur	Mineralisation, sulfur oxidation and sulfate reduction	Redox chemistry, sulfide formation and interactions with metals
Phosphorus	Organic-phosphorus mineralisation, solubilization and microbial uptake	Plant nutrition and nutrient removal or recycling

Iron and manganese	Microbial oxidation and reduction	Mineral transformations and contaminant mobility

Photosynthetic and chemosynthetic microorganisms convert inorganic carbon into biomass.Decomposers transform organic residues into smaller molecules, while respiration returns carbon dioxide to the environment.Under suitable anaerobic conditions, methanogens produce methane.Methanotrophs consume methane and can reduce the amount reaching the atmosphere. Net greenhouse-gas exchange reflects the balance among production, consumption and transport, rather than the presence of one microbial group alone.Nitrogenfixation converts atmospheric nitrogen into biologically accessible forms. Mineralisation releases ammonium from organic nitrogen. Nitrification oxidises ammonia to nitrite and then nitrate, while denitrification reduces oxidised nitrogen compounds to gaseous products. Incomplete denitrification can release nitrous oxide.Anaerobic ammonium oxidation,oranammox, combines ammo -nium and nitrite to form nitrogen gas.

The traditional model separates ammonia oxidation and nitrite oxidation between different organisms.The discovery of complete ammonia oxidation, termed comammox, in certain *Nitrospira* demonstrated that one organism can perform both stages (Daims et al., 2015). This finding illustrates how molecular and cultivation approaches can revise established ecological models.Microorganisms mineralise organic phosphorus, take up phosphate and may mobilise phosphorus through changes in local chemistry.Such processes infl-uence plant nutrition and nutrient management.Sulfate-reducing micro-organisms produce sulfide under suitable anaerobic conditions,while sulfur -oxidisers transform reduced sulfur compounds.Ironand manganesetrans -forming micro-organisms influence mineral formation and dissolution.These changes can affect associated contaminants, emphasising the importance of measuring both microbial activity and geochemical consequences.

Environmental Pollution and Microbial Responses:

Pollution can alter community composition, reduce sensitive populations and favour organisms able to tolerate or transform particular contaminants. Responses depend on concentration, exposure duration, chemical availability and interactions among stressors.Sewage and other organic wastes supply substrates for microbial growth.Their decomposition consumes oxygen, and excessive loading may exceed the oxygen replenishment capacity of a waterbody.Nutrient enrichment can stimulate algal

and cyanobacterial growth. Subsequent decomposition of accumulated biomass may further reduce oxygen availability.Some micro-organisms use pesticides or hydrocarbons as substrates, while others transform them through cometabolism.Degradation depends on molecular structure, environmental conditions and access to the compound. Transformation products may differ in toxicity and persistence from the original contaminant. Demonstrating parent-compound disappearance is therefore insufficient to establish detoxification. Microorganisms can bind, accumulate, precipitate or change the oxidation state of metals. Metals cannot be biodegraded into non-elemental end products in the manner of organic chemicals.Microbial processes instead alter their chemical form, distribution or mobility. A successful intervention must demonstrate reduced exposure or effective removal and account for the possibility of later remobilisation.

Biodegradation and Bioremediation:

Biodegradation is the biological transformation of a substance.Minera - lisation is a more specific outcome in which an organic compound is converted to inorganic products,such as carbon dioxide under aerobic conditions.Bio-remediation applies biological processes to reduce environmental contamination or its consequences.

Natural attenuation relies on naturally occurring processes and requires evidence that the contaminant plume and risks are being controlled. Biostimulation adjusts conditions,for example nutrient or electron-acceptor avai -lability,to support useful indigenous activity.Bioaugmentation introduces sele -cted organisms or consortia, but introduced populations may fail to establish because of competition or unsuitable conditions.

Treatment can occur in situ, within the contaminated environment, or ex situ, after materials are removed. Each approach has trade-offs in access, control, cost and disturbance. Performance should be evaluated through chemical mass balances, transformation-product measurements, toxicity assessments and evidence of sustained effectiveness. Increased microbial abundance alone is not proof of remediation.

Wastewater Treatment and Resource Recovery:

Biological wastewater treatment uses microbial communities to transform organic matter and nutrients. In activated sludge, aerated microbial aggregates metabolise biodegradable material and are subsequently separated from treated water. Attached-growth systems use biofilms on supporting

surfaces.Anaerobic digesters convert suitable organic wastes into biogas through interacting hydrolytic,fermentative, acetogenic and methanogenic commu -nities.Nitrogen removal can combine nitrification and denitrification, while some engineered systems use anammox.Enhanced biological phosphorus removal exploits organisms that accumulate phosphate under managed operating conditions.Composting uses aerobic decomposition to stabilise suitable organic wastes.Resource recovery may include biogas, stabilized organic amendments and recovered nutrients. Treatment must also address residual contaminants and hygienic quality.Wastewater facilities can receive antibiotic-resistant organisms and resistance genes, so treatment performance should be assessed beyond conve -ntional measures of organic-matter removal (Berendonk et al., 2015).

Environmental Microbiology and One Health:

One Health recognises connections among human, animal and environmental health. Water, soil, food-production systems and waste streams can connect microbial populations across these domains. Environmental monitoring helps characterise contamination pathways and identify opportunities to reduce exposure.Faecal indicator organisms are used to assess evidence of faecal contamination. They are indicators rather than a complete inventory of pathogens. Their absence from a limited sample cannot guarantee that all pathogenic bacteria, viruses or protozoa are absent.Antimicrobial resistance also has an environmental dimension. Resistance occurs naturally, but human activities can introduce resistant organisms, resistance determinants and selective pressures into environmental settings. Gene detection alone does not demonstrate transfer to a pathogen or establish clinical risk. Interpretation requires consideration of hosts, mobile genetic elements, exposure pathways and resistance phenotypes (Berendonk et al., 2015).

Methods in Environmental Microbiology:

A study should begin with a defined question, appropriate sites, independent biological replication and a sampling schedule matched to environmental variability. Water, soil, sediment and biofilm samples require suitable collection and preservation. Field metadata, blanks and controls are essential. Seasonal surveys should avoid treating repeated measurements from the same site as fully independent observations.Cultivation provides organisms for identification, physiological testing and experimental work. Colony counts represent the fraction able to grow under the selected conditions, not the entire microbial community. Microscopy helps assess abundance, morphology and

spatial organization. Fluorescence-based methods can reveal structures and selected groups, although staining and image interpretation require appropriate controls.PCR and quantitative PCR detect selected genetic targets. Marker-gene sequencing, including 16S rRNA gene analysis for bacteria and archaea and ITS analysis for fungi, characterizes aspects of community composition. Shotgun metagenomics examines community DNA and can reveal functional potential beyond cultivated organisms (Handelsman, 2004). Metatranscriptomics investigates community RNA, metaproteomics examines proteins and metabolomics measures metabolites. These methods provide complementary evidence, but none independently captures all ecosystem activity. DNA may persist after cell deathgene presence does not establish gene expressionand relative sequence abundance need not correspond to absolute cell abundance.Respiration, nutrient-transformation rates, enzyme activities and stable-isotope approaches can connect organisms with processes. Chemical measurements and experimental controls are necessary to distinguish biological transformations from adsorption, volatilisation, or other abiotic changes. Statistical associations generate hypo -theses; controlled experiments or carefully designed causal analyses are needed to establish mechanisms.

Emerging Research Themes:

Warming, altered rainfall, drought, flooding and changing ocean condi - tions can influence microbial activity and community structure. Microorganisms also produce and consume greenhouse gases. These relationships create feedbacks that differ among ecosystems and require direct process measurements rather than assumptions based solely on community composition (Cavicchioli et al., 2019).Plastic surfaces support microbial biofilms collectively termed the plastisphere. Zettler et. al. (2013) documented communities asso -ciated with marine plastic debris. Colonization must be distinguished from actual polymer degradation. Plastic-associated micro-organisms may affect transport and ecological interactions but their presence does not establish pathogenicity or a quantified exposure risk.

Yoshida et.al. (2016) reported a bacterium capable of degrading and assimilating particular PET material under experimental conditions.Such findings support research on biological recyclingbut they should not be generalized to rapid degradation of all plastics in natural environments. Polymer type, crystallinity, temperature and accessibility influence performance.Research increasingly combines community profiling with chemical analysis, process measurements and ecological modelling. Potential applications include improved biological treatment, pollutant-transforming

enzymes and microbial approaches to nutrient recovery. Translation to practical use requires reproducibility, field validation and evidence that benefits persist under variable environmental conditions.

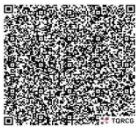
Conclusion

Environmental microbiology demonstrates that microorganisms are fundamental to ecosystem stability, environmental quality and the health of living organisms.Their activities drive decomposition,nutrient recycling, primary production and the transformation of pollutants. Understanding these processes provides a scientific foundation for protecting natural resources and managing environments affected by human activities. Microbial communities offer valuable applications in agriculture, waste-water treatment, bioremediation and resource recovery. Their metabolic diversity enables the degradation of many contaminants and supports technologies that reduce waste and improve environmental sustainability. Nevertheless, microbial activities can also create challenges through disease transmission, harmful algal blooms and the spread of antimicrobial resistance. Reliable assessment requires the integration of culture-based techniques, microscopy, molecular assays, sequencing and measurements of microbial activity. Identifying microorganisms or detecting functional genes alone does not establish that a particular ecological process is occurring. Biological observations must therefore be interpreted alongside chemical measurements, environmental conditions and evidence of actual microbial functioning. Future research should address microbial responses to climate change, interactions within plastic-associated biofilms and the movement of resistance genes across environmental compartments.Greater attention to community interactions and local environmental conditions will improve predictions of microbial behaviour and strengthen the design of effective environmental interventions. Ultimately, environmental microbiology connects ecosystem conservation with agricultural productivity, animal welfare and human health. A multidisciplinary approach, supported by monitoring and carefully validated microbial technologies, can translate scientific knowledge into practical solutions.Responsible application of these insights will help restore contaminated environments, conserve resources and promote sustainable development while maintaining the ecological processes essential for life.

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One Health Approach for Infectious Diseases

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Introduction

The health of human beings cannot be separated from the health of animals or the environment in which they live. Infectious diseases emerge and spread through complex interactions involving people, domestic animals, wildlife, microorganisms, ecosystems, climate, food systems, and patterns of human behaviour. The COVID-19 pandemic demonstrated how a pathogen emerging at the human–animal–environment interface can rapidly develop into a global health, social, and economic crisis. However, the emergence of infectious diseases is not limited to pandemics. Rabies, influenza, brucellosis, leptospirosis, dengue, malaria, Nipah virus disease, Kyasanur Forest disease, tuberculosis, and antimicrobial-resistant infections all demonstrate the close relationship between human health, animal health, and environmental conditions.

The One Health approach provides a framework for understanding and managing these interconnected risks. It is defined as an integrated and collaborative approach that seeks to sustainably balance and optimise the health of people, animals, plants, and ecosystems. Instead of addressing human, veterinary, and environmental health separately, One Health brings these sectors together for joint surveillance, risk assessment, research, prevention, outbreak investigation, and response. This approach is particularly important for India, where rapid urbanisation, population growth, intensive livestock production, wildlife–human contact, climate variability, environmental degradation, and unequal access to healthcare create conditions that can facilitate the emergence and spread of infectious diseases.

India has a particularly important role in the development and implementation of One Health strategies. The country contains diverse ecological zones, extensive livestock populations, dense urban settlements, large forested areas, long coastlines, and considerable human movement

between rural, peri-urban, and urban regions. Livestock, poultry, companion animals, wildlife, and humans often live in close proximity. In many communities, people depend on animals for food, income, transport, and cultural practices. These interactions are important for livelihoods but may also create opportunities for pathogen transmission when animal health services, sanitation, vaccination, food safety, and environmental protection are inadequate.

The One Health approach is therefore not merely an international policy concept; it is directly relevant to everyday public health practice in India. A dog-bite victim treated in a hospital, for example, is part of a wider rabies-control system that also includes vaccination of stray and owned dogs, management of dog populations, laboratory confirmation of animal cases, municipal services, veterinary care, and community education. Similarly, the prevention of avian influenza requires collaboration between public-health departments, veterinary authorities, poultry farmers, laboratory networks, wildlife agencies, and local communities. These examples demonstrate that infectious-disease control is more effective when the source, host, environment, and social context of transmission are considered together.

Conceptual Foundations of One Health

The modern One Health movement developed from the recognition that many infectious diseases cross species boundaries and that health problems cannot always be solved within a single professional discipline. Although the term has gained prominence in recent decades, the underlying idea has historical roots in medical, veterinary, ecological, and public-health practice. The modern movement was strengthened by the “One World, One Health” principles promoted in the early 2000s and later formalised through collaboration among the World Health Organization, the Food and Agriculture Organization, the World Organisation for Animal Health, and the United Nations Environment Programme.

The One Health framework is commonly represented by three interconnected domains: human health, animal health, and environmental health. The area of overlap represents the interfaces where zoonotic infections, vector-borne diseases, foodborne infections, antimicrobial resistance, and ecological disturbances occur. In practice, this framework also includes plant health, food systems, occupational health, social sciences, economics, public policy, and community participation. Its central message is that improving health in one sector while neglecting the others may provide only temporary

benefits. For example, treating human cases of brucellosis without controlling infection in cattle, goats, or sheep may reduce symptoms in individual patients but will not interrupt transmission in the community.

The One Health approach is based on several principles. First, human, animal, and ecosystem health are interdependent. Second, infectious-disease prevention requires collaboration among physicians, veterinarians, microbiologists, epidemiologists, ecologists, environmental scientists, laboratory specialists, social scientists, policymakers, and community workers. Third, disease prevention should be equitable and should address the needs of vulnerable communities, including rural populations, livestock keepers, tribal communities, migrant workers, healthcare workers, and people living in informal urban settlements. Fourth, prevention and early detection are generally more effective and less costly than responding to large outbreaks. Finally, One Health interventions should be sustainable and should protect ecological systems rather than creating new environmental risks.

Conceptual Framework of One Health

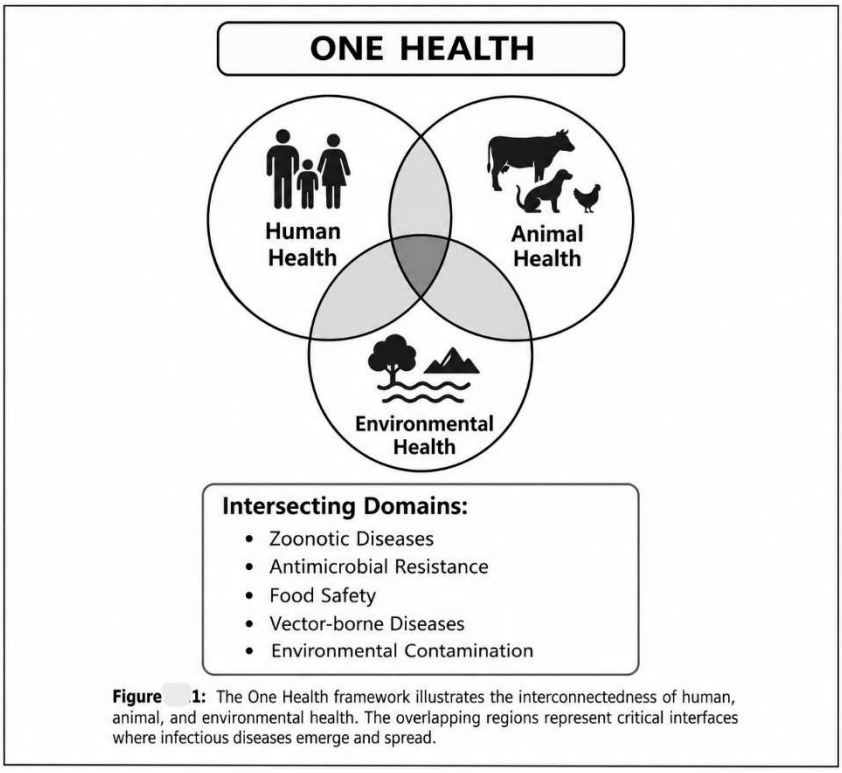


Figure 1: The One Health framework illustrates the interconnectedness of human, animal, and environmental health. The overlapping regions represent critical interfaces where infectious diseases emerge and spread.

Why One Health Is Important in India

India's geographical and ecological diversity influences the distribution of infectious diseases. The Himalayan region, tropical forests, coastal ecosystems, agricultural landscapes, urban settlements, and livestock-producing areas each present different disease risks. Environmental changes can alter the distribution of vectors and animal reservoirs. For example, changes in temperature, rainfall, water storage, and urban development can affect the transmission of dengue and malaria. Deforestation and fragmentation of forest habitats may increase contact between people, livestock, and wildlife. Agricultural expansion, mining, road construction, and settlement growth may create new interfaces between humans and wildlife.

India has experienced several infectious-disease events that demonstrate the value of a One Health perspective. Nipah virus outbreaks in Kerala highlighted the importance of understanding fruit bats, domestic animals, human behaviour, healthcare-associated transmission, and community communication. The outbreak response required coordination among clinicians, public-health specialists, veterinary authorities, laboratories, local administration, and communities. Early identification of cases, contact tracing, infection-prevention measures, risk communication, and monitoring of potential animal reservoirs were all essential components of the response.

Kyasanur Forest disease, reported mainly in parts of Karnataka and neighbouring regions, is another important example. The virus is maintained in forest ecosystems and is associated with ticks and wildlife, including monkeys and other animals. Human cases may occur when people enter or work in forested areas. Effective prevention therefore requires more than clinical treatment. It involves tick surveillance, monitoring of wildlife deaths, vaccination where appropriate, use of protective clothing, occupational education, environmental observation, and coordination between health, forest, veterinary, and local administrative authorities.

Rabies remains one of India's most important zoonotic diseases. Most human rabies cases are associated with dog bites, particularly from free-roaming dogs. The disease is almost invariably fatal after the onset of symptoms, but it is preventable through prompt wound washing, post-exposure prophylaxis, and sustained vaccination of dogs. A One Health rabies programme must therefore combine human healthcare services with veterinary vaccination, laboratory surveillance, responsible pet ownership, humane dog-population management, municipal action, and community education. Focusing

only on treatment after a bite does not address the source of transmission. The long-term goal requires coordinated dog vaccination and strengthened access to post-exposure prophylaxis, particularly in rural and underserved areas.

India's large livestock and dairy sectors also make brucellosis, leptospirosis, anthrax, and other zoonoses important One Health concerns. Farmers, veterinarians, slaughterhouse workers, dairy workers, animal handlers, and laboratory personnel may face occupational exposure. Control measures include animal vaccination where indicated, safe handling of livestock and animal products, pasteurisation of milk, personal protective equipment, occupational education, and rapid reporting of suspected cases. Human and animal laboratories should share relevant information so that clusters can be identified and investigated promptly.

Disease	Primary Reservoir	Transmission Route	One Health Interventions
Avian influenza (H5N1 and other strains)	Poultry (chickens, ducks), wild birds	Direct contact, aerosols, contaminated environment	Surveillance in poultry, biosecurity, vaccination, culling when needed, public awareness
Japanese encephalitis	Pigs, wading birds (herons, egrets)	Mosquito vectors (Culex species)	Vector control, pig vaccination, surveillance, community education
Leptospirosis	Rodents (rats), other wild animals	Contaminated water/soil, direct contact	Rodent control, safe water & sanitation, awareness, occupational health
Rabies	Dogs (major), wildlife (jackals, foxes, etc.)	Bite exposure, saliva	Mass dog vaccination, PEP, stray dog control, community education
Crimean-Congo Haemorrhagic Fever (CCHF)	Livestock (cattle, goats, sheep), wildlife (ticks)	Tick bite, direct contact, contaminated blood/tissues	Tick control, livestock management, PPE for high-risk workers, health education
Brucellosis	Cattle, goats, sheep, other farm animals	Unpasteurized dairy, direct contact, aerosols	Livestock vaccination, food safety, occupational health, awareness
Monkeypox	Rodents (rope squirrels), primates	Direct contact, respiratory droplets, contaminated materials	Surveillance, isolation, contact tracing, smallpox vaccine (where indicated), public awareness
Snakebite envenoming (with bacterial co-infections)	Venomous snakes (e.g., Russell's viper, cobra)	Bite (and secondary infection)	Antivenom availability, first aid training, habitat management, community education

Table 12.1: Eight priority zoonotic diseases identified in India through the One Health approach. Effective control requires coordinated action across human, animal, and environmental health sectors.

Land-Use Change and Disease Emergence

Land-use change is one of the major factors influencing the emergence of infectious diseases. Deforestation, mining, road construction, agricultural expansion, urban growth, and habitat fragmentation can bring people and domestic animals into closer contact with wildlife. When natural habitats are disturbed, wildlife may move into farms, villages, or urban areas in search of food and shelter. These changes can create new opportunities for pathogens to move between wildlife, livestock, and humans.

In India, this issue is relevant in areas where settlements, plantations, farms, and infrastructure projects are located near forest ecosystems. The expansion of agriculture and livestock production may increase contact at the forest edge. Urbanisation can also create indirect risks by increasing waste accumulation, reducing drainage, and encouraging the growth of vector populations. Environmental assessments and development planning should therefore consider infectious-disease risks in addition to economic and ecological effects.

Biodiversity loss may further influence disease transmission. Healthy ecosystems contain a range of species that can affect pathogen maintenance, vector abundance, and host behaviour. When ecosystems are simplified, some adaptable reservoir hosts or vectors may become more dominant. This can potentially increase exposure to certain pathogens. However, the relationship between biodiversity and disease transmission is complex and must be evaluated according to the specific pathogen, host, vector, and ecological setting.

Pathways from Land-Use Change to Zoonotic Disease Emergence

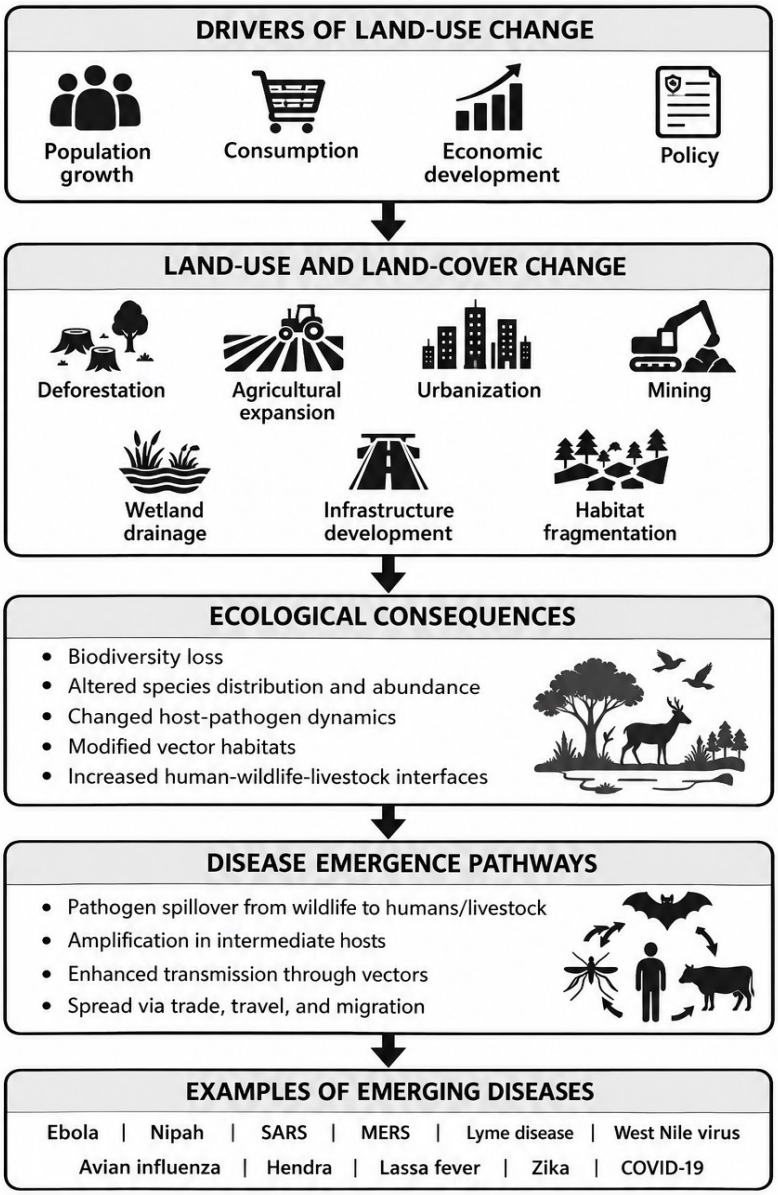


Figure 2: This diagram illustrates the causal chain from socioeconomic drivers through land-use change, ecological consequences, and ultimately disease emergence. The model links demographic and societal factors to land-use change, whose associated ecological factors help explain disease emergence.

Climate Change and Infectious Diseases

Climate change is altering the distribution and intensity of several infectious diseases. Temperature affects the survival, reproduction, and geographic range of mosquitoes, ticks, and other vectors. Changes in rainfall may increase mosquito breeding sites or contribute to flooding and water contamination. Drought may force animals and people to gather around limited water sources, increasing contact and transmission. Heatwaves, floods, cyclones, and other extreme events can disrupt health services, displace communities, damage sanitation systems, and delay disease detection.

In India, climate-sensitive diseases such as dengue, malaria, chikungunya, Japanese encephalitis, leptospirosis, and acute diarrhoeal diseases require surveillance that combines clinical, laboratory, meteorological, environmental, and vector data. For example, dengue prevention in urban areas cannot depend only on hospital-based case reporting. It also requires monitoring of mosquito breeding sites, rainfall, temperature, water-storage practices, construction sites, waste management, and community behaviour. Municipal authorities, public-health departments, laboratories, schools, housing societies, and residents must participate in source reduction and prevention.

Climate change may also influence diseases at the human–animal interface. Changes in animal movement, vector distribution, food availability, and habitat conditions may modify the risk of zoonotic transmission. Consequently, climate information should be incorporated into disease forecasting, early-warning systems, vaccination planning, emergency preparedness, and health-system resilience.

Zoonotic Diseases and One Health Control

Zoonotic diseases are infections that are naturally transmitted between vertebrate animals and humans. They may be caused by viruses, bacteria, parasites, fungi, or prions. Transmission can occur through direct contact, bites, scratches, inhalation, contaminated food or water, animal products, vectors, or environmental exposure. Because zoonotic diseases involve more than one host species, their prevention requires coordinated action across sectors.

Rabies illustrates the need for an integrated approach. Human health departments provide post-exposure prophylaxis and clinical care, while veterinary authorities support dog vaccination, animal diagnosis, and surveillance. Municipal bodies are responsible for waste management and implementation of dog-population-management programmes, and communities

contribute by reporting suspected animal cases, vaccinating pets, avoiding high-risk contact, and seeking immediate medical attention after exposure. The effectiveness of rabies control depends on the functioning of all these components together.

Nipah virus disease illustrates another aspect of One Health. Fruit bats are considered important natural hosts, while transmission may occur through contact with infected animals, contaminated food, or infected people. In India, particularly in Kerala, outbreak control has involved rapid case recognition, laboratory confirmation, contact tracing, isolation, infection prevention and control, risk communication, and close monitoring of contacts. The response also demonstrates the importance of avoiding misinformation and unnecessary fear of wildlife. Public-health measures should reduce risky contact while supporting evidence-based conservation and community livelihoods.

Avian influenza requires cooperation between poultry producers, veterinarians, wildlife authorities, laboratories, public-health agencies, and local administrations. Unusual bird deaths or increased illness in poultry should be reported quickly. Control measures may include testing, movement restrictions, farm biosecurity, safe handling of birds, appropriate personal protective equipment, and protection of exposed workers. Human surveillance is also essential because some avian influenza viruses can infect people.

Antimicrobial Resistance

Antimicrobial resistance is one of the clearest examples of a One Health problem. Resistant bacteria and resistance genes circulate among humans, animals, food, water, soil, wastewater, and healthcare environments. Antibiotic use in hospitals and clinics, veterinary practice, poultry and livestock production, aquaculture, and pharmaceutical manufacturing can contribute to the selection and dissemination of resistance when medicines are misused or waste is inadequately managed.

India faces significant challenges related to antimicrobial resistance because of its large population, varied healthcare systems, high infectious-disease burden, extensive pharmaceutical production, and widespread use of antimicrobials in human and animal health. The National Action Plan on Antimicrobial Resistance provides a framework for coordinated action, but successful implementation requires strong state-level programmes, laboratory capacity, antimicrobial stewardship, infection prevention, vaccination, regulation of antimicrobial sales, and improved surveillance.

An effective One Health AMR surveillance system should collect and analyse information from hospitals, community laboratories, veterinary laboratories, farms, food products, wastewater, pharmaceutical manufacturing sites, and the wider environment. Human and animal laboratories should use harmonised methods and quality-assurance systems. Genomic sequencing can help determine whether resistant organisms or resistance genes are related across hospitals, farms, food chains, and environmental sites. Such information can guide targeted interventions and identify important transmission pathways.

In human healthcare, antimicrobial stewardship involves prescribing antibiotics only when clinically indicated, selecting appropriate drugs and doses, and reviewing treatment when laboratory results become available. In animal health, antibiotics should be used under veterinary guidance and supported by vaccination, improved husbandry, hygiene, nutrition, and biosecurity. Environmental measures include treatment of hospital and pharmaceutical wastewater, responsible disposal of unused medicines, and monitoring of contaminated sites.

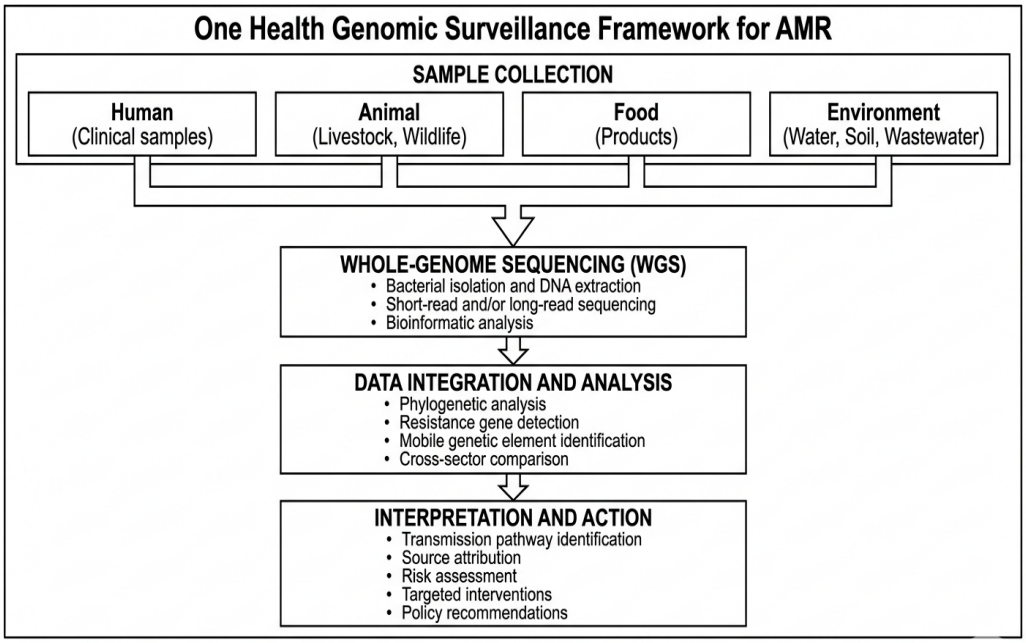


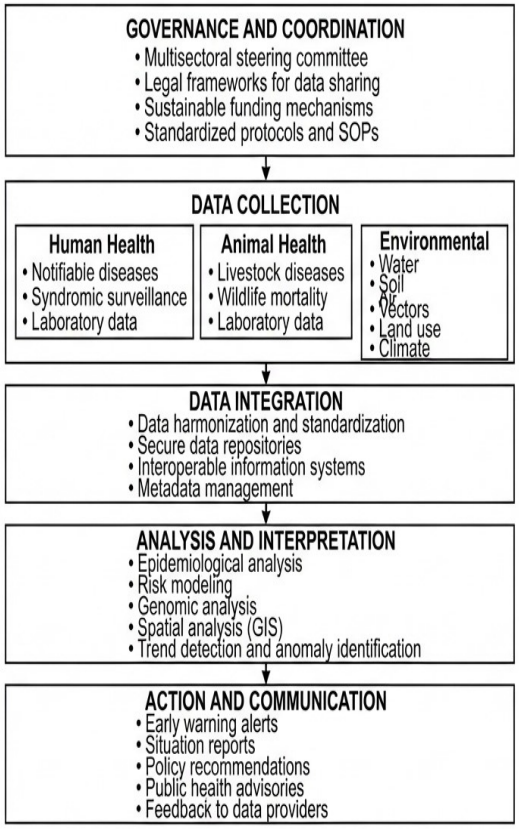
***Figure 3:** Integration of a One Health approach is paramount for comprehensive genomic surveillance and AMR management. This framework illustrates sample collection from multiple sectors, genomic analysis, data integration, and translation into public health action. *(Black and white flowchart for textbook reproduction)*

One Health Surveillance and Outbreak Investigation

Surveillance is the foundation of infectious-disease prevention. Traditional surveillance systems often operate separately: hospitals report human cases, veterinary departments report animal disease, and environmental agencies collect information on water, climate, pollution, vectors, or wildlife. A One Health surveillance system connects these sources so that patterns can be recognised earlier and interpreted more accurately.

In India, integrated surveillance can combine data from the Integrated Disease Surveillance Programme, the National Centre for Disease Control, the National Centre for Vector Borne Diseases Control, the National Animal Disease Reporting System, veterinary laboratories, wildlife authorities, food-safety agencies, meteorological services, and academic institutions. The aim should not simply be to collect more data. Information must be standardised, shared in a timely manner, analysed jointly, and translated into action.

A One Health outbreak investigation begins by confirming that an unusual increase in disease has occurred. The diagnosis should be verified through appropriate laboratory testing, and standard case definitions should be developed for humans and animals. Investigators should describe cases according to time, place, person, and species, while also assessing environmental exposures, animal contact, food and water sources, vectors, occupational risks, and healthcare-associated transmission. Samples may need to be collected from humans, animals, food, water, soil, vectors, or other environmental sources. Once the likely transmission pathways are identified, control measures should be implemented and the findings communicated to all relevant sectors and communities.



ealth approach to disease surveillance requires infrastructure for coordinating, collecting, integrat
This framework illustrates the flow from governance through data collection, integration, analysis, ar

Governance and Implementation in India

The success of One Health depends on governance arrangements that clarify responsibilities and support collaboration. In India, effective implementation requires coordination among the Ministry of Health and Family Welfare, the Department of Animal Husbandry and Dairying, the Ministry of Environment, Forest and Climate Change, the Indian Council of Medical Research, the National Centre for Disease Control, state health departments, veterinary services, municipal authorities, wildlife agencies, academic institutions, and community organisations.

National coordination must be supported by functional mechanisms at the state, district, and local levels. District-level One Health committees can bring together medical officers, veterinary officers, forest officials, laboratory

representatives, municipal authorities, agricultural officers, epidemiologists, and community organisations. These committees can develop local risk maps, conduct joint outbreak investigations, plan vaccination campaigns, coordinate public communication, and review disease events.

One Health implementation is often limited by separate budgets, unclear mandates, unequal laboratory capacity, inadequate data-sharing systems, and professional silos. Medical, veterinary, and environmental professionals may use different terminologies, reporting systems, and priorities. These barriers can be reduced through joint training, shared standard operating procedures, intersectoral exercises, interoperable information systems, and regular communication.

Community participation is equally important. People are more likely to adopt preventive measures when messages are culturally appropriate, locally relevant, and communicated through trusted health workers, veterinarians, teachers, local leaders, and community organisations. In India, frontline workers such as Accredited Social Health Activists, auxiliary nurse midwives, multipurpose health workers, veterinary field staff, and community-based organisations can play an important role in reporting unusual illness, promoting vaccination, encouraging safe animal handling, and guiding people to appropriate services.

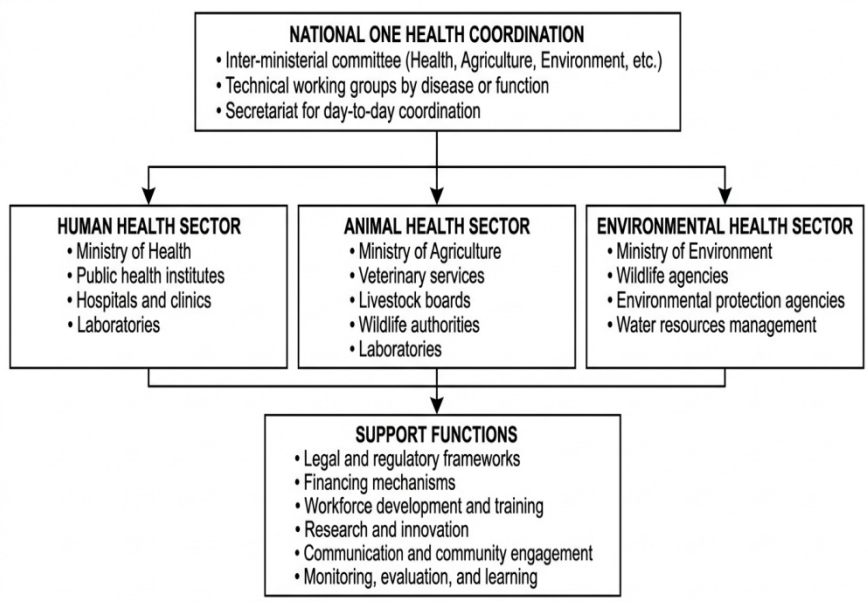


Figure.5: Constructing a One Health governance architecture requires coordination across sectors with clear mandates, sustainable resources, and support functions. This diagram illustrates the structure of national One Health coordination.

Education, Research, and Future Directions

One Health education should begin during the training of medical, veterinary, public-health, environmental, agricultural, and laboratory professionals. Students should learn through shared case discussions, field visits, outbreak simulations, and interdisciplinary projects. A medical student studying rabies, for example, should understand not only wound management and post-exposure prophylaxis but also dog vaccination, animal surveillance, municipal services, community behaviour, and vaccine access.

Research priorities in India include the study of pathogen transmission across human and animal populations, climate-sensitive diseases, wildlife–livestock interfaces, antimicrobial resistance, genomic epidemiology, environmental reservoirs, health-seeking behaviour, cost-effectiveness, and community-based interventions. Research should include rural, tribal, coastal, forest-fringe, and urban populations so that national strategies reflect India's diversity.

Digital technologies can strengthen One Health surveillance through mobile reporting, geographic information systems, satellite-based land-use monitoring, electronic laboratory systems, genomic databases, and artificial intelligence. These technologies should, however, be introduced with attention to data privacy, interoperability, affordability, digital literacy, and equitable access. Technology should support public-health workers and communities rather than replace local knowledge or human judgement.

Conclusion

The One Health approach recognises that infectious diseases arise from interactions among human beings, animals, microorganisms, and the environment. It provides a practical framework for preventing zoonotic diseases, controlling antimicrobial resistance, responding to outbreaks, strengthening surveillance, and preparing for future pandemics. For India, the approach is especially relevant because of the country's ecological diversity, large human and animal populations, rapid urbanisation, changing climate, and frequent interactions between people, livestock, wildlife, and shared environments.

Examples such as rabies, Nipah virus disease, Kyasanur Forest disease, avian influenza, dengue, leptospirosis, brucellosis, and antimicrobial resistance demonstrate that disease prevention cannot be achieved by the health sector alone. It requires coordinated action among medical, veterinary, environmental,

agricultural, wildlife, laboratory, administrative, and community stakeholders. Strong governance, sustained financing, integrated surveillance, laboratory networking, responsible antimicrobial use, public education, and community participation are essential for success.

One Health should therefore be viewed not only as a response to emerging infections but also as a long-term strategy for building resilient health systems and healthier communities. By linking human health with animal welfare and environmental sustainability, India can improve its capacity to detect infectious threats early, prevent avoidable outbreaks, and protect the health of present and future generations.

Key Points

- One Health is an integrated approach that recognizes the interdependence of human, animal, and environmental health. Approximately 60% of known human infectious diseases and 75% of emerging infectious diseases are zoonotic.
- Land-use change, climate change, agricultural intensification, and wildlife trade are major drivers of disease emergence.
- Antimicrobial resistance is a quintessential One Health challenge requiring integrated surveillance and action across sectors.
- Governance, financing, and workforce capacity are critical enablers of One Health implementation.
- Despite progress, barriers including sectoral silos, limited data sharing, and inadequate resources hinder One Health implementation.
- Future directions include integrating Planetary Health, leveraging digital technologies, addressing climate change, and promoting equity.

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Diagnostic Stewardship and Antimicrobial Stewardship: Synergistic Approaches to Optimizing Patient Care

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1. Introduction

The global rise of antimicrobial resistance (AMR) has emerged as one of the foremost threats to modern healthcare, jeopardizing the effective prevention and treatment of an ever-expanding range of infections caused by bacteria, fungi, viruses, and parasites. Inappropriate and excessive use of antimicrobial agents, coupled with delayed or inaccurate diagnosis of infectious syndromes, has accelerated the emergence and spread of resistant organisms across hospital and community settings. In response, two complementary disciplines have taken centre stage in efforts to safeguard antimicrobial efficacy: antimicrobial stewardship (AMS) and diagnostic stewardship (DS). While antimicrobial stewardship programs focus on optimizing the selection, dosing, route, and duration of antimicrobial therapy, diagnostic stewardship addresses the upstream processes of test ordering, sample collection, and result interpretation that ultimately inform those therapeutic decisions.

Historically, these two domains have often been implemented in isolation, with clinical microbiology laboratories and infectious disease pharmacists or physicians operating as separate silos. However, emerging evidence increasingly demonstrates that the greatest gains in patient outcomes, cost savings, and resistance containment occur when diagnostic and antimicrobial stewardship are integrated into a single, coordinated framework. This chapter explores the conceptual foundations of both stewardship domains, examines their points of synergy, discusses practical strategies for integration, reviews the role of rapid diagnostic technologies, and outlines the challenges

and future directions of combined diagnostic-antimicrobial stewardship programs in optimizing patient care.

2. Understanding Diagnostic Stewardship

Diagnostic stewardship refers to a coordinated set of interventions designed to improve the appropriate use of microbiological and other diagnostic testing in order to guide clinical decision-making(National Academies of Sciences et al., 2023). It encompasses the entire testing pathway, commonly conceptualized in three phases: the pre-analytical phase (appropriate test selection and specimen collection), the analytical phase (accurate and timely laboratory processing), and the post-analytical phase (correct interpretation and communication of results to clinicians)(Schinas et al., 2023).

2.1 Pre-analytical Stewardship

Pre-analytical stewardship involves ensuring that the right test is ordered for the right patient at the right time, using properly collected specimens. Common pitfalls at this stage include ordering urine cultures for asymptomatic patients, obtaining blood cultures from contaminated peripheral sites, or requesting broad respiratory pathogen panels without clinical correlation. Clinical decision support tools embedded within electronic health records, reflexive testing algorithms, and restrictive test ordering menus have all been shown to reduce unnecessary testing and downstream inappropriate antimicrobial use(Ines Staneloni et al., 2026).

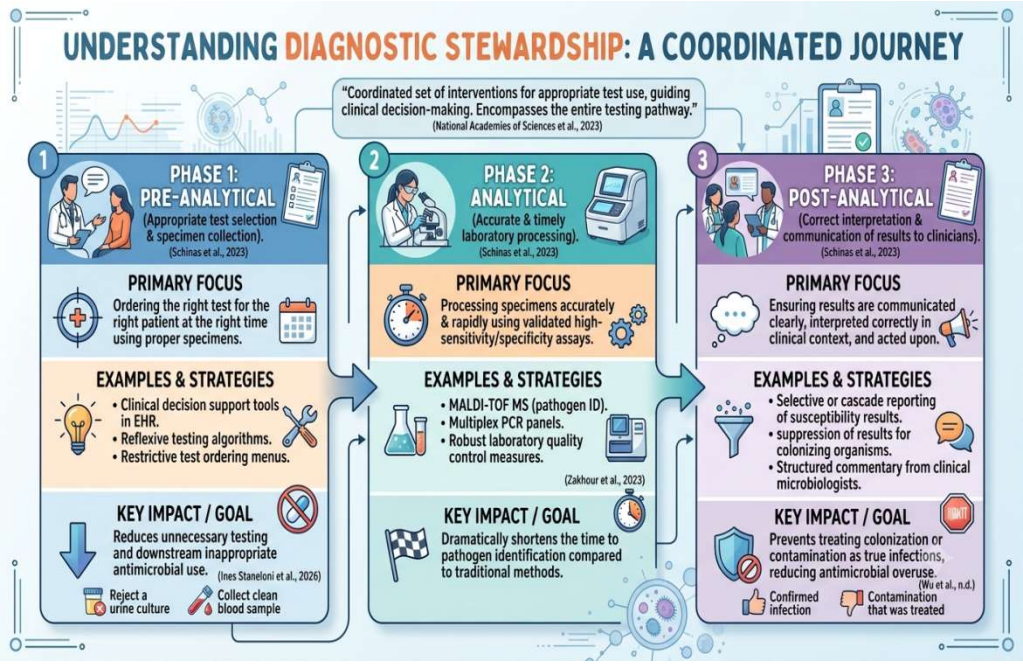
2.2 Analytical Stewardship

Analytical stewardship centres on the laboratory's capacity to process specimens accurately and rapidly. This includes the adoption of validated, high-sensitivity and high-specificity assays, appropriate quality control measures, and technologies such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and multiplex polymerase chain reaction (PCR) panels that dramatically shorten the time to pathogen identification compared with conventional culture-based methods(Zakhour et al., 2023).

2.3 Post-analytical Stewardship

Post-analytical stewardship focuses on ensuring that results are communicated clearly, interpreted correctly in clinical context, and acted upon appropriately. Selective or cascade reporting of antimicrobial susceptibility results, suppression of results for colonizing organisms, and structured commentary from clinical microbiologists all help prevent the treatment of

colonization or contamination as though it were true infection, a common driver of antimicrobial overuse(Wu et al., n.d.).



3. Understanding Antimicrobial Stewardship

Antimicrobial stewardship programs (ASPs) are coordinated interventions designed to improve and measure the appropriate use of antimicrobials by promoting the selection of the optimal drug regimen, including dosing, duration of therapy, and route of administration. The core goals of ASPs are to achieve optimal clinical outcomes while minimizing toxicity and other adverse events, reducing the selective pressure that drives resistance, and containing healthcare costs(Shah et al., 2024).

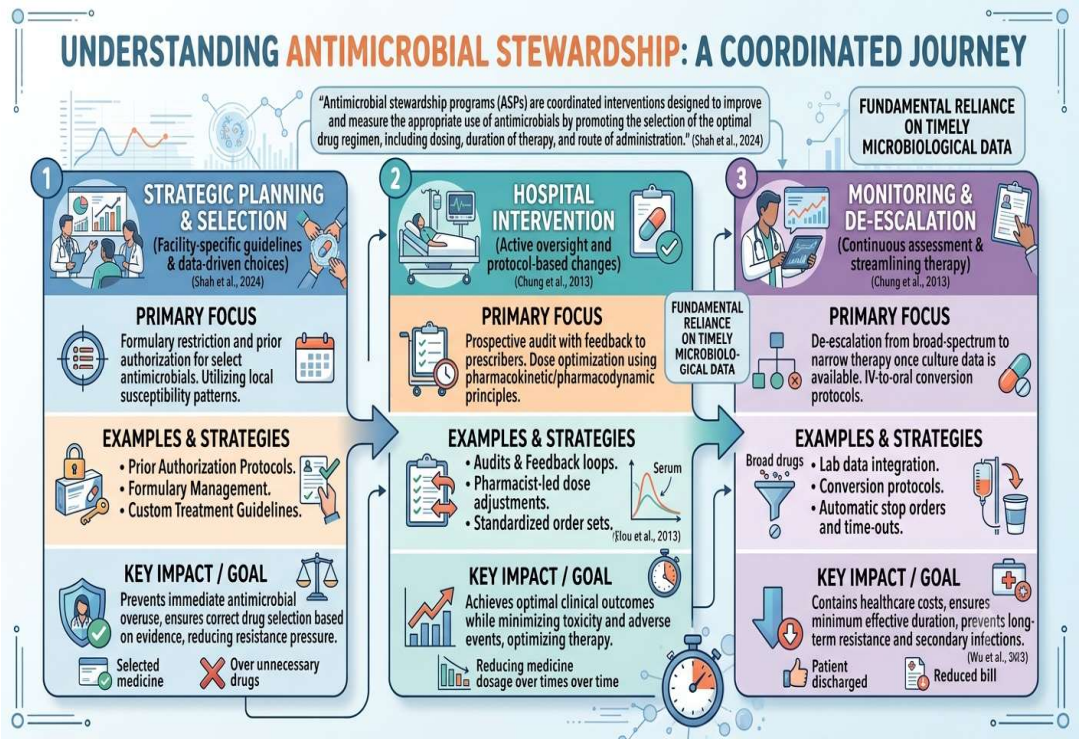
3.1 Core Strategies of Antimicrobial Stewardship

- Prospective audit with intervention and feedback to prescribers.
- Formulary restriction and prior authorization for select antimicrobials.
- Facility-specific treatment guidelines aligned with local susceptibility patterns.
- De-escalation of empiric broad-spectrum therapy once culture data are available.

IV-to-oral conversion protocols and dose optimization based on pharmacokinetic/pharmacodynamic principles.

Duration optimization, including automatic stop orders and time-outs.

These interventions rely fundamentally on the availability of timely and accurate microbiological data. Without robust diagnostic input, even the most sophisticated stewardship program is limited to empiric, broad-spectrum decision-making, underscoring the intrinsic dependency of antimicrobial stewardship on diagnostic stewardship(Chung et al., 2013).



4. The Synergy Between Diagnostic and Antimicrobial Stewardship

Diagnostic stewardship and antimicrobial stewardship are best understood not as parallel but as interlocking disciplines. Diagnostic stewardship shapes the quality and timeliness of the information that antimicrobial stewardship teams use to make prescribing decisions, while antimicrobial stewardship provides the clinical framework that determines which diagnostic information is actionable and how it should influence

therapy. When diagnostic tests are ordered indiscriminately, or when results are reported without clinical context, antimicrobial stewardship teams may find themselves reacting to noise rather than signal, potentially prompting unnecessary escalation of therapy for colonizing or contaminant organisms(Ku et al., 2023).

Conversely, when diagnostic stewardship principles are absent, rapid molecular tests intended to accelerate appropriate therapy can paradoxically increase antimicrobial use if positive results, including detection of colonizing flora or non-viable nucleic acid, are treated as automatic triggers for treatment. It is only when diagnostic and antimicrobial stewardship activities are coordinated, typically through shared leadership, joint protocols, and integrated informatics, that the full benefit of rapid diagnostics is realized in the form of earlier targeted therapy, de-escalation of unnecessary broad-spectrum agents, and reduced length of hospital stay(Claeys & Johnson, 2023).

5. Core Strategies for Integration

5.1 Multidisciplinary Stewardship Teams

Effective integration requires a multidisciplinary team comprising clinical microbiologists, infectious disease physicians, stewardship pharmacists, infection preventionists, and information technology specialists. Regular joint case review meetings, sometimes referred to as antimicrobial stewardship and diagnostic stewardship rounds, allow real-time correlation of laboratory findings with clinical status and facilitate rapid, informed decision-making at the bedside(Han et al., 2025).

5.2 Clinical Decision Support Systems

Electronic clinical decision support systems (CDSS) that link diagnostic ordering, result reporting, and prescribing alerts within a unified interface represent a powerful mechanism for embedding stewardship principles into routine workflow. For example, algorithms that flag a positive blood culture result and simultaneously suggest a narrow-spectrum regimen based on organism identification and local antibiogram data can shorten the time to optimal therapy considerably(Alexiuk et al., 2023).

5.3 Rapid Diagnostic Technologies

Technologies such as multiplex PCR panels for bloodstream infections, respiratory pathogen panels, procalcitonin-guided algorithms, and MALDI-TOF MS for rapid organism identification have transformed the speed at which actionable results become available. However, the clinical and economic

benefit of these technologies is maximized only when paired with an active antimicrobial stewardship response protocol; rapid diagnostics without a corresponding stewardship intervention frequently fail to translate into improved antimicrobial use or patient outcomes(Raycheva et al., 2026).

5.4 Selective and Cascade Reporting

Cascade reporting, in which susceptibility results for broad-spectrum agents are withheld unless the organism is resistant to first-line narrow-spectrum agents, has been shown to reduce unnecessary use of last-resort antimicrobials such as carbapenems and vancomycin, without compromising clinical outcomes. Similarly, suppression of urine culture results in asymptomatic patients who do not meet criteria for testing helps prevent unnecessary treatment of asymptomatic bacteriuria(Werneburg & Rhoads, 2022).

5.5 Education and Feedback

Ongoing education of prescribers regarding appropriate test indications, interpretation of results in clinical context, and the rationale behind stewardship interventions is essential to sustaining behavior change. Regular audit-and-feedback cycles, benchmarking against peer institutions, and transparent reporting of stewardship metrics reinforce a culture of judicious testing and prescribing(Rajendran et al., 2025).

6. Frameworks and Models for Integration

Several professional bodies have proposed structured frameworks to guide the integration of diagnostic and antimicrobial stewardship. The Society for Healthcare Epidemiology of America (SHEA) has articulated core principles of diagnostic stewardship emphasizing appropriate test selection, accurate and timely result generation, and effective communication to guide clinical action. The Centers for Disease Control and Prevention's Core Elements of Antibiotic Stewardship Programs likewise identifies leadership commitment, accountability, pharmacy expertise, action, tracking, reporting, and education as foundational pillars, several of which intersect directly with laboratory and diagnostic processes(Fabre et al., 2023).

A practical model for integration involves establishing a joint governance structure in which diagnostic stewardship and antimicrobial stewardship committees report to a shared oversight body, ensuring alignment of priorities, shared data infrastructure, and coordinated policy development. Institutions that have adopted such integrated governance structures report improved

concordance between laboratory testing practices and antimicrobial prescribing patterns, along with measurable reductions in overall antimicrobial consumption(Dik et al., 2017).

7. Challenges and Barriers to Implementation

- Resource constraints, particularly in low- and middle-income settings, limiting access to rapid diagnostic platforms and dedicated stewardship personnel.
- Fragmented electronic health record systems that do not seamlessly integrate laboratory and prescribing data.
- Resistance to change among prescribers accustomed to established ordering and prescribing habits.
- Variable local expertise in interpreting complex molecular diagnostic results.
- Difficulty demonstrating short-term return on investment despite long-term benefits in resistance containment.

Overcoming these barriers requires sustained institutional commitment, phased implementation strategies, and investment in both technological infrastructure and human capital. Engaging hospital administration and demonstrating the cost-effectiveness of integrated stewardship, through reduced length of stay, decreased adverse drug events, and lower rates of Clostridioides difficile infection, can help secure the necessary resources for program sustainability(Divecha et al., 2024).

8. Future Directions and Innovations

8.1 Artificial Intelligence and Machine Learning

Artificial intelligence and machine learning algorithms are increasingly being explored to predict infection risk, flag inappropriate test orders, and recommend antimicrobial regimens based on patient-specific data and institutional resistance patterns. These tools hold promise for further tightening the feedback loop between diagnostic results and prescribing decisions, although rigorous validation and attention to algorithmic bias remain essential(Khare & Shahane, 2026).

8.2 Point-of-Care and Genomic Diagnostics

The continued miniaturization and cost reduction of point-of-care molecular diagnostics, along with advances in whole-genome sequencing and

metagenomic next-generation sequencing, are expected to further accelerate pathogen identification and resistance gene detection, extending stewardship capabilities beyond the hospital into outpatient and resource-limited settings(Elbehiry & Abalkhail, 2025).

8.3 One Health Integration

Recognizing that antimicrobial resistance spans human, animal, and environmental domains, future stewardship frameworks are likely to increasingly incorporate One Health perspectives, coordinating diagnostic and antimicrobial stewardship efforts across veterinary medicine, agriculture, and environmental monitoring alongside human healthcare(Al-Khalaifah et al., 2025).

9. Conclusion

Diagnostic stewardship and antimicrobial stewardship represent two sides of the same coin in the global effort to preserve antimicrobial efficacy and improve patient outcomes. Neither discipline functions optimally in isolation: diagnostic stewardship supplies the accurate, timely, and clinically relevant information upon which sound antimicrobial decisions depend, while antimicrobial stewardship provides the clinical governance necessary to translate diagnostic data into appropriate action. As antimicrobial resistance continues to escalate as a global public health threat, healthcare institutions must move beyond siloed approaches and embrace integrated, multidisciplinary stewardship models. Through coordinated governance, shared informatics infrastructure, rapid diagnostic technologies, and sustained education, synergistic diagnostic-antimicrobial stewardship programs offer a powerful pathway toward optimizing patient care, curbing the spread of resistant organisms, and safeguarding the antimicrobial arsenal for future generations.

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Microbial Pathogens and the Dynamics of Emerging and Reemerging Disease Threats

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Abstract

Emerging and reemerging infections arise through interactions among microbial evolution, host susceptibility, environmental change and social conditions. An emerging infection may involve a newly recognised agent, an established pathogen entering a new population, or a rapid increase in geographical distribution. A reemerging infection returns as a major health problem after a period of decline. Microbiology explains these processes by investigating pathogen structure, replication, virulence, genetic exchange, antimicrobial resistance and survival within hosts and environments. This chapter examines the major groups of infectious agents and the mechanisms that permit their emergence or resurgence. Representative examples include SARS-CoV-2, influenza A viruses, Nipah virus, monkeypox virus, dengue viruses, measles virus, toxigenic *Vibrio cholerae*, drug-resistant *Mycobacterium tuberculosis* and *Candida auris*. Particular attention is given to zoonotic spillover, changing land use, vector ecology, vaccination gaps and the spread of resistance determinants. Conventional microbiological methods are considered alongside molecular diagnostics, whole-genome sequencing, metagenomics and wastewater surveillance. The chapter also discusses prevention, outbreak investigation, laboratory biosafety and research priorities within a One Health framework. Effective preparedness requires accessible diagnostics, resilient surveillance, appropriate antimicrobial use, vaccination, environmental management and coordination across human, animal and environmental health sectors.

Keywords: emerging infections; reemerging pathogens; microbial evolution; zoonotic spillover; antimicrobial resistance; genomic surveillance; One Health.

Introduction

Microbiology investigates microorganisms and infectious agents, their biological properties and their relationships with hosts and environments. Its contribution to infectious-disease control extends from recognising an organism in a clinical specimen to explaining how its genes, transmission routes and ecological associations influence disease. Viruses are acellular agents; bacteria, fungi and protozoa have cellular organisation. Their differences determine which diagnostic tests, preventive measures and treatments are appropriate. Infectious threats change even when the causative organisms are familiar. A pathogen may enter a new host population, expand through trade and travel, acquire resistance, or return where vaccination and sanitation have weakened. Conversely, improved testing can reveal an infection that has circulated undetected for years. Increased recognition must therefore be distinguished from a true increase in incidence [1,2]. Historical analyses show that emergence is associated with ecological, demographic and socioeconomic conditions, while zoonotic agents constitute an important component of recognised emerging infections. These observations support targeted surveillance at interfaces among people, livestock, wildlife and ecosystems. They do not imply that every wildlife-associated organism will infect humans or produce an epidemic [3,4].

Emerging, reemerging and endemic infections

An emerging infectious disease appears in a population for the first time or increases rapidly in incidence or geographical range. The agent need not be newly evolved: a previously established organism can become emerging when it reaches a new region or host population. A reemerging disease becomes an important health problem again after a substantial reduction in occurrence [1,2]. Endemic infection refers to continuing presence within a population or geographical area. An outbreak is an occurrence above the expected level in a defined setting; an epidemic is a larger increase, while a pandemic involves extensive international spread. These terms describe different features of disease occurrence. An endemic disease can cause outbreaks, and the same infection can be emerging in one region while reemerging in another. Classification requires a stated place, population and time period. Drug-resistant tuberculosis, for example, combines a longstanding infectious disease with emerging resistance profiles. Dengue may represent geographical emergence where transmission becomes established and resurgence where recurrent epidemics intensify. Table 1 summarises these distinctions.

Table 1. Concepts used to describe infectious-disease occurrence

Concept	Defining feature	Illustrative example	Interpretive caution
Emerging infection	New recognition, host involvement, geographical establishment or rapid increase	SARS-CoV-2 recognised in 2019; dengue entering a new transmission area	Newly detected does not always mean newly evolved
Reemerging infection	Return to public-health importance after decline	Measles resurgence in an underimmunised population	Requires evidence of an earlier decline
Endemic infection	Continuing presence within a defined population or area	Tuberculosis in many populations	Endemic does not mean mild or unchanging
Emerging resistance	New or expanding resistance in an established pathogen	Carbapenemase-producing Enterobacterales	Resistance and virulence are different properties
Outbreak or epidemic	Cases above the expected level in a specified setting	Cholera following disruption of safe-water services	Increased testing can change observed case counts

Microbiological foundations of emergence

Diversity of infectious agents

Bacteria are prokaryotic cells with diverse metabolic and structural properties. Viruses depend on host cells for replication and contain DNA or RNA genomes. Pathogenic fungi include yeasts and filamentous forms. Protozoan parasites are unicellular eukaryotes, often with complex life cycles involving vectors or environmental stages. A common clinical syndrome, such as fever, can result from agents in several of these groups; symptoms alone rarely establish the organism's identity. The structure of an agent influences detection and intervention. Molecular assays detect selected nucleic-acid sequences; microscopy reveals cellular or parasitic forms; culture can recover viable bacteria and fungi. Antibacterial drugs do not treat viral infections, and antifungal agents target features different from those targeted by antibacterial drugs. Figure 1 illustrates representative structures without implying that all members of a group share the same morphology.

Pathogenicity, virulence and host susceptibility

Pathogenicity is the capacity to cause disease, whereas virulence describes the degree of pathogenicity under defined conditions. Adhesion, invasion, toxin production, nutrient acquisition and evasion of host defence can contribute to bacterial disease. The outcome also depends on exposure dose, entry route, immune status and underlying illness. Colonisation means the presence of an organism without necessarily producing tissue damage or clinical disease [5]. A virulence factor can alter disease severity without changing transmission efficiency. Likewise, antimicrobial resistance can make treatment difficult without intrinsically increasing tissue damage. These distinctions prevent the mistaken assumption that a more resistant organism must be more virulent.

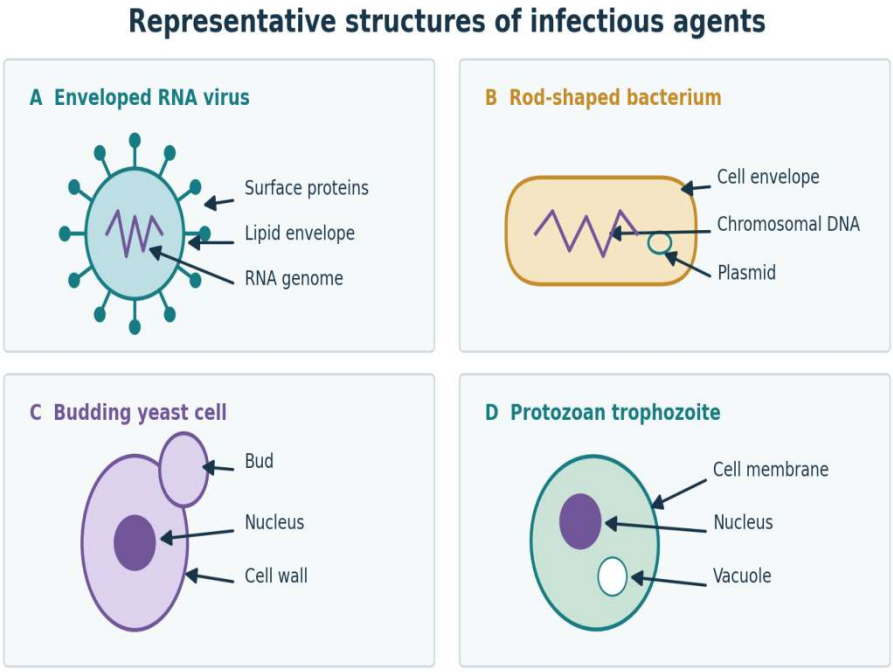


Figure 1. Representative structures of an enveloped RNA virus, a rod-shaped bacterium, a budding yeast and a protozoan trophozoite. Original educational schematics; structures are simplified and not to scale. The plasmid is illustrative and is not present in every bacterial cell.

Mutation, recombination and genetic exchange

Mutations introduce genetic variation. Selection changes the frequency of variants according to their reproductive success in a particular environment. Bacteria also exchange genetic material by transformation, transduction and conjugation. Plasmids and other mobile elements can carry resistance determinants across strains and species [6]. Viruses undergo mutation and, in appropriate circumstances, recombination. Segmented viruses such as influenza A can exchange genome segments through reassortment when compatible viruses infect the same cell. Genomic analysis of the 2009 H1N1 pandemic virus identified a complex history involving swine influenza lineages, illustrating why animal surveillance is relevant to human preparedness [7]. Evolution has no predetermined direction: greater transmissibility, greater severity and immune escape need not develop together.

Ecological and social drivers

Zoonotic spillover and land-use change

Zoonotic spillover occurs when an animal-associated pathogen infects a human. Successful spillover requires exposure, access to susceptible tissues and compatibility with the new host. Continued human transmission introduces additional requirements and does not follow automatically from a single spillover event. Agricultural expansion, fragmented habitats and changes in settlement can alter the abundance and distribution of reservoir hosts. Gibb and colleagues found that known zoonotic hosts were relatively more abundant and species-rich in human-managed ecosystems than in nearby less disturbed sites. This association helps identify interfaces for investigation; it does not show that every disturbed habitat has the same disease risk [8]. Figure 2 presents a conceptual spillover framework.

Climate, vectors and environmental exposure

Temperature, rainfall and humidity affect vectors, reservoirs and pathogen survival. The direction and magnitude of these effects depend on the species and local conditions. Dengue transmission, for example, is influenced by *Aedes* distribution, urban water storage, human mobility and vector control as well as climate [9]. Modelling by Carlson and colleagues projected that climate-driven shifts in mammalian ranges could create new opportunities for viral sharing. These are scenario-dependent predictions, not observations of future human outbreaks. Surveillance must test such predictions against field data and account for uncertainty [10].

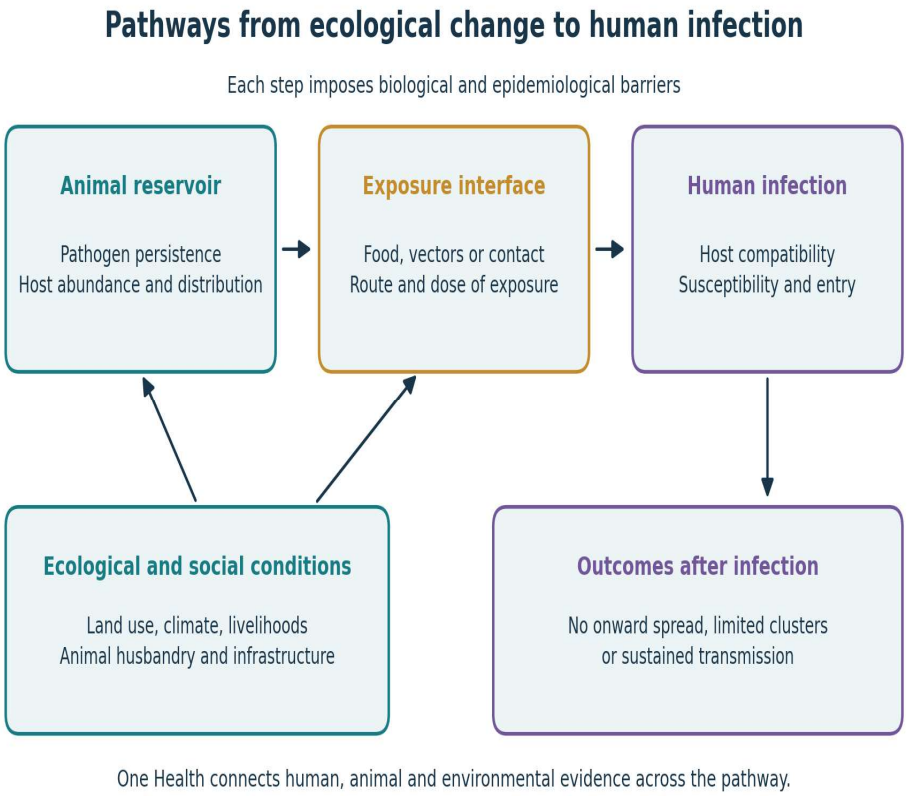


Figure 2. Conceptual pathways to zoonotic spillover. Exposure, biological compatibility and onward transmission are separate stages. Ecological and social conditions modify the pathway. Original schematic informed by references 3, 4, 8, 10 and 29.

Human mobility, inequality and disrupted services

Travel can move infected people and organisms across borders, but establishment requires suitable transmission conditions. Crowded housing, unsafe water and unequal access to diagnosis can increase exposure or delay care. Conflict and displacement can interrupt vaccination and disease-control programmes. Healthcare facilities can amplify infection when equipment, surfaces or patient contacts allow transmission. These social conditions interact with microbial properties rather than acting independently of them [11,12].

Representative emerging viral pathogens

SARS-CoV-2

SARS-CoV-2 is an enveloped positive-sense RNA coronavirus recognised during the investigation of pneumonia in China in 2019. Initial characterisation combined sequencing, isolation and imaging to establish a new coronavirus associated with the illness [13]. Its emergence demonstrates the value of integrating clinical recognition with microbiological investigation.

The spike glycoprotein mediates host-cell attachment and membrane fusion. Structural work on the prefusion spike helped guide the development of medical countermeasures [14]. Subsequent viral evolution illustrates the need to evaluate genotype alongside laboratory measurements and epidemiological findings. A sequence change alone does not prove altered virulence or vaccine failure.

Table 2. Selected pathogens and their emergence or resurgence

Agent	Microbial group	Important transmission pathway	Reason for concern
SARS-CoV-2	RNA virus	Infectious respiratory particles	Novel recognition followed by global spread and continuing evolution
Influenza A viruses	Segmented RNA viruses	Respiratory transmission; animal exposure for zoonotic strains	Reassortment, antigenic change and animal-human interfaces
Nipah virus	RNA virus	Animal exposure, contaminated food and close contact	Recurrent spillover and severe neurological or respiratory disease
Monkeypox virus	DNA virus	Close contact, infected materials and animal exposure	Expansion into new populations and transmission networks

Agent	Microbial group	Important transmission pathway	Reason for concern
Dengue viruses	RNA viruses	*Aedes* mosquitoes	Geographical expansion and recurrent epidemics
Measles virus	RNA virus	Airborne transmission	Resurgence where immunity gaps accumulate
Toxigenic *V. cholerae*	Bacterium	Contaminated water or food	Outbreaks linked to unsafe water and disrupted services
Drug-resistant *M. tuberculosis*	Bacterium	Airborne transmission	Persistence and spread of difficult-to-treat infection
Carbapenemase-producing Enterobacterales	Bacteria	Healthcare, contact and relevant environmental pathways	Spread of resistant organisms and mobile resistance genes
Candida auris	Yeast	Healthcare-associated contact and contaminated equipment	Invasive infection, difficult identification and antifungal resistance
Plasmodium falciparum	Protozoan	*Anopheles* mosquitoes	Resurgence in some settings and emerging drug resistance

Sources: [7,9,11–13,15–23]. Classification depends on location and time; the table is illustrative rather than a ranking of current outbreak severity.

Nipah virus

Nipah virus is a zoonotic RNA virus associated with fruit bats. Human infection can occur through infected animals, contaminated food and direct contact with infected people. It can produce encephalitis and respiratory disease. Different outbreaks have involved different exposure pathways, including pig-associated transmission and food contaminated by bats [15]. Its relevance to India and Bangladesh makes coordinated clinical, veterinary and ecological investigation particularly valuable. An animal reservoir should be

understood through evidence-based surveillance; indiscriminate destruction of wildlife is not a substitute for identifying and reducing exposure pathways.

Monkeypox virus and mpox

Monkeypox virus is an enveloped double-stranded DNA orthopoxvirus. Mpox spreads through close contact with infected people, contaminated materials and infected animals. The international outbreak beginning in 2022 showed that established pathogens can expand through changing contact networks and geographical movement [16]. Laboratory confirmation and respectful contact assessment are central to investigation. Communication should explain exposure routes without identifying an entire community as a biological source of infection. Viral clade, host factors and access to care all contribute to observed differences in disease outcome.

Reemerging infections

Measles is a highly contagious airborne viral infection. Its resurgence demonstrates that a familiar organism can return without requiring a fundamentally new microbial mechanism. When susceptible individuals accumulate through missed vaccination or interrupted services, introduction of the virus can initiate an outbreak. Maintaining high and equitable two-dose vaccination coverage and identifying underimmunised communities are central preventive measures [11]. Cholera is caused by toxigenic *Vibrio cholerae*, especially epidemic-associated O1 and O139 serogroups. Exposure usually occurs through contaminated water or food. Outbreaks can follow breakdowns in sanitation, displacement and limited access to safe drinking water. Cholera toxin produces intestinal fluid loss, while the severity of illness depends on dehydration and timely care [12]. Control combines water, sanitation and hygiene with surveillance, prompt case management and appropriately targeted oral cholera vaccination. Vaccination complements infrastructure; it cannot provide reliable long-term protection against all consequences of unsafe water. Tuberculosis is a longstanding airborne bacterial infection that remains important in many populations. The emergence and spread of resistant strains complicate its control. Multidrug-resistant tuberculosis is resistant to at least isoniazid and rifampicin. Resistance can arise during treatment and can also be transmitted directly to another person [17].

Microbiological confirmation and drug-susceptibility testing guide treatment decisions. Molecular resistance markers and phenotypic susceptibility results should be interpreted with clinical and programme information. Calling all tuberculosis reemerging is imprecise where it has

remained continuously endemic. Malaria resurgence may follow interruptions in prevention, vector-control failure or reduced access to diagnosis and treatment. Resistance in *Plasmodium falciparum* adds another challenge. Partial artemisinin resistance is associated with delayed parasite clearance; it does not automatically mean that every artemisinin-based combination treatment will fail, because partner-drug activity also matters [18].

Antimicrobial resistance and emerging fungi

Antimicrobial resistance involves reduced susceptibility of an organism to a drug. Selection can favour resistant variants, while transmission spreads those variants between hosts and settings. Mobile genes can also spread between bacterial populations. Inappropriate antimicrobial exposure contributes to selection, but poor infection prevention and inadequate sanitation contribute to dissemination [19]. NDM-type metallo-beta-lactamases illustrate the importance of transferable resistance. A molecular and epidemiological investigation documented NDM-1-associated resistance in Enterobacteriaceae, including isolates linked across countries. The example connects laboratory characterisation with patient movement and surveillance [20]. The WHO bacterial priority pathogens list guides research and investment in resistant bacterial infections. It is not a prediction of the next pandemic or a universal treatment algorithm. Local susceptibility data remain essential for clinical and public-health decisions [21].

Candida auris, also known as *Candidozyma auris*, is an emerging yeast capable of causing invasive infection and spreading in healthcare settings. Colonised patients can contribute to transmission without having symptomatic invasive disease. Identification, screening in appropriate circumstances, hand hygiene and effective cleaning of equipment and surfaces are important control components [22]. Whole-genome sequencing demonstrated distinct clonal populations associated with emergence on several continents, showing why geographically separated cases should not automatically be assigned to a single transmission chain [23]. The WHO fungal priority list identifies gaps in surveillance, diagnosis and antifungal development across multiple pathogens [24]. Climate-related explanations for fungal emergence should be treated as hypotheses requiring organism-specific evidence rather than as settled causes of every outbreak.

Laboratory diagnosis and microbial characterisation

Diagnostic investigation begins with the clinical syndrome, exposure history and selection of an appropriate specimen. Collection timing, transport

conditions, specimen quality and prior antimicrobial exposure influence test performance. A negative result can reflect absence of the organism, low abundance, sampling of the wrong site or a test that does not detect the relevant target. Culture, microscopy, antigen assays and serology retain important roles. Nucleic-acid amplification permits rapid targeted detection; sequencing can provide strain and resistance information. The WHO genomic strategy emphasises that sequencing must be supported by specimen collection, diagnostics, analysis and data sharing rather than treated as an isolated technology [25]. Table 3 compares the information supplied by these approaches.

Table 3. Diagnostic and surveillance methods

Method	Main information obtained	Principal strength	Important limitation
Microscopy	Morphology or identifiable parasitic forms	Rapid assessment in suitable specimens	Sensitivity and specificity depend on organism and expertise
Culture	Recovery of viable organisms	Supports identification and phenotypic susceptibility testing	Slow or unsuitable for some organisms; prior treatment can reduce yield
Antigen assay	Detection of pathogen-associated components	Rapid and practical for selected infections	Performance varies with disease stage and test design
Serology	Host antibody response	Useful for selected recent or past exposures	Early infection may be negative; cross-reactivity may occur
PCR or RT-PCR	Selected DNA or RNA targets	Rapid, sensitive targeted detection	Does not establish viability; unexpected agents may be missed
Whole-genome sequencing	Genome variation and selected resistance determinants	Strain comparison and molecular surveillance	Sequence similarity alone does not establish direct transmission

Method	Main information obtained	Principal strength	Important limitation
Metagenomic sequencing	Broad nucleic-acid content of a specimen	Can identify agents outside a targeted panel	Contamination, cost and uncertain clinical significance
Wastewater surveillance	Population-level environmental signal	Complements clinical reporting	Cannot identify individual patients or directly count infections

Metagenomic sequencing analyses nucleic acids without restricting detection to one suspected organism. In a multicentre study of meningitis and encephalitis, it identified infections missed by routine clinical testing, but also missed infections detected by other methods. Findings required confirmation and clinical interpretation [26]. Detection of microbial DNA or RNA alone does not prove that the detected organism caused the illness. Figure 3 shows how microbiological evidence supports an integrated investigation.

Surveillance and outbreak investigation

Surveillance involves systematic collection, analysis and interpretation of information followed by action. Useful signals include unusual clinical clusters, laboratory findings, increases in mortality, animal illness, vector changes and community reports. An alert becomes useful when it triggers verification and an appropriate response, rather than merely accumulating in a database. Genomic surveillance can help describe lineages, detect resistance determinants and assess possible links between cases. Incomplete sampling and uneven sequencing access can distort apparent transmission patterns. Genomic similarity must therefore be evaluated alongside dates, geography and contact information [25]. Wastewater and environmental surveillance can detect population-level signals in sewage and other human-impacted waters. Its value depends on the target, sampling design and ability to link findings to action. Rainfall, dilution, shedding and population movement complicate interpretation, and a positive environmental signal does not by itself demonstrate viable organisms or quantify clinical disease [27]. In India, the Integrated Disease Surveillance Programme uses syndromic, presumptive and laboratory-confirmed reporting through the Integrated Health Information Platform. Its laboratory network includes district public-health laboratories and state referral laboratories for confirmation and outbreak investigation [28].

Local preparedness depends on timely reporting, quality specimens and functional referral pathways as well as advanced testing capacity.

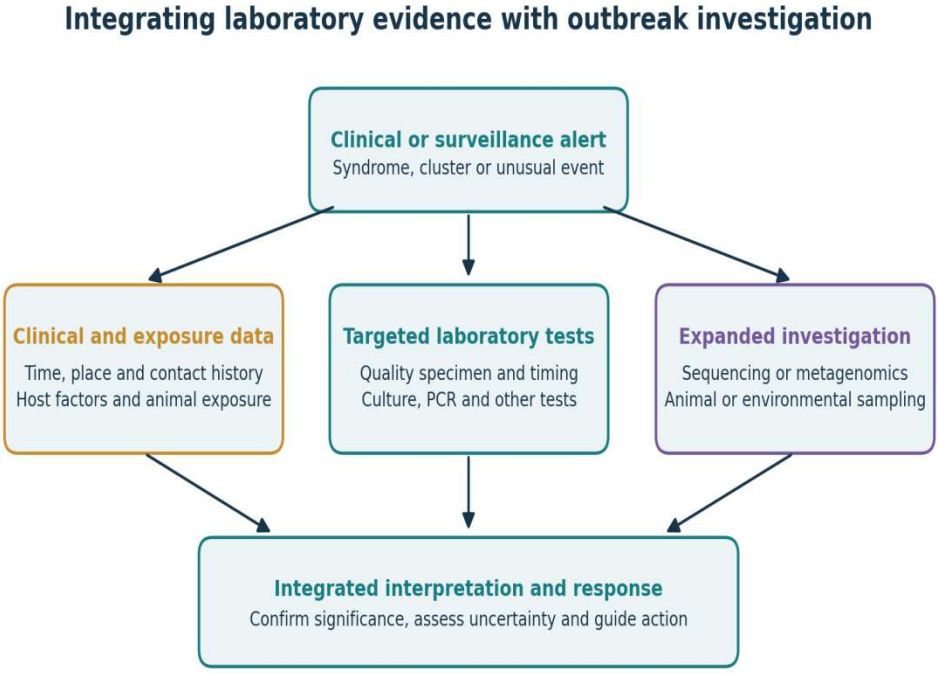


Figure 3. Laboratory investigation within an integrated clinical and epidemiological response. Expanded testing is selected according to the problem and available evidence. Original schematic informed by references 25–28.

Prevention and response through One Health

One Health recognises the interdependence of human, animal, plant and environmental health. The Quadripartite One Health Joint Plan of Action provides a framework for coordinated prevention and response across these sectors. Its practical value lies in shared investigations and linked decisions, such as combining human case findings with animal exposure histories and environmental assessment [29]. Prevention should address demonstrated transmission routes. Vaccination closes susceptibility gaps where effective vaccines are available. Safe water and food systems reduce enteric exposures. Vector control requires knowledge of the local vector and its habitat. Healthcare infection prevention interrupts transmission through hands,

equipment, surfaces and relevant respiratory pathways. Appropriate antimicrobial use reduces avoidable selection pressure.

An effective outbreak response integrates case detection, care, laboratory confirmation, contact assessment where indicated, risk communication and monitoring of control measures. Measures should be proportionate to the organism and setting. Maintaining routine health services is also essential, because disruption of immunisation and other programmes can permit additional infections to reemerge.

Conclusion

Emerging and reemerging infections reflect changing relationships among infectious agents, susceptible hosts and their environments. Newly recognised viruses attract attention, but resurgence of measles and cholera, persistent tuberculosis, resistant bacteria and invasive fungi can be equally consequential. Classification should always be linked to a defined population, region and period. Microbiology explains how pathogens replicate, evade defence, exchange genes and respond to antimicrobial pressure. It also provides the evidence needed to distinguish colonisation from disease, identify transmission patterns and guide intervention. Molecular diagnostics, genomics and metagenomics expand this capability, but their findings remain dependent on specimen quality, validation and clinical interpretation. Environmental surveillance adds another perspective without replacing patient-based investigation.Sustained prevention requires vaccination, safe water and food, infection prevention, responsible antimicrobial use and accessible healthcare. A One Health approach connects these measures with animal and environmental surveillance. The central goal is to recognise meaningful changes early and convert reliable microbiological evidence into timely, locally appropriate action.

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Microbiology and Epidemiology in Understanding and Controlling Infectious Diseases

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Abstract

Microbiology and epidemiology are complementary disciplines that explain the biological basis and population distribution of infectious diseases. Microbiology investigates the structure, physiology, genetics and pathogenic mechanisms of microorganisms, whereas epidemiology examines the frequency, distribution and determinants of health events in populations. Their integration connects pathogen identification with transmission pathways, risk factors, outbreak detection and prevention. This chapter discusses microbial agents of infection, host–pathogen interactions, the epidemiological triad, disease transmission, measures of disease occurrence and major epidemiological study designs. It explains the contribution of culture, microscopy, immunological assays, nucleic acid testing and genome sequencing to surveillance and outbreak investigation. Particular attention is given to antimicrobial resistance, zoonotic infections, environmental reservoirs and the One Health approach. Emerging applications include genomic epidemiology, metagenomics, wastewater surveillance and the integration of laboratory information with population data. The chapter also addresses diagnostic uncertainty, sampling bias, ethical responsibilities and unequal access to laboratory services. Effective disease control requires interpretation of microbiological evidence within its clinical, ecological and social context. Coordinated laboratory and epidemiological systems support timely detection, reliable assessment of disease burden and interventions that protect human, animal and environmental health.

Keywords: Microbiology; epidemiology; infectious diseases; surveillance; outbreak investigation; antimicrobial resistance; genomic epidemiology; One Health.

Introduction

Infectious diseases arise through interactions among microbial agents, susceptible hosts and the environments in which exposure occurs. Understanding them requires both biological investigation and population analysis. Microbiology explains how microorganisms survive, multiply, interact with hosts and cause damage. Epidemiology establishes who becomes affected, where and when disease occurs, and which exposures or conditions influence risk.The two disciplines answer different but connected questions. Identifying a bacterium in a patient specimen helps establish a possible diagnosis; determining whether similar infections share a food source, water supply or healthcare exposure requires epidemiological investigation. Conversely, an unusual cluster of illness can direct laboratories towards particular pathogens and specimen types. Their integration transforms individual laboratory results into evidence for public health action (Bonita et al., 2006; Murray et al., 2020).This relationship extends beyond human disease. Animal infections, agricultural practices, wastewater, wildlife and environmental change can influence microbial circulation. An integrated understanding is therefore relevant to zoology, veterinary science, medicine and environmental health.Bacteria are cellular prokaryotes with diverse metabolic and structural properties. Pathogenic examples include Mycobacterium tuberculosis, Vibrio cholerae and Salmonella enterica. Viruses contain DNA or RNA within a protein capsid, sometimes surrounded by a lipid envelope, and depend on host cells for replication. Influenza viruses, rabies virus and dengue virus illustrate their diversity of transmission pathways.

Fungi are eukaryotic organisms occurring as yeasts or filamentous forms; some cause superficial infections, whereas others produce invasive disease in susceptible hosts. Protozoan parasites include Plasmodium species and Giardia duodenalis. Medical parasitology also examines helminths, although adult worms are generally not microorganisms. Prions are infectious misfolded proteins and differ fundamentally from cellular organisms and viruses.Most microorganisms do not cause disease. Resident microbial communities may support digestion, immune development and resistance to colonisation. Some members become opportunistic pathogens when host defences weaken or when they enter normally sterile sites.Colonisation is the establishment of microorganisms without necessarily producing tissue damage or symptoms. Infection involves establishment and multiplication of an infectious agent in a host and may remain asymptomatic. Infectious disease occurs when infection produces clinically meaningful dysfunction or

injury.These distinctions affect epidemiological interpretation. A positive test may indicate asymptomatic infection, colonisation, contamination or residual microbial material. Its significance depends on the specimen, assay and clinical setting.Pathogenicity describes an agent's capacity to cause disease, while virulence concerns the degree of damage or severity it produces in a particular host context. Adhesion, invasion, toxin production, immune evasion and biofilm formation can contribute to pathogenic mechanisms. Host age, nutrition, immunity, genetic characteristics and underlying illness influence outcomes. Infectiousness and clinical severity should be evaluated separately: severe disease does not automatically imply efficient transmission.

Epidemiological Foundations

The agent–host–environment triad provides a useful framework for analysing infection. Agent characteristics include infectious dose, survival outside the host and capacity for immune evasion. Host characteristics include susceptibility, vaccination, occupation and behaviour. Environmental conditions include sanitation, housing, ventilation, climate and contact with animals or vectors.The same organism may produce different disease patterns under different conditions. Crowding can increase opportunities for respiratory exposure, while unsafe water can increase exposure to enteric pathogens. The triad should be supplemented by consideration of social inequalities, healthcare access and interconnected ecological systems.A reservoir is the habitat in which an infectious agent normally persists, lives or multiplies. Reservoirs may include humans, animals or environmental settings. The source is the immediate origin from which a host acquires an agent. A reservoir and a source may coincide, but contaminated food can be the immediate source of an infection originating from an animal reservoir.

Transmission can occur through direct contact, respiratory particles, contaminated food or water, blood exposure, sexual contact, vectors or maternal transmission. The importance of each route depends on the pathogen and circumstances. A vector transmits an agent between hosts; a biological vector also supports part of the agent's development or multiplication.The incubation period is the interval between infection and symptom onset. The latent period, in transmission modelling, is the interval between infection and becoming infectious. When infectiousness begins before symptoms, symptom-based detection alone may miss transmission opportunities.Endemic disease has an expected or persistent occurrence in a defined population. An epidemic involves occurrence above the expected level; an outbreak commonly refers to a more localised event. A pandemic is an epidemic with extensive international

spread. These terms describe distribution and occurrence rather than clinical severity.

Foundations of Infectious Disease Epidemiology and Transmission Dynamics

Population denominators are essential for comparing disease patterns. Counts alone cannot distinguish a large population with low individual risk from a small population with high risk.

Measure	Definition	Main application
Incidence proportion	New cases during a period divided by the initially disease-free population at risk	Estimating risk over a defined interval
Incidence rate	New cases divided by accumulated person-time at risk	Comparing occurrence with unequal follow-up
Point prevalence	Existing cases divided by the population at a specified time	Assessing current burden
Attack proportion	New outbreak cases divided by the relevant population at risk	Comparing outbreak exposures
Case fatality proportion	Deaths attributed to a disease divided by cases of that disease	Assessing severity among detected cases

Attack proportion is often called attack rate, although it is a proportion. Prevalence depends on both new disease occurrence and duration. Case fatality estimates depend on case ascertainment, follow-up and the definition of disease-associated death (CDC, 2012).For illustration, if 40 of 200 initially unaffected participants develop illness after a shared exposure, the attack proportion is 20%. This hypothetical calculation describes that defined group and interval.The basic reproduction number, R_0, is the expected number of secondary infections caused by a typical infectious individual in a wholly susceptible population under specified conditions. The effective reproduction number reflects transmission under current immunity and interventions. These measures depend on population conditions and modelling assumptions, rather than being fixed biological constants.Descriptive studies characterise illness by person, place and time. Case reports, case series, epidemic curves and spatial descriptions can identify unusual patterns and generate hypotheses.Cross-sectional studies assess exposure and outcome at approximately the same time.

They are useful for measuring prevalence, but often cannot establish whether an exposure preceded disease. Case–control studies compare prior exposures among cases and controls drawn appropriately from the population giving rise to the cases. They are useful for uncommon outcomes and usually estimate odds ratios.Cohort studies follow or reconstruct outcomes in exposed and unexposed groups. They can estimate incidence and relative risk, but may be affected by loss to follow-up or differences between groups. Experimental studies allocate interventions and assess outcomes; randomisation can reduce confounding when ethically and practically appropriate.In every design, selection bias, measurement error and confounding require attention. An association between an exposure and a positive test is insufficient by itself to demonstrate causation. Temporal sequence, plausible mechanisms and consistency with other evidence strengthen interpretation (Bonita et al., 2006).

Microbiological Methods in Epidemiological Investigation

Laboratory evidence begins with an appropriate specimen collected at an informative stage of illness. Collection technique, transport conditions, storage and prior antimicrobial treatment can affect results. Specimen records should link laboratory data with relevant clinical and exposure information while protecting personal identifiers.Microscopy can provide rapid information about microbial morphology or parasites. Culture demonstrates recoverable viable organisms and supplies isolates for further characterisation. However, growth requirements differ, some agents cannot be cultured routinely, and a negative culture does not exclude infection.Antigen assays detect microbial components, while antibody assays assess host immune responses. Antibody detection may reflect previous infection or vaccination rather than current disease. Timing, cross-reactivity and the purpose of testing influence interpretation.PCR and other nucleic acid amplification methods detect selected genetic targets, often rapidly and sensitively. They may remain positive after viable organisms have declined. Detection therefore does not invariably establish active infection, infectiousness or the cause of a patient's symptoms.Sensitivity describes the proportion of affected individuals correctly identified; specificity describes the proportion of unaffected individuals correctly classified. Predictive values also depend on disease prevalence or the pre-test probability in the tested population. Test accuracy should be evaluated in settings relevant to its intended use.Microbial typing distinguishes strains within a species. Whole-genome sequencing offers detailed comparisons that can support recognition of related isolates and investigation of possible transmission clusters. Genetic relatedness must be interpreted alongside

sampling dates, patient contacts and exposure histories; closely related genomes alone do not prove direct person-to-person transmission (Didelot et al., 2012).

Surveillance is the continuing collection, analysis, interpretation and communication of health information for action. Passive systems receive routine reports; active systems seek cases directly. Sentinel systems collect information from selected facilities, while syndromic systems monitor patterns of symptoms before full diagnostic confirmation.Laboratory surveillance tracks confirmed agents, strain characteristics and resistance patterns. Changes in testing access, referral practices or diagnostic methods can create apparent changes in disease occurrence. Reliable interpretation requires awareness of who was tested, which specimens were examined and how reporting changed.Genomic surveillance connects sequence data with epidemiological information. Its usefulness depends on representative sampling, timely processing and the ability to translate findings into response. WHO's 2022–2032 strategy places genomics within broader laboratory and surveillance capacities, including specimen collection, diagnostics and data analysis (WHO, 2022).

An outbreak investigation combines field observations, clinical assessment and microbiological evidence. Its main activities include verifying the diagnosis and unusual occurrence; defining cases by clinical, laboratory, time and place criteria; identifying additional cases; describing patterns; developing and testing exposure hypotheses; and evaluating control measures.Laboratories help distinguish agents that produce similar syndromes and compare patient, food, animal or environmental samples. Epidemiologists establish whether observed similarities correspond to plausible shared exposures. Control measures should begin when sufficient evidence supports action and need not await completion of every analytical step (CDC, 2012).For example, a hypothetical cluster of diarrhoea after a community meal could be examined by comparing food consumption among ill and unaffected participants, testing suitable specimens and inspecting food handling. Agreement among exposure associations, laboratory findings and environmental observations would strengthen the source assessment. A negative food sample would not necessarily rule out the suspected source if contaminated portions were no longer available.Antimicrobial resistance arises when microorganisms can withstand drugs that would otherwise inhibit or kill them. Mechanisms include drug inactivation, altered targets, reduced entry and increased efflux. Mutation and horizontal gene transfer contribute to resistance,

while antimicrobial exposure can favour resistant populations.Microbiology identifies susceptibility patterns and resistance determinants. Epidemiology examines their distribution across patients, facilities, animals and environmental settings. A resistance gene may circulate through mobile genetic elements among different strains, so surveillance must distinguish transmission of organisms from movement of resistance determinants.WHO's GLASS programme supports standardised surveillance of resistance and antimicrobial use. Interpretation requires attention to specimen type, patient population and testing practices. Hospital samples may overrepresent severe or previously treated infections and should not automatically be treated as representative of the whole community (WHO, 2021). Prevention combines infection control, vaccination, appropriate antimicrobial use, sanitation and effective surveillance.

Zoonotic infections pass naturally between vertebrate animals and humans, requiring investigation of animal reservoirs, occupational exposure, food systems and environmental contamination. One Health integrates human, animal, plant and environmental health to address shared risks. Freshwater research can connect animal health, water quality, microbial contamination and human exposure, while distinguishing fish pathogens, human pathogens and indicator organisms. Detecting microorganisms or resistance genes does not establish transmission or identify infection sources. Metagenomic sequencing analyses genetic material in mixed samples but requires contamination controls, reliable databases and contextual interpretation; detected sequences do not necessarily represent viable pathogens. Wastewater surveillance complements clinical reporting by providing population information, although it cannot identify infected individuals or determine exact case numbers. Spatial analysis and machine learning can reveal patterns, but require validation and cannot independently establish causation. Priorities include inclusive sampling, rapid laboratory response, coordinated research, staff training, quality assurance and sustained operational support across participating sectors.Surveillance can involve sensitive clinical histories, locations and genomic information. Appropriate consent or public health authority, controlled access and proportionate reporting are necessary. Small-area reporting can stigmatise communities if uncertainty and context are neglected.Unequal access to diagnostics affects both care and the accuracy of disease estimates. Shared case definitions, quality assurance and compatible data systems improve comparisons. Laboratory and field teams also require appropriate biosafety, communication and professional training. Credible findings depend on scientific quality and public trust.

Conclusion

Microbiology and epidemiology together explain how infectious agents behave and how infections are distributed within populations. Laboratory investigation identifies microbial agents and their characteristics, while epidemiological analysis connects those findings with exposures, transmission patterns and disease burden. Their integration supports more reliable diagnosis, earlier outbreak recognition and informed prevention.The value of a laboratory result depends on its context. Detection of microbial material does not always establish viable infection, and genetic similarity does not independently prove direct transmission. Representative sampling, appropriate denominators, reliable case definitions and consideration of bias are therefore essential.Genomic sequencing, metagenomics and wastewater surveillance expand investigative capacity, but their benefits depend on interpretation and timely public health action. Antimicrobial resistance and zoonotic infections further demonstrate the need to connect human health with animal populations and environmental conditions.Future progress requires coordinated surveillance, strong laboratory services, trained personnel and ethical data sharing. For researchers in zoology and related disciplines, this integrated approach creates opportunities to examine disease at the interfaces among hosts, microorganisms and ecosystems. Combining biological evidence with careful population analysis provides a sound foundation for protecting health and preventing infectious disease.

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Antimicrobial Resistance in the Twenty-First Century: Emerging Challenges, Novel Therapeutics and One Health Perspectives

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Abstract

Antimicrobial resistance (AMR) has emerged as one of the most serious global public health challenges of the twenty-first century, threatening the effectiveness of antimicrobial therapy and compromising the management of infectious diseases. The rapid emergence and dissemination of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) pathogens have been driven by the inappropriate use of antimicrobials in human medicine, veterinary practice, agriculture, and environmental settings. This review highlights the global burden, epidemiology, and clinical implications of AMR, emphasizing its growing impact on healthcare systems, economies, and patient outcomes. The molecular mechanisms underlying antimicrobial resistance are comprehensively discussed, including enzymatic degradation and modification of antibiotics, target-site alterations, active efflux pumps, reduced membrane permeability, biofilm-mediated tolerance, and horizontal gene transfer through conjugation, transformation, and transduction. Particular attention is given to the dissemination of resistance genes via mobile genetic elements and the emergence of clinically significant resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), carbapenem-resistant Enterobacterales (CRE), and other ESKAPE pathogens. The review further underscores the importance of global surveillance, antimicrobial stewardship, rapid diagnostics, and infection prevention strategies. Emerging therapeutic approaches, including bacteriophage therapy, CRISPR-Cas-based antimicrobials, antimicrobial peptides, nanotechnology-assisted drug delivery, artificial intelligence-driven drug discovery, and plant-derived antimicrobial compounds, are highlighted as promising alternatives to conventional antibiotics. Finally, the review emphasizes that sustainable mitigation of AMR requires coordinated multidisciplinary efforts integrating human, animal, and environmental health

through the One Health framework to preserve the effectiveness of existing and future antimicrobial therapies.

Key Words: Antimicrobial resistance, Multidrug-resistant bacteria, One Health, Antimicrobial stewardship, CRISPR-Cas, Bacteriophage therapy.

Introduction

The discovery of antibiotics in the early twentieth century revolutionized modern medicine and transformed the management of infectious diseases, significantly reducing morbidity and mortality worldwide. The introduction of penicillin by Alexander Fleming in 1928, followed by the development of numerous antimicrobial agents, enabled effective treatment of bacterial infections that were previously considered fatal. Antibiotics became indispensable for modern healthcare by facilitating complex surgical procedures, organ transplantation, cancer chemotherapy, intensive care management, and neonatal medicine (Davies & Davies, 2010; Ventola, 2015). However, the remarkable success of antimicrobial therapy has been progressively undermined by the emergence and rapid spread of antimicrobial resistance (AMR), a phenomenon in which microorganisms evolve mechanisms to survive exposure to antimicrobial agents that were once effective against them. Today, AMR is recognized as one of the greatest threats to global public health, food security, and sustainable development (World Health Organization [WHO], 2026; Antimicrobial Resistance Collaborators, 2022).

Antimicrobial resistance is a natural evolutionary process driven by spontaneous genetic mutations and horizontal gene transfer among microorganisms. Nevertheless, human activities have substantially accelerated its emergence and dissemination. The inappropriate and excessive use of antibiotics in human medicine, self-medication, poor patient adherence, indiscriminate use of antimicrobials in livestock production and aquaculture, inadequate infection prevention and control measures, and environmental contamination with antimicrobial residues have collectively intensified the selection pressure favoring resistant microorganisms (Munita & Arias, 2016; WHO, 2026). Consequently, multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) pathogens have emerged across healthcare and community settings, compromising the effectiveness of existing antimicrobial therapies and limiting treatment options for common infectious diseases.

The global burden of AMR has increased dramatically over the past two decades, affecting both developed and developing countries. Resistant bacterial infections are responsible for a substantial proportion of hospital admissions, prolonged illnesses, treatment failures, and infection-related deaths. The landmark Global Research on Antimicrobial Resistance (GRAM) study estimated that 4.95 million deaths were associated with bacterial AMR in 2019, including approximately 1.27 million deaths directly attributable to resistant bacterial infections, making AMR one of the leading infectious causes of mortality worldwide (Antimicrobial Resistance Collaborators, 2022). More recent estimates indicate that bacterial AMR was associated with more than 4.7 million deaths globally in 2021, with projections suggesting a continued increase if effective interventions are not implemented (GBD 2021 Antimicrobial Resistance Collaborators, 2024; WHO, 2026).

The burden of AMR is disproportionately higher in low- and middle-income countries, where limited diagnostic capacity, unrestricted access to antibiotics, inadequate sanitation, weak surveillance systems, and insufficient antimicrobial stewardship programs contribute to the rapid emergence and spread of resistant pathogens (Laxminarayan et al., 2016; WHO, 2026). The World Health Organization has identified AMR as one of the top global public health threats. Particularly concerning is the increasing prevalence of the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp.), which exhibit remarkable abilities to evade multiple classes of antimicrobial agents and account for a significant proportion of healthcare-associated infections worldwide (Tacconelli et al., 2018). The evolution of antimicrobial resistance closely followed the introduction of antibiotics into clinical practice. Shortly after penicillin became widely available during the 1940s, penicillin-resistant strains of *Staphylococcus aureus* were reported, illustrating the remarkable adaptive capacity of bacteria (Davies & Davies, 2010). Subsequent decades witnessed the emergence of resistance against virtually every major antibiotic class soon after its clinical introduction, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), extended-spectrum β-lactamase (ESBL)-producing Enterobacterales, carbapenem-resistant Enterobacterales (CRE), and multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) (Ventola, 2015; Munita & Arias, 2016). Initially, pharmaceutical innovation compensated for emerging resistance through the continuous discovery of novel antimicrobial agents. However, antibiotic discovery has slowed considerably during the past three decades because of

scientific, regulatory, and economic challenges, while the dissemination of resistance genes through plasmids, transposons, integrons, and other mobile genetic elements has accelerated worldwide (O'Neill, 2016; WHO, 2026). The epidemiology of AMR is highly dynamic and varies geographically depending on antimicrobial consumption patterns, healthcare infrastructure, infection control practices, and socioeconomic conditions. High levels of resistance have been reported among Gram-negative pathogens such as *Klebsiella pneumoniae, Escherichia coli, Acinetobacter baumannii,* and *Pseudomonas aeruginosa*, particularly against third-generation cephalosporins, fluoroquinolones, and carbapenems. Similarly, Gram-positive pathogens including MRSA and VRE continue to represent major causes of healthcare-associated infections globally (WHO, 2026). The widespread availability of whole-genome sequencing, molecular epidemiology, and global surveillance networks has greatly enhanced understanding of resistance evolution and transmission dynamics. Furthermore, international travel, medical tourism, food trade, migration, and environmental dissemination have facilitated the rapid cross-border spread of antimicrobial resistance determinants. The interconnectedness of human, animal, and environmental health has reinforced the importance of adopting the One Health approach to combat AMR through integrated surveillance and coordinated intervention strategies (WHO, 2026; Chindelevitch et al., 2022).

The consequences of antimicrobial resistance extend far beyond microbiological and clinical concerns, imposing enormous economic and societal burdens worldwide. Resistant infections are associated with delayed initiation of effective therapy, prolonged hospitalization, increased intensive care admissions, higher treatment costs, elevated mortality, and poorer clinical outcomes (Ventola, 2015; Prestinaci et al., 2015). Patients infected with multidrug-resistant organisms frequently require more expensive second-or third-line antimicrobial agents, longer treatment durations, and additional diagnostic investigations. Beyond direct healthcare costs, AMR contributes substantially to indirect economic losses through reduced workforce productivity, long-term disability, caregiver burden, and premature mortality. Recent WHO estimates indicate that, if current trends continue, the annual global cost of treating resistant bacterial infections alone could exceed US$ 412 billion, accompanied by approximately US$ 443 billion in annual productivity losses over the coming decade (WHO, 2026). Furthermore, long-term projections suggest that AMR could become one of the leading causes of death globally by 2050 unless comprehensive mitigation strategies are implemented (GBD 2021 Antimicrobial Resistance Collaborators, 2024; O'Neill, 2016).

The escalating burden of AMR has prompted governments, healthcare organizations, researchers, and international agencies to prioritize antimicrobial stewardship, surveillance systems, rapid diagnostic technologies, infection prevention strategies, and the development of innovative therapeutics. Emerging approaches—including bacteriophage therapy, CRISPR-Cas-based antimicrobials, antimicrobial peptides, nanotechnology-assisted drug delivery, artificial intelligence-driven drug discovery, and plant-derived antimicrobial compounds—offer promising alternatives to conventional antibiotics (Shim, 2022; Chindelevitch et al., 2022). Nevertheless, sustainable mitigation of antimicrobial resistance requires coordinated multidisciplinary efforts integrating microbiology, clinical medicine, veterinary sciences, environmental health, public policy, and global collaboration under the One Health framework

Molecular Mechanisms of Antimicrobial Resistance

Antimicrobial resistance (AMR) is a complex biological phenomenon that enables microorganisms to survive exposure to antimicrobial agents that would normally inhibit their growth or kill them. Resistance may be intrinsic, resulting from inherent structural or physiological characteristics of microorganisms, or acquired, arising through spontaneous genetic mutations or the acquisition of resistance genes from other microorganisms. The widespread and often inappropriate use of antibiotics in human medicine, veterinary practice, agriculture, and aquaculture has accelerated the evolution and dissemination of resistant microorganisms, making AMR one of the greatest threats to global health (Munita & Arias, 2016; Blair et al., 2015; World Health Organization [WHO], 2025). Bacteria have evolved several sophisticated molecular mechanisms to evade the action of antimicrobial agents. These mechanisms include enzymatic degradation or modification of antibiotics, alteration of antimicrobial targets, active efflux of antibiotics, reduced membrane permeability, biofilm-mediated resistance, and horizontal gene transfer (HGT). These mechanisms often coexist within a single bacterial isolate, resulting in multidrug-resistant (MDR), extensively drug-resistant (XDR), or pan-drug-resistant (PDR) phenotypes that significantly compromise clinical treatment (Bush & Bradford, 2020).

Enzymatic Degradation or Modification of Antimicrobial Agents

Enzymatic degradation is one of the most prevalent and clinically significant mechanisms of antimicrobial resistance. In this mechanism, bacteria synthesize enzymes capable of hydrolysing or chemically modifying antimicrobial agents before they reach their intracellular targets. The most important example is the production of β-lactamases, which hydrolyse the β-lactam ring of penicillins, cephalosporins, monobactams, and carbapenems, thereby rendering these antibiotics ineffective (Bush & Bradford, 2020). β-Lactamases are broadly classified into narrow-spectrum β-lactamases, extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, and carbapenemases. Among carbapenemases, Klebsiella pneumoniae carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), Verona integron-encoded metallo-β-lactamase (VIM), and OXA-type carbapenemases represent major global threats because they confer resistance to last-resort β-lactam antibiotics (Bush & Bradford, 2020). Apart from hydrolysis, several bacterial enzymes chemically modify antibiotics by acetylation, phosphorylation, or adenylation. Aminoglycoside-modifying enzymes, including aminoglycoside acetyltransferases (AACs), phosphotransferases (APHs), and nucleotidyltransferases (ANTs), alter aminoglycoside molecules and prevent their interaction with the bacterial ribosome. Similar enzymatic modifications contribute to resistance against chloramphenicol, macrolides, rifamycins, and streptogramins (Ramirez & Tolmasky, 2017).

Target Modification

Target modification enables bacteria to evade antimicrobial action by altering the structure or function of antibiotic-binding sites while preserving essential cellular functions. Such modifications reduce the affinity of antimicrobial agents for their molecular targets without significantly affecting bacterial viability (Munita & Arias, 2016). One of the best-known examples is methicillin-resistant *Staphylococcus aureus* (MRSA), in which the mecA gene encodes an altered penicillin-binding protein (PBP2a) that exhibits very low affinity for β-lactam antibiotics, thereby conferring resistance to nearly all β-lactam drugs (Chambers & DeLeo, 2009). Similarly, vancomycin-resistant enterococci (VRE) acquire vanA or vanB gene clusters that replace the terminal D-Ala-D-Ala residues of peptidoglycan precursors with D-Ala-D-Lac, reducing vancomycin binding affinity by nearly 1,000-fold

(Arthur et al., 1996). Resistance to fluoroquinolones commonly results from mutations in the gyrA, gyrB, parC, and parE genes encoding DNA gyrase and topoisomerase IV, whereas resistance to macrolides frequently occurs through methylation of the 23S rRNA mediated by *erm* genes, preventing antibiotic binding to the bacterial ribosome (Blair et al., 2015).

Efflux Pumps

Efflux pumps are membrane-associated transport proteins that actively expel antimicrobial agents from bacterial cells, thereby reducing intracellular drug concentrations below inhibitory levels. These transport systems contribute to both intrinsic and acquired resistance and frequently exhibit broad substrate specificity, allowing bacteria to resist multiple classes of antibiotics simultaneously (Du et al., 2018).Bacterial efflux pumps are categorized into five major families: the Major Facilitator Superfamily (MFS), Resistance-Nodulation-Division (RND) family, ATP-Binding Cassette (ABC) transporters, Small Multidrug Resistance (SMR) family, and the Multidrug and Toxic Compound Extrusion (MATE) family. Among Gram-negative bacteria, the AcrAB–TolC efflux system of *Escherichia coli* and the MexAB–OprM system of *Pseudomonas aeruginosa* are among the best-characterized multidrug efflux pumps. Their overexpression contributes to resistance against β-lactams, quinolones, tetracyclines, chloramphenicol, and several other antimicrobial classes (Du et al., 2018).The increasing recognition of efflux pumps as major contributors to multidrug resistance has stimulated research into efflux pump inhibitors (EPIs), which may restore bacterial susceptibility when used in combination with conventional antibiotics.

Reduced Membrane Permeability

Reduced membrane permeability represents another important mechanism of antimicrobial resistance, particularly among Gram-negative bacteria. The outer membrane acts as an effective permeability barrier that limits antibiotic penetration into bacterial cells. Hydrophilic antibiotics generally enter Gram-negative bacteria through porin proteins such as OmpF, OmpC, and OprD (Delcour, 2009). Mutations, deletion, or downregulation of porin proteins decrease antibiotic uptake, thereby reducing intracellular antimicrobial concentrations. Loss of the OprD porin in *Pseudomonas aeruginosa*, for example, is strongly associated with carbapenem resistance. Reduced membrane permeability frequently acts synergistically with β-lactamase production and multidrug efflux pumps, resulting in high-level resistance that is difficult to overcome clinically

(Delcour, 2009).Changes in membrane lipid composition and alterations in cell envelope architecture may further reduce susceptibility to polymyxins, antimicrobial peptides, and several hydrophobic antimicrobial agents.

Biofilm-Mediated Resistance

Biofilms are structured microbial communities enclosed within a self-produced extracellular polymeric substance (EPS) matrix consisting of polysaccharides, proteins, extracellular DNA, and lipids. Biofilm formation represents one of the most effective survival strategies employed by pathogenic microorganisms and is responsible for numerous chronic and recurrent infections (Hall & Mah, 2017).Within biofilms, bacteria exhibit markedly increased tolerance to antimicrobial agents through multiple mechanisms. The EPS matrix acts as a physical barrier that limits antibiotic diffusion, while nutrient limitation and oxygen gradients create metabolically inactive or slow-growing bacterial populations that are inherently less susceptible to antibiotics. Furthermore, biofilms harbour persisted cells, a small subpopulation of dormant bacteria capable of surviving prolonged antimicrobial exposure without possessing genetic resistance (Hall & Mah, 2017).Biofilms also facilitate quorum sensing, oxidative stress responses, and horizontal gene transfer, enhancing both antimicrobial tolerance and the dissemination of resistance genes. Clinically significant biofilm-associated pathogens include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Candida* species, particularly in chronic wounds, indwelling medical devices, prosthetic implants, urinary catheters, and respiratory infections (Flemming et al., 2016).

Horizontal Gene Transfer

Horizontal gene transfer (HGT) is the movement of genetic material between microorganisms independent of parent-to-offspring inheritance. HGT is considered one of the primary driving forces responsible for the rapid dissemination of antimicrobial resistance genes among bacterial populations. Resistance determinants are frequently carried on mobile genetic elements, including plasmids, transposons, integrons, insertion sequences, integrative conjugative elements, and bacteriophages, allowing rapid dissemination across bacterial species and even genera (von Wintersdorff et al., 2016).Conjugation is the most important mechanism of horizontal gene transfer in bacteria. During conjugation, donor bacteria transfer plasmid DNA directly to recipient cells through specialized conjugative pili encoded by self-transmissible plasmids. Conjugative plasmids frequently carry multiple antimicrobial resistance genes,

allowing simultaneous dissemination of resistance to β-lactams, aminoglycosides, quinolones, sulfonamides, tetracyclines, and carbapenems (Partridge et al., 2018). Globally important carbapenemase genes, including *blaNDM*, *blaKPC*, *blaOXA-48,* and blaVIM, are predominantly disseminated through conjugative plasmids, contributing substantially to the worldwide spread of multidrug-resistant Gram-negative pathogens. Transformation involves the uptake of free extracellular DNA released from lysed bacterial cells into naturally competent recipient bacteria. Once internalized, the DNA may integrate into the bacterial chromosome through homologous recombination or persist as extrachromosomal DNA. Naturally competent bacterial species such as *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, *Haemophilus influenzae*, and *Acinetobacter baumannii* frequently acquire resistance genes through transformation, thereby accelerating the evolution of resistant populations (Johnston et al., 2014). Environmental DNA present in hospital wastewater, soil, aquatic ecosystems, and biofilms serves as an important reservoir of antimicrobial resistance genes available for transformation.

Transduction

Transduction is mediated by bacteriophages (bacterial viruses), which inadvertently package fragments of bacterial DNA during viral replication and subsequently transfer these genes into newly infected bacterial cells. Both generalized and specialized transduction contribute to the dissemination of antimicrobial resistance genes, virulence determinants, and pathogenicity islands (Penadés et al., 2015). Although transduction generally contributes less to the dissemination of antimicrobial resistance than conjugation, it plays a crucial role in the evolution of *Staphylococcus aureus* and several other clinically important pathogens. Increasing evidence suggests that bacteriophage-mediated gene transfer contributes significantly to bacterial adaptation and the emergence of novel multidrug-resistant clones (Penadés et al., 2015).

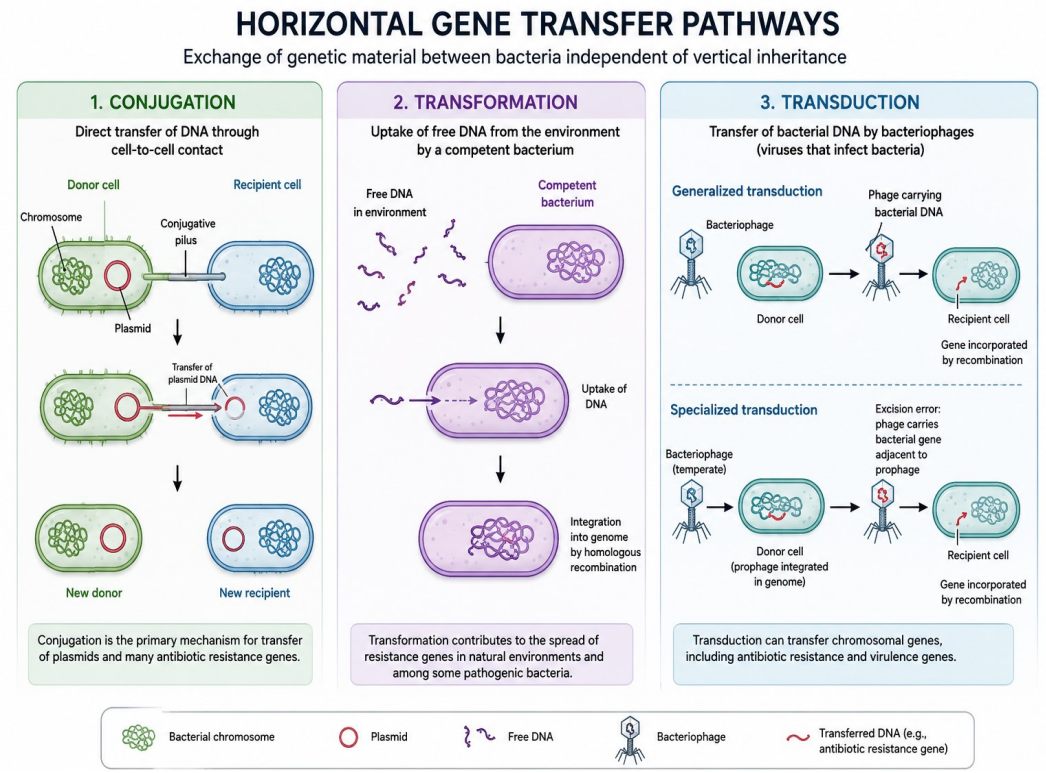


Figure 1: Various horizontal gene transfer pathways, these possibly contributes significantly to bacterial adaptation and the emergence of novel multidrug-resistant clones.

Conclusion

Antimicrobial resistance (AMR) is a rapidly growing global health challenge that threatens the effectiveness of existing antimicrobial therapies. The evolution and spread of resistance through multiple molecular mechanisms, together with the emergence of multidrug-resistant pathogens, have significantly complicated the treatment of infectious diseases. Combating AMR requires antimicrobial stewardship, effective surveillance, rapid diagnostics, infection prevention, and the development of novel therapeutic strategies. A coordinated One Health approach integrating human, animal, and environmental health is essential to preserve the efficacy of existing antimicrobials and ensure sustainable management of antimicrobial resistance.

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Microbiology and Zoonotic Diseases with Emphasis on Transmission, Prevention and the One Health Approach

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Abstract

Zoonotic diseases are infections naturally transmitted from vertebrate animals to humans. They are caused by diverse biological agents, including bacteria, viruses, fungi and parasites, and represent an important interface between microbiology, zoology, veterinary medicine and public health. Their transmission may occur through direct animal contact, bites, contaminated food or water, inhalation, or arthropod vectors. Microbial characteristics, animal reservoirs, human susceptibility and environmental conditions collectively determine whether exposure results in infection. This chapter discusses the major groups of zoonotic pathogens, their transmission pathways, mechanisms of disease development, and microbiological methods used for diagnosis and surveillance. Particular attention is given to livestock-associated, wildlife-associated, foodborne and aquatic infections. The chapter also examines environmental change, antimicrobial resistance and the emergence of infections at the human–animal interface. Prevention requires coordinated measures involving animal health, food safety, sanitation, occupational protection, vaccination and timely diagnosis. The One Health approach provides an integrated framework for these activities by recognising the interdependence of human, animal and environmental health. Improved laboratory capacity, collaborative surveillance and locally appropriate prevention strategies are essential for reducing zoonotic disease risks.

Keywords: Microbiology; zoonoses; microbial pathogens; animal reservoirs; spillover; antimicrobial resistance; One Health; public health.

Introduction

Microbiology investigates microorganisms and infectious agents, including their structure, physiology, genetics, ecological relationships and disease-causing properties. Its relationship with zoonotic diseases arises because animals can maintain infectious agents that subsequently reach humans through biological, environmental or food-related pathways.The World Health Organisation defines a zoonosis as a disease or infection naturally transmissible from vertebrate animals to humans. Transmission does not always require direct contact with an animal: contaminated animal products, water, soil and vectors can connect animal reservoirs with human populations (World Health Organisation [WHO], 2020a). Human interactions with animals provide food, livelihoods, companionship and opportunities for scientific research. However, these interactions can also create exposure to pathogens. Farmers, veterinarians, slaughterhouse workers, wildlife personnel, laboratory staff and fish handlers may encounter particular occupational risks.Microbiology contributes to zoonotic disease control by identifying causative agents, explaining their survival and transmission, detecting infections and supporting effective interventions. Zoology contributes knowledge of host biology, animal behaviour, population dynamics and vector ecology. Integrating these disciplines helps explain disease transmission across the human–animal–environment interface.

A pathogen is a biological agent capable of causing disease. A host is an organism that supports the agent during part or all of its life cycle. A reservoir is a population or ecological system in which a pathogen is maintained and from which infection can reach a susceptible population. A reservoir animal may show illness, mild symptoms or no obvious disease. An amplifying host allows a pathogen to multiply sufficiently to increase opportunities for onward transmission. An intermediate host, particularly in parasitic infections, supports a developmental stage required within the parasite's life cycle. These terms describe different biological roles and should not be used interchangeably. An animal associated with a human infection is not necessarily the pathogen's long-term reservoir. Spillover occurs when a pathogen passes from an animal host to a human. Successful spillover requires several conditions: the pathogen must be present in a source population, leave that source, reach a person through an effective route and overcome barriers to infection. An animal-to-human infection does not automatically lead to sustained human-to-human transmission. Some zoonoses mainly produce

isolated infections, whereas others can generate transmission chains after introduction into humans (Plowright et al., 2017).

Infection means that an infectious agent has established itself within a host. Disease involves resulting damage or disturbance of normal function. Carriage refers to harbouring an agent, sometimes without symptoms. Apparently healthy animals may carry pathogens capable of causing human illness. Consequently, animal appearance alone cannot establish microbiological safety (Centres for Disease Control and Prevention.

Major Groups of Zoonotic Agents

Zoonotic agents differ in cellular organisation, replication, environmental survival and response to treatment. These differences influence diagnosis and control.

Agent group	Representative diseases	Examples of associated animals or sources	Common transmission pathways
Bacteria	Brucellosis, leptospirosis	Livestock, rodents and other infected mammals	Animal tissues, raw dairy products, urine-contaminated water or soil
Viruses	Rabies, zoonotic influenza	Infected mammals; infected birds or other animals, depending on the virus	Bites, saliva exposure, close contact and contaminated environments
Fungi	Zoonotic dermatophytosis	Infected companion animals or livestock	Contact with infected skin, hair or contaminated materials
Protozoa	Toxoplasmosis	Felids; tissue cysts in infected food animals	Ingestion of oocysts or tissue cysts
Helminths	Taeniasis, echinococcosis and some fish-borne parasitic infections	Hosts vary with the parasite	Ingestion of eggs or larval stages

Helminths are multicellular animals rather than microorganisms. Nevertheless, they are commonly discussed alongside microbial zoonoses within medical and veterinary parasitology.

Bacteria can cause local infections, gastrointestinal disease or systemic illness. Their zoonotic potential depends on their host range, route of entry, virulence and ability to survive outside a host. Brucellosis is caused by bacteria of the genus *Brucella*. Human infection can follow consumption of contaminated unpasteurised dairy products, contact with infected tissues and body fluids, or inhalation of infectious material. Animal workers and laboratory personnel are important occupational risk groups. Clinical manifestations can include fever, sweating, fatigue and joint or back pain (CDC, 2026a). Leptospirosis is caused by pathogenic *Leptospira*. Infected animals shed bacteria in urine, contaminating water or soil. Human exposure often occurs when contaminated material contacts damaged skin or mucous membranes. Flooding can increase exposure opportunities. Severe disease may involve kidney damage, liver dysfunction, meningitis or respiratory complications (CDC, 2026b). Other important bacterial zoonoses include anthrax, zoonotic tuberculosis, salmonellosis and campylobacteriosis. Their prevention requires attention to the particular animal source, food pathway and environmental conditions involved.

Viruses replicate within living cells. Their ability to infect another species depends partly on receptor compatibility, intracellular replication conditions and the host's immune defences. Rabies is a viral infection affecting the central nervous system. Transmission usually occurs through saliva from an infected animal, commonly through bites. Once clinical symptoms develop, rabies is almost invariably fatal. Prompt wound washing and appropriate post-exposure prophylaxis can prevent disease following exposure (WHO, n.d.). Zoonotic influenza involves animal influenza viruses infecting humans. Influenza A viruses circulate in several animal populations, especially birds, and some can infect additional species. Close exposure to infected animals or contaminated materials can provide opportunities for human infection. Viral evolution and reassortment make coordinated animal and human surveillance particularly valuable (CDC, n.d.-b).

Some dermatophyte fungi pass from animals to humans and infect keratinised tissues, including skin and hair. Transmission may occur through direct contact or contaminated grooming equipment, bedding and other materials.Fungal disease associated with an animal environment is not necessarily zoonotic. When humans and animals acquire a fungus

independently from soil, their infections may reflect a shared environmental exposure rather than transmission between them.*Toxoplasma gondii* illustrates how animal hosts, food and the environment interact in zoonotic transmission. Felids are definitive hosts and can shed oocysts in faeces. Humans may become infected by ingesting oocysts from contaminated soil, water or food, or by consuming tissue cysts in undercooked meat. Serious consequences can occur in congenital infection and in people with impaired immunity (CDC, n.d.-c). Helminth zoonoses frequently depend on complex life cycles involving multiple hosts. Prevention therefore requires identifying the infective stage and interrupting the relevant food, sanitation or animal-management pathway.

Routes of Transmission

Infectious agents may enter through animal bites, scratches, damaged skin or mucous membranes. Exposure to blood, saliva, reproductive tissues and other body fluids can also transmit infection.The risk depends on the organism, amount of infectious material, condition of the exposed tissue and susceptibility of the individual.Animal products may become contaminated during production, slaughter, processing, storage or preparation. Raw milk and inadequately cooked animal products are important examples of potential exposure pathways.Cross-contamination can also transfer pathogens from raw animal products to ready-to-eat foods. Food safety therefore requires control throughout the production chain.Animal excreta can contaminate water, soil and equipment. Environmental persistence varies among infectious agents and is influenced by temperature, moisture, sunlight and other conditions.Leptospirosis provides a clear example of an animal-origin infection transmitted through a contaminated environment.Handling infected tissues or disturbing contaminated material can generate infectious aerosols. Such exposure may occur in animal facilities, slaughterhouses and laboratories.The likelihood of infection depends on the agent, activity, ventilation and effectiveness of protective measures.Ticks, mosquitoes, fleas and other arthropods can connect animal hosts with humans. In biological transmission, the pathogen develops or multiplies within the vector.Vector-borne disease and zoonotic disease are overlapping categories. Classification as zoonotic requires an animal component in the infection’s natural transmission cycle.These pathways explain why preventing zoonoses requires attention to animal contact, food, water, work practices and environmental hygiene (CDC, n.d.-a).

Microbial Pathogenesis and Host Responses

The outcome of exposure reflects interactions between the infectious agent and the host. Important stages include entry, attachment, multiplication, spread, immune evasion and tissue damage.Skin and mucosal surfaces form major barriers to infection. A pathogen must reach a suitable site and survive local conditions before it can establish infection.Different routes can produce different outcomes. An agent entering through a wound may cause local disease, whereas ingestion or inhalation may expose other organs.These mechanisms vary substantially among bacterial, viral, fungal and parasitic agents.Innate defences include physical barriers, phagocytic cells, complement and inflammatory responses. Adaptive immunity involves antigen-specific antibodies and T lymphocytes.Immune responses can restrict infection, but excessive or poorly regulated inflammation can also contribute to tissue damage. Age, pregnancy, nutritional status, underlying illness and immunosuppressive treatment can influence disease severity.

Microbiological Diagnosis

Accurate diagnosis combines exposure history, clinical examination and appropriate laboratory investigation. Relevant history includes animal contact, occupation, bites, raw-food consumption, freshwater exposure and travel.Microscopy can demonstrate organisms, parasite stages or tissue changes. Its sensitivity depends on the agent, specimen and stage of infection.Culture can recover viable bacteria or fungi and support further identification and antimicrobial susceptibility testing. However, some zoonotic agents require specialised facilities or pose substantial occupational hazards.Serological tests detect antibodies against an infectious agent. They are useful in diagnosis and population studies, especially when direct detection is difficult.Interpretation must consider the timing of sampling, possible cross-reactions, previous exposure and vaccination. Antibody detection does not always establish active infection.Polymerase chain reaction and related techniques detect selected genetic targets. They can provide rapid, specific evidence of infection when suitable specimens are available.A positive molecular test demonstrates detection of the target sequence. It does not invariably establish organism viability, infectiousness or the precise source of transmission.Genome sequencing can help compare isolates and identify relationships among infections in humans, animals and environmental samples.Genetic similarity must be interpreted alongside exposure information, sampling dates and epidemiological evidence. Closely related sequences alone do not necessarily establish transmission direction.Laboratory precautions

should follow an assessment of the agent, specimen, procedure and potential exposure routes. Appropriate containment, staff training, protective equipment and waste management are integral to reliable diagnostic work (WHO, 2020b).

Emergence and Environmental Change

Zoonotic emergence is influenced by changes in pathogen biology, host populations and opportunities for contact.Land-use change, agricultural expansion, urbanisation, animal movement and wildlife trade can alter the frequency and nature of interactions among wildlife, domestic animals and humans. Research on emerging infections demonstrates that ecological and demographic conditions are important for understanding where disease emergence may occur (Jones et al., 2008). Environmental effects are pathogen-specific. Flooding may increase contact with contaminated water, while changes in temperature and rainfall can affect vectors and microbial persistence. These relationships require local investigation rather than assuming that every environmental change produces the same disease response.Spillover research is most useful when it examines the complete pathway from an infected animal population to human exposure. Detecting a pathogen in wildlife establishes its presence but does not, by itself, demonstrate a high probability of human infection.Antimicrobial resistance occurs when microorganisms become less susceptible to medicines intended to control them.Resistant bacteria and resistance genes may circulate among humans, animals, food and environmental compartments. Selection pressure from antimicrobial use can contribute to this process.The presence of the same resistance gene in different settings does not automatically establish the route of transmission. Stronger conclusions require microbiological, genomic and epidemiological evidence.Prevention includes responsible prescribing, veterinary oversight, infection prevention, vaccination where appropriate and improved sanitation. The One Health framework incorporates antimicrobial resistance because its causes and consequences extend across sectors (WHO, 2022; FAO et al., 2022).

Prevention and the One Health Approach

One Health recognises that human, animal and environmental health are interdependent. It supports collaboration among clinicians, veterinarians, microbiologists, zoologists, environmental scientists and public-health authorities.The joint framework developed by FAO, UNEP, WHO and WOAH provides a basis for coordinated action at this interface (FAO et al., 2022). Surveillance should connect relevant information from human infections,

animal illness, laboratory findings and environmental investigations.Unusual animal mortality or clusters of illness among animal workers may warrant joint investigation. Shared reporting and agreed responsibilities help convert observations into timely action.Veterinary care, appropriate vaccination, disease testing and improved husbandry can reduce infection at its source.Rabies prevention demonstrates the value of combining animal vaccination, public education and accessible human post-exposure care.Important measures include pasteurisation, adequate cooking, hygienic food handling, safe drinking water and proper management of animal waste.These interventions reduce exposure to several diseases and can be implemented at household, institutional and production levels.Protective measures should match the work performed. Gloves and wound protection are relevant to fish handling; eye protection and appropriate clothing may be necessary when handling animal tissues; aerosol-generating work requires additional assessment.Workers should receive training to recognise exposures and report symptoms promptly.Prevention programmes are more effective when communities understand transmission pathways and can access practical alternatives.In agricultural and peri-urban settings, education can address safe milk consumption, animal-bite response, floodwater exposure, slaughter hygiene and animal-waste management. Local surveillance evidence should guide priorities.

Further progress requires research that connects microbial findings with actual transmission pathways. Research should distinguish pathogen detection, human exposure, established infection and onward transmission. These represent different stages and require different evidence.

Conclusion

Microbiology provides the scientific foundation for understanding zoonotic diseases by revealing the identity, survival, transmission and pathogenic mechanisms of infectious agents. However, microbial characteristics alone cannot explain disease occurrence. Animal reservoirs, human activities, environmental conditions and host susceptibility collectively determine whether exposure leads to infection.Zoonoses encompass bacterial, viral, fungal and parasitic diseases with diverse transmission pathways. Effective diagnosis therefore requires suitable laboratory methods supported by clinical and exposure information. Culture, serology, molecular detection and genome sequencing each contribute valuable evidence, but their limitations must be recognised.Prevention is most effective when it reduces infection and exposure throughout the transmission pathway. Animal health services,

vaccination, food safety, sanitation, occupational protection and accessible medical care are complementary components of control. Fish and aquatic environments also deserve attention because they can expose workers and researchers through wounds, contaminated water and food.The One Health approach connects these activities through collaboration among human health, veterinary and environmental sectors. Its practical value lies in shared surveillance, joint investigation and coordinated prevention. Strengthening these systems can reduce established zoonoses, improve responses to emerging infections and support healthier relationships among people, animals and their environments.

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Zoonotic Diseases and Emerging Pathogens: Challenges for Global Health

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Abstract

Zoonotic diseases and emerging pathogens represent a continuing and growing challenge for global health. Zoonotic diseases infections that naturally spread between humans and other vertebrate and may be caused by viruses, bacteria, fungi, parasites, or other types of infectious agents. More than 60% of emerging infectious diseases are zoonotic, while many serious diseaseoutbreaks have involved pathogens capable of rapid adaptation spread from one person to another. Studies shows that spillover is a complex multistage process requiring pathogen exposure, compatibility, host susceptibility, and suitable environmental conditions, making prediction difficult.Evidence also shows that human-dominated landscapes may contribute to the relative abundance of known zoonotic hosts.This chapter explores major drivers of zoonotic disease emergence, evaluates the role of environmental and socioeconomic changes, and examines current challenges in disease surveillance, accurate diagnosis, prevention, and outbreak response. The chapter further emphasizes the importance of One Health approach, which integrates human, animal, plant, and ecosystem health. Strengthening integrated surveillance system, advances in genomic technologies, laboratory capacity, community participation, environmental protection, responsible antimicrobial use, and international cooperation is essential for reducing the risk of future pandemic. A preventive and multidisciplinary approach is essential forsafeguarding global health while simultaneously supporting ecological sustainability and socioeconomic resilience.

Keywords: zoonotic diseases, emerging pathogens, one Health, infectious diseases, global health, disease surveillance,

Introduction

Zoonotic diseases have significantly influenced human societies throughout history, but their public health significance has become more pronounced with the expansion of human populations, intensive livestock production, urbanization, and global connectivity. A zoonosis is an infectious disease that is naturally be transmissible between vertebrate animals and humans andcaused by a diverse range of infectious agents, including viruses, bacteria, fungi, parasites, and other pathogens. More than 200 zoonotic diseases are recognized, with transmission occurring through direct animal contact, contaminated food and water, vectors, or environmental pathways (WHO, 2020). While some remain endemic inparticular regions, others may emerge when pathogens overcome ecological or species barriers, potentially resulting in localized outbreaks or widespread epidemics.

Consequently, zoonotic diseases have emerged as a major concern for global health security and are increasingly addressed through the integrated framework of One Health (Karesh et al., 2012; World Health Organization [WHO], 2023). Recent World Health Organization guideline similarly highlights the interdependence of human, animal, and ecosystem health, emphasizing that environmental and socioeconomic changes may create new opportunities for the emergence of infectious diseases (World Health Organization [WHO], 2026)

The global health implications of zoonotic diseases extend beyond their direct effects on morbidity and mortality. Major outbreaks such as SARS, MERS, Ebola, Nipah virus disease, zoonotic influenza, Zika virus disease, mpox, and COVID-19 highlight the diverse pathways through which pathogens associated with animals can emerge as significant public health threats. These outbreaks also illustrate that zoonotic disease emergence is neither confined to a particular group of pathogens nor limited to specific geographical regions.

Environmental degradation represents another major factor of disease emergence. The IPBES (2020) assessment that human-induced changes in biodiversity and ecosystem functioning are associated with an increased risk of pandemics. Deforestation, wildlife exploitation, agricultural expansion, climate change and other environmental pressures can create opportunities for pathogens to come into contact with novel hosts. Although these environmental changes do not inevitably result in disease outbreaks, they can increase the frequency and range of circumstances under which pathogen spillover becomes possible.

Agricultural intensification, particularly the expansion of large-scale livestock production, increases animal densities and contact among wildlife, livestock, humans, and consumers, creating opportunities for pathogen transmission. The emergence of highly pathogenic H5N1 influenza virus in dairy cattle demonstrates the potential for unexpected host associations and highlights emerging challenges for disease surveillance. Furthermore, global travel and trade can rapidly spread pathogens beyond their areas of origin, enabling localized spillover events to evolve into broader global public health concerns.

Another emerging concern is antimicrobial resistance (AMR)that poses an additional One Health challenge. Resistant organisms and resistance determinants can spread among humans, animals, food systems, and environmental reservoirs. This makes antimicrobial stewardship relevant not only in clinical medicine but also in agriculture, veterinary medicine, food safety, wastewater management, and environmental health.

The One Health approach offers a comprehensive framework for integrating these interconnected dimensions. It acknowledges that human health is closely linked to the health of animals and ecosystems and encourages collaboration among public health, veterinary, environmental, agricultural, laboratory, social science, and other relevant sectors (Adisasmito et al., 2022; WHO, 2026). However, the implementation of the One Health approach remains uneven and fully integrated systems are still relatively rare.

This chapter therefore explores zoonotic diseases and emerging pathogens from a broad global health perspective,focusing particularly on disease burden, key pathogens, factors driving emergence, spillover processes, environmental influences, surveillance, diagnostics, genomics, antimicrobial resistance, One Health approaches, prevention strategies, and future research priorities.

Conceptual Framework of Zoonotic Emergence

Zoonotic emergence results from the interaction of several biological, ecological, and socioeconomic processes operating across different levels. A useful conceptual pathway can be represented as follows:

Environmental and socioeconomic changes → alterations in reservoir and pathogen ecology → increased human–animal interactions → pathogen exposure → spillover → establishment of infection → onward transmission → outbreak amplification.

This pathway should not be regarded as a linear process in which progression through one stage inevitably leads to the next. Rather, each stage represents a potential barrier that must be crossed. Plowright et al. (2017) emphasized that spillover depends on the convergence of multiple conditions in both space and time. Therefore, the detection or persistence of a pathogen within an animal reservoir does not automatically indicate a high risk of infection in humans.

At the **reservoir level**, factors such as pathogen prevalence, shedding intensity, host density, immunity, demographic structure, and environmental stress can influence pathogen pressure. At the **interface level**, human behavior, occupational exposure, animal handling practices, food-related practices, vector abundance, and environmental contamination can determine the likelihood and intensity of pathogen contact. At the **recipient-host level,** characteristics including age, immunity, genetic susceptibility, underlying health status, and the dose of pathogen encountered can determine whether exposure produces infection. Finally**, pathogen-specific characteristics** can determine whether an infection remains a sporadic zoonosis or develops into sustained human-to-human transmission (Plowright et al., 2017; Morse et al., 2012).

This multilevel framework is important for prevention because interventions can be implemented at multiple points along the emergence pathway. Habitat conservation and the reduction of high-risk human–animal interactions may decrease opportunities for exposure, while vaccination and animal-health measures may reduce pathogen prevalence in wildlife or livestock populations. Strengthening diagnostic and integrated surveillance systems can detect spillover rapidly, whereas timely public health interventions can interrupt transmission after human infection occurs.

Emerging Pathogens of Global Health Concern

Emerging pathogens encompass newly identified infectious agents, established pathogens that expand into new geographical areas or infect novel host species, and previously known pathogens whose epidemiological patterns or clinical significance undergo substantial changes. Viral pathogens have received particular attention because many major emerging infectious diseases are caused by viruses with a high capacity for rapid evolution, adaptation, and host switching.

Examples of such pathogens include SARS-CoV, MERS-CoV, SARS-CoV-2, Ebola virus, Nipah virus, zoonotic influenza viruses, rabies virus, and emerging arboviruses. These infectious agents vary substantially in their reservoir hosts, modes of transmission, severity, and potential for sustained human transmission

The recent emergence of highly pathogenic avian influenza A(H5N1) illustrates the dynamic nature of viral host range. The detection of H5N1 virus in dairy cattle showed that a virus historically linked predominantly to avian hosts could establish transmission within a mammalian agricultural production system (Caserta et al., 2024). Such events highlightthe importance of surveillance systems capable of identifying unexpected host transitions

Emerging pathogens should not be assessed only on the basis of their current number of human cases. A comprehensive assessment of their potential risk should also consider factors such as host range, evolutionary potential, transmissibility, pathogenicity, environmental persistence, geographic distribution, reservoir prevalence, and opportunities for human exposure.

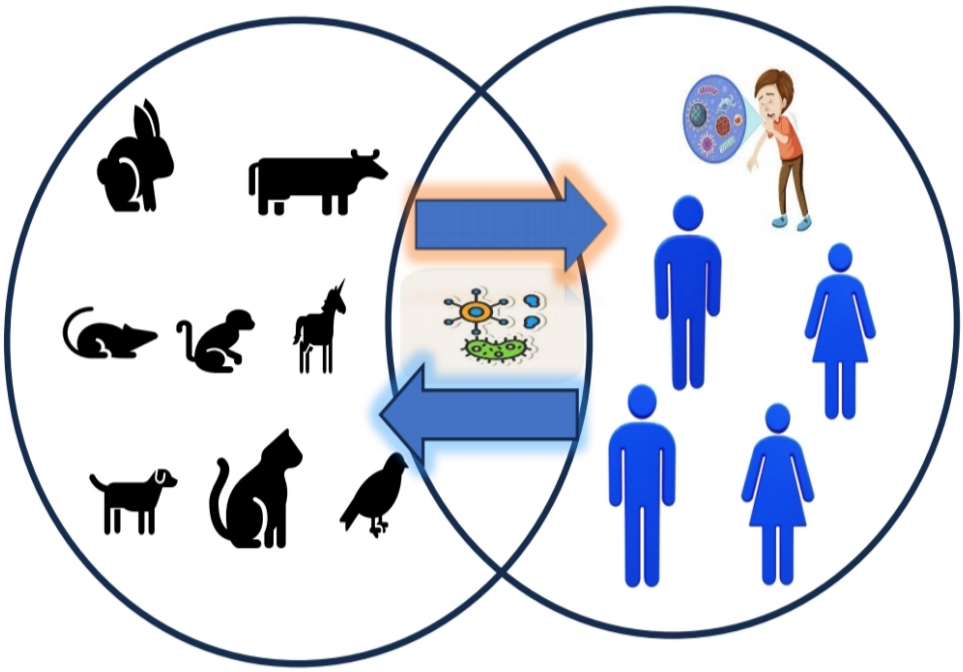


Fig: Zoonotic disease occurs amongst closely interacting species

Major Drivers of Zoonotic Disease Emergence

Land-use Change

Land-use change through deforestation, agricultural expansion, infrastructure development, and mining can alter habitats and increase wildlife–livestock–human interactions. Human-dominated landscapes can contain increased relative abundance of known zoonotic host species (Gibb et al. 2020).Recent research emphasizes the need to understand the specific mechanisms through which habitat loss contributes to pathogen spillover, rather than assuming that all forms of land-use change pose equivalent risks.

Deforestation and habitat fragmentation

Deforestation and habitat fragmentation can increase human–wildlife contact and alter animal movement. Ecological countermeasures have consequently been proposed as an important component of pandemic prevention. These include maintaining habitat integrity, reducing unnecessary wildlife disturbance, protecting ecological barriers, and designing land-use policies that minimize high-risk contact.

Wildlife Trade

Wildlife capture, transport, markets, and consumption can facilitate pathogen transmission by increasing contact among animals and humans Risk reduction requires disease surveillance, traceability, biosecurity, appropriate regulation, and context-specific interventions rather than simply focusing on individual animal species.

Agricultural intensification

Intensive livestock production can increase host density, facilitate pathogen amplification, and opportunities for pathogen evolution. Agricultural systems should therefore be considered important components of One Health surveillance, particularly where livestock production overlaps with wildlife habitats.

Urbanization

Urban expansion can increase contact with peri-urban wildlife, rodents, mosquitoes and vectors. Population density, poor sanitation, inadequate waste management, and limited healthcare may further enhance diseasetransmission.

Climate change

Climate change modifies ecological conditions that determine pathogen and vector survival. Changes in temperature, rainfall, and humidity can affect pathogens and vectors, while species redistribution may create new ecological contacts. Recent research emphasizes that climate change should be incorporated into zoonotic risk modelling rather than treated as an isolated environmental variable.

Biodiversity loss

Biodiversity loss can alter host communities and disease transmission. Reduced biodiversity may increase transmission of some infectious diseases, although effects vary among ecosystems (Keesing et al., 2010).

Recent planetary-health research indicates that biodiversity loss and pathogen emergence are interconnected consequences associated with global environmental change.

Human population growth and mobility

Population growth increases pressure on natural resources, while travel and trade enhance global connectivity, facilitating disease emergence and dissemination.

Socioeconomic inequality

Communities dependent on livestock, forests, or wildlife may experience greater exposure, while countries with limited laboratory and surveillance capacity may detect outbreaks later.. Strengthening surveillance in vulnerable regions is therefore essential for global health security.

Antimicrobial Resistance as a One Health Challenge

Antimicrobial resistance (AMR) poses a major challenge to the effective management of infectious diseases because resistant microorganisms can circulate across humans, animals, food systems, and the environment. Although AMR is not synonymous with zoonotic disease emergence, the two are closely interconnected at human–animal and environmental interfaces.

The use of antimicrobials in livestock, aquaculture, companion animals, and medicine creates selective pressure that promotes the emergence and persistence of resistant microorganisms. Wastewater, manure, contaminated food products, and polluted environmental matrices can provide pathways for dissemination.

A One Health approach is particularly appropriate because AMR cannot be effectively controlled through human healthcare interventions alone. Veterinary antimicrobial stewardship, infection prevention in animal production, food safety, environmental monitoring and responsible pharmaceutical use are required to limit the emergence and spread of resistance.

Future zoonotic disease surveillance should therefore integrate antimicrobial susceptibility testing with pathogen detection and epidemiological data. Such an integrated framework would enable health authorities to determine not only where pathogens are circulating but also whether emerging antimicrobial resistance could complicate prevention and treatment.

One Health as a Prevention Framework for Zoonotic Disease Prevention

The One Health approach acknowledges the interconnectedness of human, animal, and ecosystem health and promotes coordinated, multisectoral strategies to prevent zoonotic diseases (Adisasmito et al., 2022). An effective prevention framework should address zoonotic risks across the entire pathway of emergence, beginning with environmental drivers and extending to early detection, rapid response, and long-term risk reduction.

Environmental and Interface-Level Prevention

Primary prevention should focus on reducing opportunities for pathogen spillover through ecosystem conservation, sustainable land-use practices, biodiversity protection, improved livestock biosecurity, management of wildlife livestock interfaces, food safety, animal health, prudent antimicrobial use, and reduction of high-risk wildlife exposure. Preventing spillover at its source may be more effective than relying primarily on detection and intervention after pathogen emergence (OHHLEP, 2023).

Integrated Surveillance and Early Detection

Early detection requires coordinated surveillance across human, livestock, wildlife, vector, and environmental health sectors. Risk-based surveillance should focus on areas where ecological disturbance, pathogen prevalence, host abundance, climate variability, and human exposure overlap. Advanced tools such as PCR, metagenomics, whole-genome sequencing, wastewater surveillance, digital epidemiology, and artificial intelligence can strengthen early warning and pathogen detection capacities.

Risk Assessment and Rapid Response

Zoonotic risk assessment should integrate pathogen characteristics, host ecology, land-use change, climate, environmental factors, and pattern of human mobility. Once a potential zoonotic threat is identified, rapid laboratory confirmation, genomic characterization, epidemiological investigation, contact tracing, infection control, and effective risk communication are essential to limit further transmission.

Community Participation and Governance

Community participation is essential for zoonotic disease prevention because local populations frequently interact with wildlife, livestock, forests, markets, and water systems. Incorporating local and contextualknowledge can facilitate the identification of exposure pathways and strengthen prevention measures (Kuhn et al., 2024). Effective One Health implementation also requires sustained coordination among public health, veterinary, wildlife, agricultural, environmental, and research institutions, supported by interoperable databases and standardized data-sharing protocols.

Long-Term One Health Prevention

Long-term prevention requires policy development and reform, ecosystem conservation, community engagement, health-system strengthening, international collaboration, and continuous surveillance. Thus, the integrated One Health framework follows a continuum of environmental prevention → interface management → integrated surveillance → advanced detection → risk assessment → rapid response → long-term prevention, shifting zoonotic disease control from a predominantly reactive approach toward anticipatory integrated, and prevention-oriented action.

Conclusion

Zoonotic diseases and emerging pathogens constitute a dynamic global health challenge because their emergence occurs at the intersection of biological, ecological, environmental, socioeconomic, and technological systems. The evidence reviewed here demonstrates that zoonotic emergence cannot be explained through pathogen biology alone. Land-use change, habitat fragmentation, agricultural intensification, wildlife trade, urbanization, climate change, biodiversity loss, human mobility, antimicrobial resistance and health-system weaknesses can alter the probability that pathogens encounter susceptible hosts.

Zoonotic spillover is a multistage process involving pathogen pressure, exposure, biological compatibility, host susceptibility, and subsequent transmission. This complexity makes it difficult to predict individual spillover events with precision. However, recognizing recurringecological and socioeconomic drivers creates opportunities for prevention.The most important implication is that global health policy should shift from a predominantly reactive approach toward greater emphasis on prevention. Although detecting outbreaks after their establishment remains essential,but preventing high-risk interactions before pathogen spillover occurs is potentially more sustainable. Environmental protection, responsible land-use planning, wildlife-risk management, food safety, livestock biosecurity, antimicrobial stewardship, and meaningful community participation should therefore be incorporated into pandemic prevention.

Surveillance systems require equally fundamental transformation. Human, animal, wildlife, vector, and environmental surveillance should be interconnected through interoperable platforms to facilitate coordinated data sharing and risk assessment. Genomic sequencing, metagenomics, wastewater surveillance, artificial intelligence, remote sensing, and digital epidemiology can strengthen early warning and outbreak detection capabilities, but their effectiveness depends on availability of data, trained personnel, laboratory infrastructure, and equitable access to these technologies. Antimicrobial resistance further demonstrates the interconnected nature of health systems. Resistant organisms and resistance determinants can circulate across human, animal, food, and environmental compartments, making sector-specific approaches insufficient.

The One Health framework provides the appropriate conceptual basis for addressing these challenges; however, its effective implementation must extend beyond multidisciplinary terminology. Genuine One Health approach requires shared objectives, cross-sectoral governance, interoperable information systems, meaning community participation, environmental integration, and sustained investment.

The future of zoonotic disease prevention should progress beyond a predominantly “detect and respond” toward “understand, prevent, detect early, and respond rapidly.” Prevention at source should complement conventional preparedness and response mechanisms. The ultimate goal should be to identify and mitigate high-risk ecological and social conditions before they

facilitate sustained human transmission. A globally coordinated, scientifically rigorous, equitable, and prevention-oriented One Health strategy will be crucial for minimizing zoonotic spillover, improving pandemic preparedness, safeguarding ecosystems, and strengthening global health security.

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Earthworm Microbiota as a Source of Regenerative Medicine

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Abstract

Regenerative medicine is an interdisciplinary field concerned with restoring damaged cells, tissues and organs through the coordinated application of cells, biomolecules, biomaterials, genes and biological signals. Increasing evidence indicates that microorganisms can contribute to tissue repair by producing antimicrobial compounds, enzymes, metabolites, immunomodulatory molecules and biomaterials. In this context, earthworm-associated microbiota represent an emerging and relatively unexplored biological resource. Earthworms continuously interact with soil, decomposing organic matter, plant residues and diverse microorganisms. Their gut, body surface, coelomic fluid and casts support microbial communities that participate in digestion, decomposition, nutrient transformation, host protection and environmental adaptation.

Earthworm-associated microorganisms may produce enzymes, peptides, polysaccharides, antimicrobial substances, antioxidant compounds and metabolites with potential applications in wound healing, infection control, extracellular-matrix remodeling, tissue engineering and biomaterial development. However, evidence must be interpreted carefully because many reported biological effects have been obtained from whole earthworms, crude extracts, proteins, peptides or oils rather than from purified microbiota-derived products. Therefore, the contribution of microorganisms must be experimentally separated from host-derived molecules and environmental contaminants.

Modern culture-dependent and culture-independent approaches, including metagenomics, metatranscriptomics, metaproteomics, metabolomics, genome mining and synthetic biology, provide opportunities to identify microorganisms and their functional products. Functional screening using cell cultures, reconstructed tissues, organoids, organ-on-chip systems and animal

models can establish biological relevance. India has considerable potential in this emerging area because of its earthworm diversity, vermiculture and vermicomposting practices, agricultural biotechnology, natural-product research, traditional knowledge and biomedical infrastructure. A coordinated research strategy integrating microbiology, omics, natural-product chemistry, biomaterials, tissue engineering and biosafety is required to translate earthworm-associated microbial resources into reproducible biomedical products.

Keywords: Earthworm microbiota; gut microbiome; regenerative medicine; wound healing; antimicrobial compounds; tissue engineering; biomaterials; bioactive metabolites; vermiculture; India.

Introduction

Regenerative medicine seeks to restore the structure and function of tissues affected by injury, disease or degeneration. It integrates cell biology, stem-cell science, molecular medicine, immunology, microbiology, biomaterials, nanotechnology and bioengineering. Tissue restoration is a complex process involving inflammation, infection control, extracellular-matrix formation, cell proliferation and migration, angiogenesis, tissue remodeling and recovery of physiological function. Thus, effective regeneration requires coordinated biological regulation rather than simply rapid closure of an injured area.

Microorganisms have increasingly attracted attention as sources of biologically active compounds and as regulators of host responses. Microbial products can function as therapeutic molecules, enzymes, biomaterials or biological regulators. The human microbiome has demonstrated the importance of microorganisms in epithelial-barrier function, metabolism, immune regulation and inflammatory processes. These observations encourage exploration of microbial communities from non-human organisms as sources of new biological products.

Earthworms are particularly interesting because they live in microbe-rich environments and ingest large quantities of soil and organic material. Their digestive system provides selective conditions involving pH, oxygen concentration, digestive enzymes, mucus and host-derived immune factors. These conditions can enrich or modify microbial communities passing through the digestive tract. Earthworms also exhibit remarkable wound-repair capacity and, in some species, regeneration of damaged body structures, making them useful biological systems for studying tissue repair.

The concept of earthworm microbiota must, however, be carefully defined. It may include microorganisms present in the gut, associated with the gut lining, present in coelomic fluid, attached to the body surface, released in casts or associated with cocoons. Microorganisms originating from soil, manure, compost or plant residues may also occur in earthworm-associated samples. Detection of a microorganism in gut material does not necessarily demonstrate a stable host association because some organisms may simply pass through the digestive tract.

The central premise of this chapter is therefore not that earthworm microbiota already constitute an established treatment for human tissue injury. Rather, earthworm-associated microbial communities represent a bioprospecting platform for discovering molecules, enzymes, pathways and microbial systems that may contribute to regenerative medicine.

Earthworm Microbiota: Composition, Ecology and Functional Significance

Microbial Diversity and Environmental Influences

Earthworm-associated microbial communities can contain bacteria, archaea, fungi, protozoa, bacteriophages and other microorganisms. Bacteria have received considerable attention because of the availability of 16S rRNA sequencing, cultivation methods and genomic databases. Nevertheless, fungi and viruses may also contribute to decomposition, host defense and metabolite production.

Microbial composition is influenced by earthworm species, developmental stage, diet, soil chemistry, land-use history, agricultural inputs, temperature, moisture, oxygen availability, geographic location and the duration of microbial residence in the digestive tract. Laboratory and natural populations may therefore exhibit substantially different microbial profiles.

The earthworm gut should not be considered an isolated microbial ecosystem. It is strongly influenced by the surrounding soil, yet the gut community is not identical to the soil community. Passage through the digestive tract exposes microorganisms to mechanical processing, digestive enzymes, mucus, altered pH and oxygen conditions. Some microorganisms may consequently be selectively enriched or suppressed.

Earthworm casts provide a second important microbial resource. Casts contain microorganisms, enzymes, nutrients and transformed organic matter and can differ substantially from the original soil or organic substrate.

Therefore, research should distinguish between internal earthworm microbiota and environmental microbiota processed by earthworms.

Ecological Functions Relevant to Biotechnology

Earthworm-associated microorganisms participate in decomposition, cellulose degradation, nutrient transformation and adaptation to environmental stress. Their functional capabilities may provide useful enzymes and metabolites for biotechnology. Research has particularly examined their contribution to cellulose digestion, nutrient cycling and adaptation to environmental conditions.

From a biomedical perspective, the ecological interactions within the earthworm gut may select microorganisms capable of producing antimicrobial compounds, enzymes and metabolites that function under unusual environmental conditions. Such organisms could therefore represent a source of novel biomolecules. However, ecological function alone does not establish therapeutic activity, and each candidate product requires independent biological and safety validation.

Biological Features Relevant to Regenerative Medicine

Antimicrobial Defense and Infection Control

Infection is a major obstacle to tissue repair. Microbial colonization and biofilm formation can prolong inflammation, damage extracellular matrices and interfere with tissue restoration. Earthworms survive in environments containing numerous microorganisms and possess natural defense mechanisms involving physical barriers, coelomocytes, soluble factors and antimicrobial substances.

Earthworm-associated microorganisms may contribute to this protective environment by producing bacteriocins, lipopeptides, organic acids and antimicrobial enzymes. These compounds could potentially be investigated for wound dressings, antimicrobial coatings, medical-device surfaces and anti-biofilm biomaterials.

A critical scientific issue is determining the source of observed antimicrobial activity. Activity detected in an earthworm extract could originate from earthworm proteins, microbial metabolites, environmental microorganisms, residual soil substances or combinations of these components. Demonstrating a microbiota-derived effect therefore requires microbial isolation or molecular identification, genomic evidence for biosynthetic

capacity, purification of the active compound and confirmation that the microorganism or its genes produce the activity.

Inflammation and Immune Regulation

Inflammation is necessary for normal healing, but persistent inflammation can result in chronic wounds, tissue damage and excessive scarring. A regenerative product should therefore regulate inflammation rather than simply suppress immune activity.

Earthworm-associated molecules may potentially influence macrophage differentiation, neutrophil recruitment, inflammatory mediator release, complement activity, oxidative stress, fibroblast activation, epithelial migration and inflammatory signaling. Candidate pathways include Toll-like receptors, inflammasome signalling, nuclear factor-kappa B, mitogen-activated protein kinases and pro-resolving lipid pathways.

Currentevidence for anti-inflammatory effects is stronger for complex earthworm extracts than for purified microbial metabolites. Future investigations should therefore identify individual microbial products and determine their molecular targets using mechanistic assays.

Antioxidant and Cytoprotective Potential

Reactive oxygen species are important signaling molecules at controlled concentrations but excessive oxidative stress can damage cellular proteins, lipids, DNA and membranes. Antioxidant microbial metabolites may potentially protect fibroblasts, keratinocytes, endothelial cells and stem cells during tissue injury.

Possible mechanisms include radical scavenging, stimulation of endogenous antioxidant enzymes, metal chelation, reduction of lipid peroxidation, mitochondrial protection and regulation of redox-sensitive transcription factors. Chemical antioxidant assays are useful for preliminary screening but do not establish therapeutic potential. Candidate compounds should subsequently be evaluated in cell-based injury models for biological activity, stability, bioavailability and toxicity.

Extracellular-Matrix Remodeling

The extracellular matrix provides structural and biochemical support during tissue repair. Collagen, fibronectin, laminin and proteoglycans undergo carefully regulated deposition and remodeling. Excessive matrix formation

may result in fibrosis, whereas insufficient matrix production can impair tissue closure and structural recovery.

Earthworm-derived preparations have been associated with collagen-related wound-healing effects, including reported effects on collagen type III. Microorganisms associated with earthworms may potentially contribute collagen-related proteins, proteases, glycosidases, polysaccharides and metabolites capable of influencing fibroblast activity.

Importantly, increased collagen production alone should not be regarded as proof of regeneration. Studies must determine whether the newly formed tissue has appropriate architecture, mechanical properties and function.

Angiogenesis and Vascular Repair

Regenerating tissues require adequate blood supply for oxygen delivery, nutrient transport, waste removal and immune-cell recruitment. Microbial or earthworm-derived molecules may influence endothelial-cell proliferation, migration and tube formation and may regulate vascular growth factors.

Earthworm-derived proteins and peptides have been associated with wound healing and growth-factor-related responses involving epidermal growth factor and vascular endothelial growth factor pathways. These observations provide a basis for investigating whether specific microbiota-derived compounds directly influence vascular repair.

Cell Migration and Proliferation

Re-epithelialization requires migration and proliferation of keratinocytes, while fibroblasts contribute to matrix deposition and endothelial cells participate in angiogenesis. Appropriate experimental approaches include scratch-wound assays, transwell migration assays, fibroblast proliferation assays, keratinocyte models, endothelial tube formation, reconstructed skin and organoid systems.

Earthworm-derived preparations have demonstrated biological activity in wound-healing and tissue-repair models, but these observations do not establish that microorganisms are responsible. Microbiota-specific investigations should therefore use purified microbial products and appropriate source controls.

Microbial Enzymes and Biotechnological Potential

Earthworm-associated microorganisms may produce cellulases, hemicellulases, proteases, lipases, chitinases, oxidoreductases, glycosidases and other polymer-modifying enzymes. Although several of these enzymes have primarily ecological functions, they may have indirect relevance to regenerative medicine.

Potential applications include modification of biomaterial polymers, preparation of bioactive oligosaccharides, scaffold processing and development of wound-dressing materials. Metagenomic libraries can provide access to enzymes from microorganisms that cannot be cultivated under standard laboratory conditions.

The combination of enzyme discovery and biomaterial science may be particularly useful for developing biodegradable scaffolds, controlled-release systems and functional coatings. However, enzyme purity, specificity, stability, immunogenicity and compatibility with mammalian cells must be established before biomedical applications are considered.

Earthworm Microbiota as a Bioprospecting Platform

From Earthworm Sampling to Bioactive Product Discovery

Conventional natural-product research frequently begins with a whole organism or crude extract. Modern microbiome research requires a more refined approach that identifies the microbial producer, biosynthetic pathway and active molecule.

A comprehensive workflow should include:

1. Authentication of the earthworm species.
2. Documentation of soil, diet and environmental conditions.
3. Separate collection of gut, gut wall, body surface, coelomic fluid, casts and surrounding soil.
4. Microbial community characterization.
5. Identification of enriched or functionally relevant microorganisms.
6. Culture-dependent isolation.
7. Culture-independent genomic investigation.
8. Functional screening.

9. Purification and structural identification of active compounds.
10. Mechanistic and safety validation.

This strategy minimizes confusion between host-derived and microbiota-derived activities.

Culture-Dependent Approaches

Cultivation remains important because isolated microorganisms can be experimentally manipulated and tested. Anaerobic, microaerophilic, oligotrophic and selective culture conditions may increase recovery of organisms adapted to the earthworm gut.

Isolates can be screened for antimicrobial activity, anti-biofilm effects, enzyme production, antioxidant properties, cytoprotection, fibroblast migration, macrophage modulation and compatibility with hydrogels or scaffolds.

The major limitation is that many environmental microorganisms are difficult to cultivate under conventional conditions. Consequently, culture-dependent investigation should be combined with molecular and metagenomic approaches.

Multi-Omics Characterization

Amplicon sequencing can describe community composition, whereas shotgun metagenomics provides information about genes and biosynthetic pathways. Metatranscriptomics identifies actively expressed genes, metaproteomics evaluates proteins and metabolomics reveals chemical products.

An integrated approach can address several important points:

- Identification of microorganisms consistently associated with the earthworm gut.
- Determination of genes and metabolic pathways that are actively expressed.
- Identification of metabolites produced by the earthworm-associated microbiota.
- Assessment of whether these metabolites are enriched in the earthworm gut compared with the surrounding soil.

- Experimental reconstruction and validation of biosynthetic pathways.
- Evaluation of whether purified products enhance tissue repair without increasing toxicity or fibrosis.

Such multi-omics integration moves research from microbial identification toward functional understanding.

Genome Mining and Synthetic Biology

Genome mining can identify biosynthetic gene clusters associated with antimicrobial peptides, polyketides, non-ribosomal peptides, terpenes, siderophores and other bioactive molecules. When microorganisms cannot be cultivated or produce insufficient quantities of a candidate molecule, biosynthetic pathways may potentially be expressed in suitable laboratory hosts.

Synthetic biology could facilitate production of purified peptides, recombinant enzymes, defined metabolites, conditioned media and biomaterial-compatible coatings. Nevertheless, genomic screening must include assessment of antimicrobial-resistance genes, virulence-associated characteristics and other biosafety concerns.

Potential Applications in Regenerative Medicine

Chronic Wound Management

Chronic wounds associated with diabetes, vascular disease, pressure injury, aging and infection frequently exhibit prolonged inflammation and biofilm formation. Earthworm microbiota-derived compounds could potentially be incorporated into wound dressings designed to combine antimicrobial protection, moisture retention, antioxidant effects, controlled inflammation, fibroblast migration and matrix remodeling.

Hydrogels, electrospunfibers and nanofibrous scaffolds represent potential delivery platforms. A purified microbial metabolite incorporated into a defined biomaterial would be preferable to an uncharacterized earthworm preparation because composition, dose and manufacturing quality can be controlled.

Skin Regeneration

Skin regeneration requires restoration of the epidermal barrier, dermal structure, vascularization and mechanical integrity. Candidate products should

therefore be evaluated in keratinocyte and dermal-cell systems, reconstructed epidermis, full-thickness skin equivalents, infected wound models, diabetic wound models and aged-skin models.

Relevant endpoints include barrier recovery, tissue organization, tensile strength, vascularization, scar quality and microbiological safety. Accelerated wound closure alone is insufficient evidence of complete skin regeneration.

Bone and Cartilage Repair

Microbial products may potentially influence bone and cartilage repair through modulation of inflammation, mineralization, extracellular-matrix formation and stem-cell differentiation. Possible applications include osteogenic peptides, scaffold coatings, microbial enzymes for biomaterial modification, anti-biofilm implant surfaces and immunomodulatory scaffold components.

Evidence for these applications remains less developed than evidence for wound-healing-related applications, and claims should therefore remain appropriately cautious.

Nerve Regeneration

Nerve repair requires coordinated regulation of inflammation, Schwann-cell activity, axonal growth, vascularization and scar formation. Microbial metabolites with appropriate immunomodulatory or trophic properties could potentially be incorporated into nerve-guidance systems.

Future studies should include neurite outgrowth, Schwann-cell migration, neural co-culture, electrophysiological function, axonal regeneration in suitable animal models and neurotoxicity assessment.

Antimicrobial Biomaterials and Cell-Free Therapeutics

Bacterial colonization and biofilm formation are major challenges for implants and tissue-engineered constructs. Earthworm-associated microbial products may potentially serve as antimicrobial coatings, hydrogel components, nanoparticle-stabilizing agents and controlled-release additives.

Cell-free approaches involving purified peptides, enzymes, secreted metabolites, extracellular vesicles or microbial polysaccharides may provide advantages over living microbial products. They nevertheless require rigorous purification, identity testing, stability assessment, sterility and reproducible manufacturing.

Mechanistic Model of Regenerative Action

A hypothetical earthworm microbiota-derived regenerative product may influence several interconnected stages of tissue repair:

1. Antimicrobial protection – reduction of pathogenic microorganisms and biofilm formation.
2. Inflammation regulation – limitation of excessive inflammatory signaling while maintaining host defense.
3. Redox regulation – reduction of excessive oxidative damage.
4. Cell migration – promotion of movement of epithelial and connective-tissue cells.
5. Matrix formation – regulation of collagen and other extracellular-matrix components.
6. Angiogenesis – support of appropriate blood-vessel formation.
7. Tissue remodeling – maturation and strengthening of newly formed tissue while limiting excessive scarring.

These mechanisms are interconnected rather than independent. Effective tissue repair requires appropriate timing and balance. A product that accelerates wound closure but promotes fibrosis or produces poorly organized tissue should not automatically be regarded as regenerative.

Discussion

Importance of Source Attribution

One of the most important issues in this field is the distinction between earthworm-derived and microbiota-derived biological activity. “Earthworm-derived medicine” may refer to whole-earthworm preparations, crude extracts, purified proteins, oils, coelomic fluid, microbial isolates, microbial culture products, purified microbial metabolites or cast extracts.

These materials have different compositions, mechanisms, safety profiles and manufacturing requirements. Mixing them in the interpretation of experimental results can lead to incorrect attribution of biological activity.

Host–Microbe Synergy

Some biological effects may arise from interactions between earthworm molecules and microbial products. Earthworm mucus, immune proteins,

digestive enzymes and microbial metabolites may coexist during extraction or processing. Such interactions could contribute to biological activity but also make standardization difficult.

Comparative studies involving whole-earthworm extracts, microbial culture supernatants, purified microbial compounds and defined synthetic mixtures can help determine the relative contribution of host and microbial components.

Regeneration versus Repair

The distinction between repair and true regeneration is fundamental. Wound closure, reduced inflammation and increased collagen production indicate improvement but do not necessarily demonstrate restoration of normal tissue structure and function.

Regenerative studies should therefore examine tissue architecture, vascular and neural organization, mechanical strength, barrier function, long-term remodeling, scar quality and functional recovery. This distinction should guide experimental design and scientific terminology.

Ecological and Evolutionary Significance

Microorganisms living within earthworm-associated environments experience competition with other microorganisms and exposure to plant materials, soil particles, digestive conditions and host immune factors. These environmental pressures may contribute to the evolution of enzymes and antimicrobial metabolites.

The same ecological complexity can introduce risks, including pathogenic microorganisms and antimicrobial-resistance determinants. Therefore, biosafety evaluation should accompany microbial discovery rather than being postponed until late-stage development.

Sustainability and Ethical Considerations

Earthworms can be maintained under controlled culture systems, creating opportunities for sustainable research without extensive harvesting of wild populations. Sustainable approaches should emphasize captive cultivation, minimal biological damage, laboratory production of microbial products and careful characterization of waste-derived substrates.

Environmental sourcing must also consider the origin and chemical quality of organic wastes used in vermiculture. Contaminated substrates can

introduce metals, pesticides, pharmaceutical residues, pathogens or other unwanted substances into earthworm-associated materials.

Ethical research should include responsible handling of experimental organisms, protection of traditional knowledge, equitable benefit sharing, microbial containment and transparent reporting. Claims about biomedical applications should remain proportional to the evidence.

Potential Advantages

Earthworms are relatively accessible biological resources and can be maintained under controlled conditions. Their associated microorganisms perform diverse ecological functions and may produce enzymes, antimicrobial compounds and metabolites of biotechnological interest. Earthworm biology also provides useful models for studying wound repair.

India has additional advantages because of its earthworm resources, established vermiculture practices, agricultural biotechnology capabilities and expanding biomedical research infrastructure. Defined microbial products may eventually provide greater manufacturing control than complex whole-earthworm extracts.

Future Perspectives

Multi-Omics Integration

Future investigations should combine metagenomics, metatranscriptomics, metaproteomics and metabolomics to connect microbial identity with biological function. Multi-omics approaches are more informative than taxonomic identification alone because they can reveal which genes are present, which pathways are active and which metabolites are produced.

Defined Synthetic Microbial Communities

Defined microbial communities composed of selected earthworm-associated microorganisms may provide controlled systems for investigating host–microbe interactions. Such communities can help determine whether combinations of microorganisms produce biological effects that are absent in individual isolates.

Microbiota-Informed Biomaterials

Microbial products could potentially be incorporated into chitosan dressings, collagen scaffolds, alginate gels, gelatin-based matrices, electrospunfibers and implant coatings. Controlled incorporation could provide

antimicrobial, anti-inflammatory or tissue-supportive functions while reducing exposure to living microorganisms.

Precision Wound Medicine

Wounds differ in their microbial burden, inflammatory state, oxygenation, metabolic environment and tissue condition. Future microbiota-derived products could potentially be selected according to the biological characteristics of individual wounds. Such an approach would require robust biomarkers and validated relationships between wound characteristics and treatment responses.

Indian Translational Networks

India could establish interdisciplinary networks linking universities, agricultural institutions, medical colleges, microbiology laboratories, biotechnology companies, pharmaceutical industries and regulatory bodies. Shared facilities and standardized protocols could improve reproducibility, reduce duplication and accelerate translation of promising discoveries.

Conclusion

Earthworm-associated microbiota represent a promising but incompletely characterized resource for regenerative medicine. Their ecological functions suggest potential for discovery of antimicrobial compounds, enzymes, antioxidant metabolites, immunomodulatory molecules and biomaterial-associated products. Earthworm biology itself also provides a valuable model for studying wound repair and tissue regeneration.

However, the current evidence should be interpreted cautiously. Many reported biomedical effects are derived from whole earthworms, crude extracts, earthworm proteins, peptides or oils rather than from purified microbiota-derived products. Therefore, the microbial contribution must be demonstrated through source authentication, microbial characterization, genome and metabolite analysis, purification, functional validation and mechanistic studies.

The most realistic near-term applications are likely to involve defined antimicrobial or tissue-supportive molecules incorporated into wound dressings, hydrogels, scaffolds and other biomaterials. Longer-term possibilities include applications in skin, bone, cartilage and nerve regeneration, provided sufficient mechanistic and safety evidence becomes available.

India is well positioned to contribute to this emerging field because of its earthworm diversity, vermiculture infrastructure, microbial research capacity, natural-product expertise and biomedical resources. A coordinated approach combining microbiology, multi-omics, natural-product chemistry, biomaterials, tissue engineering and regulatory science could establish a strong national research program.

Overall, earthworm microbiota should be regarded as a promising discovery platform rather than an established clinical source of regenerative therapies. The principal objective should be to identify reproducible microbial products that improve tissue repair while minimizing infection, excessive inflammation, fibrosis and toxicity.

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Cardio Vascular Diseases

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Introduction

A thousand of years back, **William Harvey** (1628) – discovered the circulatory system (how blood is pumped by the heart and circulates through the body) in his work '**De Motu Cordis'**. {De Motu Cordis is a Latin – it means 'On the motion of the heart}. In modern era, focus on minimally invasive procedure and rise of robotic – assisted heart surgery and artificial hearts.

CVD includes a wide range of disorder affecting the heart and blood vessels. Globally, it remains the leading cause of death.The most common causes of CVD in the incidence of diseases and the number of death within a population.

Abbreviation:

- **CVD –**Cardio vascular diseases
- **IHD –** Ischemic heart diseases
- **HTN –** Hypertension
- **CHD –** Congenital heart diseases
- **VHD –** Valvular heart diseases
- **BP –** Blood pressure
- **CABG –** Coronary artery bypass graft
- **ABPM -** Ambulatory BP monitoring
- **HBPM -** Home BP monitoring
- **TMT –** Thread mill test
- **STEMI -** ST Elevated myocardial infraction

What is CVD?

Cardio vascular diseases is a group of disorder affecting the heart and circulatory system (which are responsible for circulating blood throughout the body) including the coronary arteries, veins and capillaries, muscles and valves and leading to reduced blood flow, oxygen delivery, tissue damage or organ failure.Some diseases are hypertension, arrhythmia, ischemic heart diseases etc.,

Hypertension:

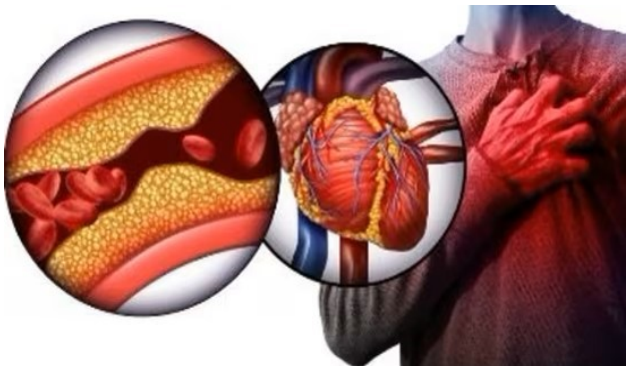
HTN is the one of the most common worldwide health problems. Across the world over 20% of all adults have hypertension. The statistics of hypertension growing among women and adolescents as well as the older adult (past two decades it has doubled).

Definition:

HTN is also known as high blood pressure, where the blood pressure in the arteries is higher than normal (figure:1).

Normal BP: 120/80 mmHg

Hypertension: 140/90 mmHg



(Fig: 1 Hypertension)

Epidemiology:

According to the 2006, Iraqi National Survey said 40.4% of the Iraqi adult population elevated BP(figure:2). Approximately 26% of the world population has hypertension and the incidence is expected to increases to 29% by 2025. Rate of hypertension dramatically increases in patients older than 60 years. In

many countries 50% of individuals have HTN in this age. World widely hypertension enhances more than 7.1 million death per head.

HTN controlled countries:

- 22% in Canada, of which 16% is controlled
- 26.3% in Egypt, of which 8% is controlled
- 13.6% in China, of which 3% is controlled.



(Fig: 2 BP)

Pathophysiology:

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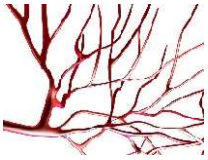
Increased cardiac output (HR×SV) Increased peripheral vascular resistance (arteriolar vasoconstriction)

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Increased sympathetic nervous system overactivity Increased RAAS activation (renin→angiotensin I→angiotensin II)

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 Increased abnormal sodium handling by kidney → 

Increased endothelial dysfunction(vascular remodelling)

Causes and risk factors:

- **Primary(essential) hypertension**:

1. 90–95% of cases.
2. Multifactorial: genetic predisposition, aging, sympathetic overactivity, endothelial dysfunction, renin–angiotensin–aldosterone system (RAAS) alterations, insulin resistance, obesity, high sodium intake, low potassium intake, alcohol, physical inactivity, chronic stress.

- **Secondary hypertension**:

when onset <30 or >55 years, resistant BP, or with features suggesting a specific cause:

1. Renal parenchymal disease (most common secondary cause)
2. Renovascular hypertension (renal artery stenosis)
3. Primary hyperaldosteronism (Conn's)
4. Obstructive sleep apnea
5. Cushing's syndrome
6. Pheochromocytoma
7. Thyroid disease

Clinical features:

Hypertension is often asymptomatic (the "**silent killer**").

- Headache
- Dizziness
- Visual blurring
- epistaxis (rare).

Symptoms from target-organ damage:

- chest pain/angina
- dyspnea
- transient ischemic attacks/stroke
- reduced urine output
- visual disturbances (hypertensive retinopathy).

Diagnosis:

1. **BP measurement**:

- **ABPM**— 24-hour average is gold standard for diagnosis.

- **HBPM** — useful for ongoing management and for diagnosis when ABPM unavailable.

2. **Baseline investigations**: CBC, fasting glucose/HbA1c, lipid profile, serum creatinine + eGFR, electrolytes (K+), urinalysis (protein)
3. **ECG**
4. **Echocardiography**(if LVH or heart disease suspected).

For resistant/secondary causes, use targeted testing (plasmaaldosterone/renin ratio, renal imaging, sleep study).

Classification (example from ESH 2023): optimal <120/80, normal 120–129/80–84, high-normal 130–139/85–89.

Treatment and management:

- ✓ ACE inhibitor (or ARB)
- ✓ Calcium channel blocker (CCB) — typically amlodipine/dihydropyridine
- ✓ Thiazide/thiazide-like diuretic (chlorthalidone or indapamide often preferred)
- ✓ beta-blockers for ischemic heart disease or heart failure
- ✓ aldosterone antagonists for resistant HTN or primary aldosteronism.
- ✓ **Resistant hypertension** (BP uncontrolled on ≥3 drugs including a diuretic): consider spironolactone or eplerenone.

Case studies:

Case 1 — Middle-aged new diagnosis (typical):

Patient: 54-year-old man, BMI 29, smoker, reports headaches in mornings.

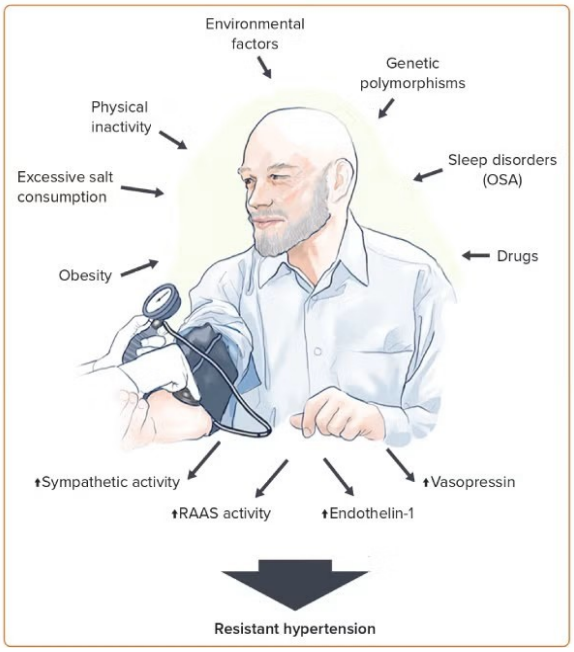
Clinic BP on 2 visits: 152/94 and 150/92. Home BP average (measured twice daily for 7 days) 148/90. eGFR normal, K+ normal, urine dipstick no protein. 10-year ASCVD risk moderate.

Assessment: Grade 1–2 hypertension confirmed by HBPM; lifestyle risk factors present.

Plan: Start lifestyle program (DASH diet, sodium restriction, weight loss, exercise), initiate combination therapy: ACE inhibitor (e.g., lisinopril 10 mg) + amlodipine 5 mg (or single pill combination). Arrange baseline labs in 2 weeks (creatinine, K+), follow up in 2–4 weeks for BP check via home readings, titrate meds to reach systolic <130 if tolerated. Counsel smoking cessation and refer to smoking program.

Case 2 — Resistant HTN prompting secondary workup:

Patient: 46-year-old woman with long-standing HTN on lisinopril 20 mg, amlodipine 10 mg, chlorthalidone 25 mg — clinic BP persistently 165/100 despite reported adherence. K+ is low-normal; creatinine normal. She reports loud snoring and daytime sleepiness. On exam: abdominal obesity. **Assessment:** Suspected resistant hypertension; obstructive sleep apnea (OSA) likely contributor; consider primary hyperaldosteronism (low-normal K+) and evaluate adherence/white coat effect with ABPM. **Plan:** Order 24-hr ABPM to confirm out-of-office BP, screen for OSA (overnight sleep study), check plasma aldosterone:renin ratio after adjusting interfering meds per local protocol, review medication adherence and dosing. If OSA confirmed — start CPAP therapy which often lowers BP; if primary hyperaldosteronism confirmed — refer for endocrinology and consider surgical/medical options (spironolactone/eplerenone)



Ischemic Heart Diseases (IHD)

Definition:

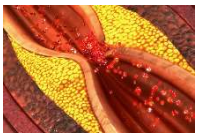
Ischemic Heart Disease (IHD), also known as **Coronary Artery Disease (CAD)**, is a condition where the **blood supply to the heart muscle**

(myocardium) is reduced due to narrowing or blockage of the coronary arteries.

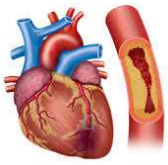
Epidemiology:

IHD is the **leading cause of death worldwide**.According to the World Health Organization (WHO), it accounts for **16% of all deaths globally**.In India, IHD prevalence is rising due to lifestyle changes, sedentary habits, and increasing diabetes and hypertension.Men are more commonly affected at an earlier age than women, though post-menopausal women are also at high risk.

Pathophysiology:

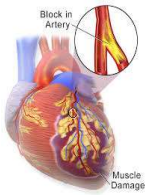
Atherosclerosis -Narrowed lumen  → reduced blood flow

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Myocardial ischemia -Causes angina (chest pain)

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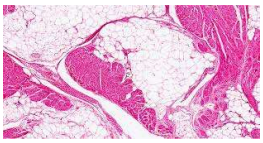


Myocardial infraction-Leads to necrosis of heart muscle

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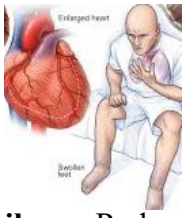
Reperfusion injury - Damage caused by sudden restoration of blood supply

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Myocardial fibrosis - Weakening of heart wall

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Chronic heart failure - Reduced pumping capacity of the heart

Causes\risk factor:

1) **Non-modifiable factors**:
- Age (above 45 years in men, 55 in women)
- Male gender
- Family history of premature IHD

2) **Modifiable factors**:

- Hypertension
- Diabetes mellitus
- High cholesterol levels (hyperlipidemia)
- Smoking & alcohol consumption
- Obesity & sedentary lifestyle
- Stress

Clinical features:

1. **Angina pectoris** (main symptom):
   - Central chest pain, pressure, or heaviness.
   - Radiates to left arm, neck, jaw, or back.
   - Triggered by exertion, relieved by rest or nitroglycerin.
2. Shortness of breath
3. Fatigue
4. Palpitations
5. In **acute MI**: severe chest pain lasting >20 minutes, sweating, nausea, and restlessness.

Diagnosis:

1. Electrocardiogram (ECG) – detects ischemic changes or arrhythmias
2. Echocardiography – evaluates wall motion and heart function
3. Exercise Stress Test (TMT) – checks for ischemia during exertion
4. Blood markers – Troponin I, CK-MB (elevated in MI)
5. Coronary Angiography – gold standard for detecting artery blockages
6. CT Coronary Angiogram – non-invasive method for coronary artery imaging

Treatment & management:

Medications

- Antiplatelet drugs: Aspirin, Clopidogrel
- Nitrates: Nitroglycerin for chest pain
- Beta-blockers: Reduce heart oxygen demand
- ACE inhibitors/ARBs: Control blood pressure
- Statins: Lower cholesterol
- Anticoagulants: In MI or high-risk patients

Interventional Management

- **Percutaneous Coronary Intervention (PCI)**: Angioplasty with stent placement
- **CABG**: Open-heart surgery to bypass blocked arteries

Rehabilitation

- Cardiac rehabilitation programs
- Stress management, yoga, meditation

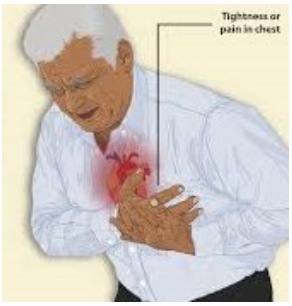
Mini case studies:

Case 1: Stable Angina

Mr. Vivek, 55-year-old businessman, presented with **chest pain on climbing stairs**, which subsided after rest.

- **History**: Diabetes for 10 years, smoker.

- **Examination**: Normal at rest.



- **Investigations**: TMT positive for ischemia, angiography showed 70% blockage in the left anterior descending (LAD) artery.
- **Management**: Started on aspirin, statins, and nitrates. Underwent angioplasty with stent placement. Improved significantly after lifestyle modification.

Case 2: Acute Myocardial Infarction

Mrs. Swetha, 62-year-old, came to emergency with **severe chest pain radiating to left arm, sweating, and breathlessness**.



- **History**: Hypertension, post-menopausal.
- **ECG**: ST elevation in anterior leads.
- **Troponin I**: Positive.
- **Diagnosis**: Acute STEMI.
- **Management**: Immediate thrombolysis with streptokinase, followed by dual antiplatelet therapy and statins. Later underwent CABG.
- **Outcome**: Stable, discharged with cardiac rehabilitation advice.

Congestive Heart Failure (CHF)



Definition:

Congestive Heart Failure (CHF) is a chronic condition in which the **heart is unable to pump blood effectively** to meet the body's oxygen and nutrient demands. This results in backward congestion, where fluid builds up in the lungs, liver, and lower limbs, along with forward failure, which causes inadequate blood supply to body tissues.

Epidemiology:

Worldwide, more than **64 million people live with CHF**, making it a significant public health issue. The condition is more common after the **age of 60** and its frequency rises as people grow older. In India, the incidence is escalating in parallel with lifestyle-related disorders such as high blood pressure, diabetes, and ischemic heart disease. Despite treatment advances, the five-year survival rate in advanced stages is still **below 50%.**

Pathophysiology:

Underlying causes (IHD/HTN/Valvular)



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Decreased cardiac output

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Neurohormonal  activation (RASS, SNS)

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Na+ & water retention + vasoconstriction



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Ventricular remodelling

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Congestion &  Edema (Lungs + Peripheral Tissues)

Causes/ risk factors:

- Ischemic heart disease (IHD)
- Hypertension
- Valvular heart disease
- Cardiomyopathies
- Arrhythmias

- Diabetes, obesity, smoking, alcohol abuse

Clinical features:

- **Left-sided failure**:
  - Dyspnea, orthopnea (breathlessness lying flat), paroxysmal nocturnal dyspnea
  - Fatigue, cough with frothy sputum
- **Right-sided failure**:
  - Peripheral edema, ascites, hepatomegaly, jugular venous distension
- **General**:
  - Reduced exercise tolerance
  - Tachycardia, palpitations

Diagnosis:

1. **Chest X-ray** – cardiomegaly, pulmonary congestion
2. **Echocardiography** – ejection fraction (EF), chamber size, wall motion
3. **ECG** – arrhythmias, ischemic changes
4. **BNP/NT-proBNP levels** – elevated in CHF
5. **Cardiac MRI / Angiography** – to find underlying cause

Treatment & management:

Medications

- Diuretics – reduce fluid overload (furosemide, spironolactone)
- ACE inhibitors/ARBs/ARNIs – improve survival, reduce remodeling
- Beta-blockers – reduce heart rate and improve function
- Aldosterone antagonists – reduce mortality
- Digitalis (Digoxin) – useful in atrial fibrillation with CHF
- Vasodilators – nitrates, hydralazine in selected patients

Advanced therapy

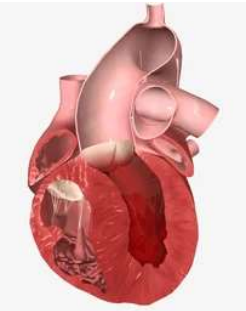
- Implantable cardioverter-defibrillator **(ICD)**
- Cardiac resynchronization therapy **(CRT)**

- Left Ventricular Assist Device **(LVAD)**
- **Heart transplantation** in end-stage cases

Mini case studies:

Case 1: Left-Sided CHF

Mr. Venugopal, 60-year-old, hypertensive for 15 years, presented with **breathlessness on exertion, difficulty lying flat, and frequent nighttime cough**.



- **Investigations**: Echo showed EF 35%, dilated left ventricle.
- **Management**: Started on ACE inhibitor, beta-blocker, and diuretics with salt restriction.
- **Outcome**: Symptomatically improved, able to walk longer distances.

Case 2: Right-Sided CHF

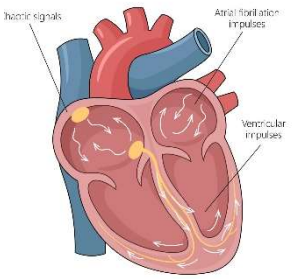
Mrs. Amutha, 65-year-old, with past history of **rheumatic heart disease**, presented with **swelling of feet, abdominal distension, and fatigue**.

- **Examination**: Elevated JVP, pedal edema, hepatomegaly.
- **Investigations**: Echo showed severe tricuspid regurgitation with RV dysfunction.
- **Management**: Diuretics, digoxin, and salt restriction. Surgical valve replacement was advised.
- **Outcome**: Edema reduced, better functional status.

Arrhythmia

Definition:

Arrhythmia is an **abnormality in the rate, rhythm, or conduction of electrical impulses in the heart**. It can cause the heart to beat too fast (tachyarrhythmia), too slow (bradyarrhythmia), or irregularly.



Epidemiology:

Arrhythmias are **very common worldwide**.The prevalence increases with **age, hypertension, ischemic heart disease, and heart failure**.**Atrial fibrillation (AF)** is the most common sustained arrhythmia.Ventricular arrhythmias are a major cause of **sudden cardiac death**.

Pathophysiology:

Arrhythmias occur due to problems in the **cardiac conduction system**:

1. **Abnormal impulse formation**

- After depolarization (e.g., sinus tachycardia)

2. **Abnormal impulse conduction**

- Conduction block (e.g.,atrial flutter, ventricular tachycardia).

3. **Combined mechanisms**

- Seen in atrial fibrillation and complex arrhythmias.

Causes/ risk factors:

- Ischemic heart disease
- Hypertension and heart failure.
- Electrolyte imbalance
- Thyroid disorders
- Drug toxicity (digoxin, antiarrhythmic drugs).
- Structural heart disease

Clinical features:

- Palpitations
- Syncope
- Chest pain
- Breathlessness
- Sudden cardiac arrest

Classification of arrhythmias:

1. **According to heart rate**

- **Tachyarrhythmia** (>100 bpm) → atrial fibrillation, atrial flutter, ventricular tachycardia.
- **Bradyarrhythmia** (<60 bpm) → sinus bradycardia, AV block.

2. **According to site of origin**

- **Supraventricular arrhythmias** → AF, atrial flutter, PSVT.
- **Ventricular arrhythmias** → VT, VF, PVCs.

Diagnosis:

- **ECG** → main tool for detecting rhythm abnormalities
- **Holter monitoring (24-48 hrs)** → for intermittent arrhythmias
- **Electrophysiological (EP) studies** → for mapping abnormal conduction
- **Echocardiography** → to detect underlying structural heart disease
- **Blood tests** → electrolytes, thyroid function

Treatment & management:

1. Medications

- **Antiarrhythmic drugs**:
  - Class I: Sodium channel blockers (quinidine, flecainide).
  - Class II: Beta-blockers (propranolol, metoprolol).
  - Class III: Potassium channel blockers (amiodarone, sotalol).
  - Class IV: Calcium channel blockers (verapamil, diltiazem).
- **Digoxin**: especially in AF with CHF.
- **Anticoagulants**: in atrial fibrillation to prevent stroke.

2. Non-Pharmacological

- **Electrical cardioversion**: for AF, atrial flutter, VT.
- **Ablation therapy**: destroys abnormal conduction pathways.
- **Pacemaker**: for bradyarrhythmias/heart block.
- **Implantable Cardioverter-Defibrillator (ICD)**: for life-threatening ventricular arrhythmias.

Mini case studies:

Case 1: Atrial Fibrillation



Mr. Subramani, 68-year-old, hypertensive, presented with **palpitations and breathlessness**.

- **ECG**: irregularly irregular rhythm, absent P waves → Atrial fibrillation.
- **Management**: Started on beta-blocker, anticoagulation with warfarin, and rate control. Improved symptoms.

Case 2: Ventricular Tachycardia

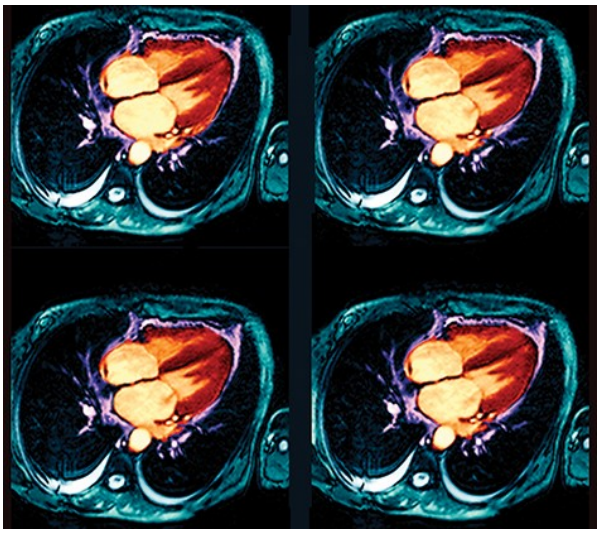


Mrs. Saraswathi, 55-year-old post-MI patient, came with **dizziness and chest discomfort**.

- **ECG**: wide QRS complex tachycardia → Ventricular tachycardia.
- **Management**: Given IV amiodarone, later ICD implanted. Condition stabilized.

Valvular Heart Disease (VHD)

Definition:



Valvular heart disease refers to damage or dysfunction of one or more of the four heart valves (mitral, aortic, tricuspid, pulmonary) leading to obstruction (stenosis) or backflow (regurgitation).

Causes:

1. **Congenital** – bicuspid aortic valve, pulmonary stenosis.

2. **Acquired**
   - Rheumatic heart disease (common in developing countries).
   - Degenerative (age-related calcification).
   - Infective endocarditis.
   - Hypertension, atherosclerosis.
   - Connective tissue disorders (Marfan syndrome).

Pathophysiology:



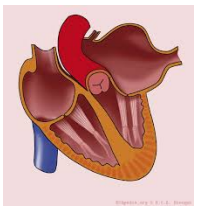
Valve stenosis → obstruction to forward blood flow → pressure overload**.**

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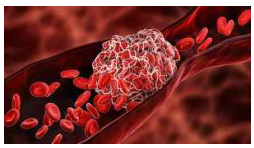
Valve regurgitation → backflow of blood → volume overload.

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Overload → ventricular/atrial dilation & hypertrophy.

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Chronic changes → heart failure, arrhythmias, pulmonary hypertension, thromboembolism.

Clinical features:

- **Mitral stenosis** – Dyspnea, orthopnea, hemoptysis, opening snap, diastolic murmur.
- **Mitral regurgitation** – Fatigue, palpitations, pansystolic murmur radiating to axilla.
- **Aortic stenosis** – Syncope, angina, dyspnea, harsh systolic murmur.
- **Aortic regurgitation** – Bounding pulse, wide pulse pressure, early diastolic murmur.
- **Tricuspid/Pulmonary valve disease** – Jugular venous distension, edema, ascites, systolic murmurs.

Diagnosis:

- **Echocardiography (gold standard)**.
- ECG (for arrhythmias, chamber enlargement).
- Chest X-ray (cardiomegaly, pulmonary congestion).
- Cardiac catheterization (severity assessment).

Treatment & management:

- **Medical** – Diuretics, ACE inhibitors, beta-blockers, anticoagulants, antibiotics (rheumatic prophylaxis).
- **Interventional** – Balloon valvotomy.
- **Surgical** – Valve repair or valve replacement (mechanical/bioprosthetic).

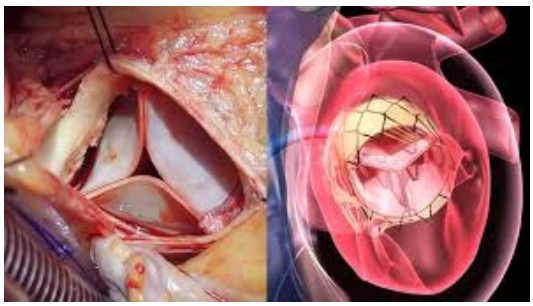
Mini case studies:

Case 1:

A 32-year-old woman with history of rheumatic fever presents with breathlessness and palpitations. Exam shows loud S1, opening snap, and mid-diastolic murmur. Echo confirms **severe mitral stenosis**. She undergoes percutaneous balloon mitral valvotomy.

Case 2:

A 65-year-old man with long-standing hypertension presents with exertional chest pain, syncope, and dyspnea. Harsh systolic murmur heard at right upper



sternal border, radiating to carotids. Echo shows **severe calcific aortic stenosis**. He undergoes **aortic valve replacement surgery**.

Clinical pearls:

Hypertension, ischemic heart disease, heart failure, arrhythmias, and valvular heart disorders share several important clinical considerations. Hypertension is often silent, making routine blood pressure screening essential, and young patients should be evaluated for secondary causes. Ischemic heart disease typically presents with exertional chest discomfort relieved by rest, though diabetics and older adults may develop silent ischemia. In heart failure, breathlessness on exertion is usually the earliest symptom, with orthopnea and nocturnal attacks being more specific indicators. Arrhythmias frequently manifest as palpitations, and atrial fibrillation remains the most common sustained rhythm disorder. In valvular heart disease, diastolic murmurs are usually pathological, and echocardiography is the gold standard for confirming severity. Across all conditions, lifestyle measures such as diet, exercise, and risk-factor control play a crucial role alongside medications.

Red flags:

Warning signs must be recognized promptly, as they indicate potentially life-threatening situations. In **hypertension**, a crisis with very high pressures and organ damage, or symptoms like severe headache and vision changes, demand urgent care. For **ischemic heart disease**, chest pain lasting longer than 20 minutes, especially if radiating to the arm or jaw and accompanied by sweating or collapse, may signal a myocardial infarction. In heart failure, sudden pulmonary edema with frothy sputum, shock with low urine output, or rapid swelling of the body are danger signs. **Arrhythmias** can present with fainting, ventricular tachycardia, or complete heart block, all of which pose a risk of sudden death**. Valvular disease** also has critical red flags such as severe aortic

stenosis causing syncope or angina, acute mitral regurgitation with pulmonary edema, or infective endocarditis leading to embolic complications. Recognizing these red flags ensures timely intervention and **improved patient outcomes**.

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Climate change and the spread of vector-borne diseases

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Introduction

Vector-borne diseases are caused by parasites, bacteria or viruses transmitted to humans or animals by living vectors. Important examples include malaria transmitted by Anopheles mosquitoes; dengue, chikungunya and Zika transmitted mainly by Aedes mosquitoes; Japanese encephalitis and West Nile disease transmitted by Culex mosquitoes; leishmaniasis transmitted by phlebotomine sandflies; and numerous bacterial, viral and parasitic diseases transmitted by ticks. WHO notes that VBDs account for more than 17% of infectious diseases and cause more than 700 000 deaths annually. Their distribution reflects interacting demographic, environmental and social determinants, including travel and trade, urbanization, climate and vector adaptation. [1]

Climate change matters because vectors, pathogens and reservoir hosts are living systems whose development and survival are environmentally constrained. IPCC assessments conclude that several climate-sensitive VBD burdens are projected to increase under continued warming without additional adaptation. Malaria transmission may increase in some areas and decrease in others, while dengue risk is projected to expand in geographical and seasonal distribution. [2,3]

Attribution requires caution. A rise in disease incidence can result from climate, vector adaptation, urbanization, population movement, changes in immunity, surveillance intensity or public-health interventions. Climate should therefore be considered a risk modifier within a broader ecological and health-system framework.

Climate determinants of vector-borne disease transmission

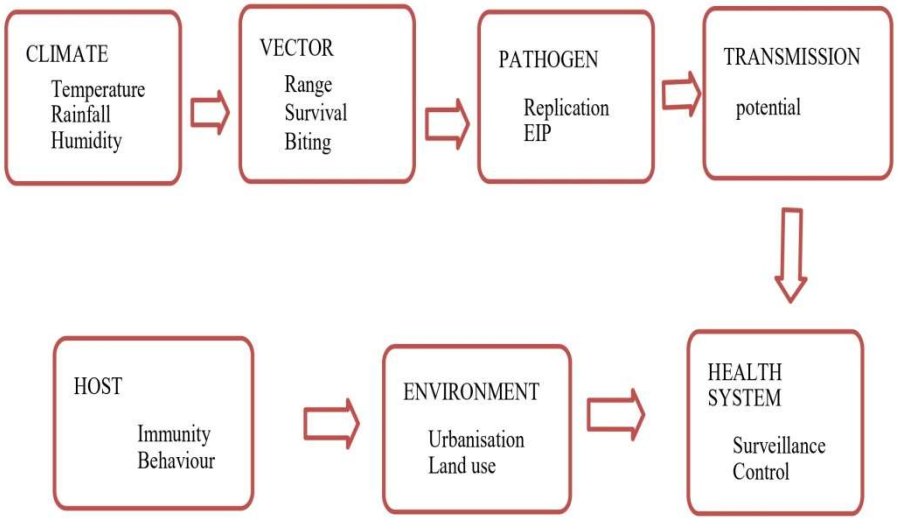
Temperature is a major determinant of vector development, adult survival, biting frequency, gonotrophic cycling and pathogen development. Within a suitable range, warming may accelerate development and shorten the extrinsic incubation period (EIP)—the interval between acquisition of a pathogen by a vector and the vector becoming infectious. Beyond upper thermal limits, however, vector survival and pathogen development can decline. Thus, temperature–transmission relationships are non-linear.

Rainfall influences the creation, persistence and flushing of aquatic breeding sites. Heavy rainfall may create habitats for mosquitoes, but intense precipitation can also wash larvae from containers. Drought can reduce natural breeding sites while increasing domestic water storage in urban settings, potentially increasing Aedes breeding. IPCC assessments document both rainfall-associated and drought-associated VBD increases depending on local ecology. [3]

Humidity can influence adult vector survival and host-seeking behaviour. Extreme weather events can alter vector habitats, human exposure and healthcare access simultaneously. Floods may increase breeding habitats while damaging health facilities and interrupting surveillance; droughts may alter water storage practices and increase household exposure.

Seasonal changes are particularly important. A longer period of suitable temperature and humidity can extend the window for transmission, whereas altered monsoon timing may shift outbreak peaks. These effects are strongly location-specific.

Figure 1. Climate–vector–pathogen–host framework. Climate interacts with vectors, pathogens, hosts, environmental conditions and health-system capacity.



Disease burden emerges from interacting biological, environmental, social and health-system determinants

Figure 2. Principal pathways linking climate variables to transmission

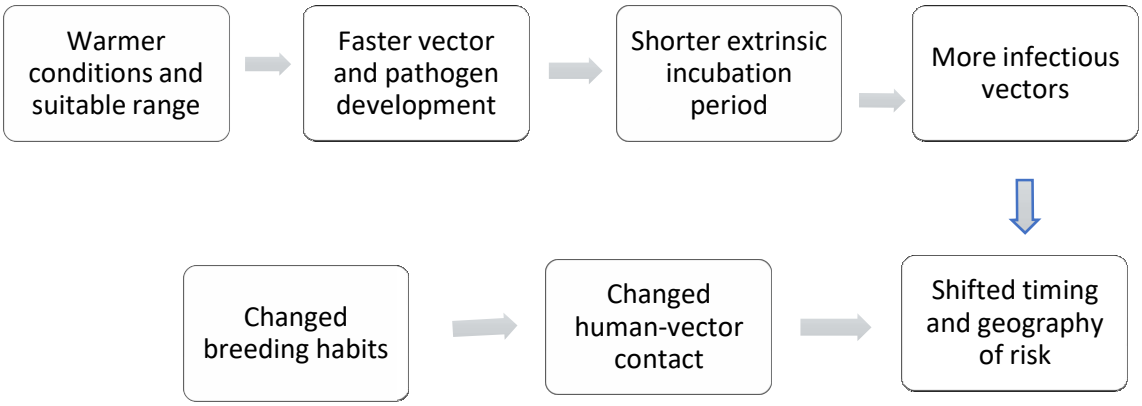


Figure 2. Effects are non-linear and modified by ecology and public-health interventions.

Climate factor	Vector-level effect	Transmission implication	Public-health relevance
Temperature	Changes development, survival, biting and geographic range	May accelerate pathogen development within physiological limits	Earlier/longer seasons or range shifts
Rainfall	Creates, sustains or flushes breeding sites	Changes vector abundance	Seasonal outbreak potential; flood-associated risk
Humidity	Often increases adult survival	Can increase infectious lifespan indirectly	Higher transmission potential in suitable settings
Drought	May remove natural habitats but increase domestic water storage	Can alter Aedes breeding	Urban dengue risk may increase
Flooding	Creates temporary habitats; disrupts control	Changes exposure and service access	Outbreak risk plus health-system disruption
Heatwaves	Can accelerate development or exceed survival thresholds	Non-linear effect on transmission	Risk may rise or fall locally
Milder winters	Improves overwinter survival of some vectors	Extends seasonal activity	Earlier seasonal onset

Vector–pathogen–host interactions

The effect of climate is expressed through a connected vector–pathogen–host system. Climate may change vector density, survival, biting frequency, geographical range and competence; pathogen development within the vector may also change. Human immunity, housing, occupation, behaviour,

mobility and access to prevention and care determine whether increased ecological suitability becomes increased disease.

Vectorial capacity is a useful conceptual measure of transmission potential and includes vector density, biting rate, vector survival and competence. Temperature can affect several components simultaneously, but not necessarily in the same direction. Consequently, a modest increase in mean temperature cannot be interpreted as a proportional increase in disease incidence.

The distinction between climate suitability and observed incidence is essential in textbook interpretation. Climate models estimate ecological opportunity; surveillance data measure disease events, which are also influenced by diagnosis, reporting, immunity, interventions and health-system performance.

Major climate-sensitive vector-borne diseases

Malaria

Malaria is caused by Plasmodium species and transmitted by female Anopheles mosquitoes. Temperature, rainfall and humidity determine the ecological limits and seasonality of transmission. Temperature affects both mosquito development and Plasmodium development within the mosquito. [2,4]

WHO's World malaria report 2025 estimates approximately 282 million malaria cases and 610 000 deaths globally in 2024. These figures represent overall burden and should not be interpreted as climate-attributable cases. [4] Climate-related changes may increase transmission at the margins of endemic zones or at higher elevations, while diagnosis, treatment, insecticide-treated nets, indoor residual spraying, vaccination and socioeconomic development can reduce burden.

Anopheles stephensi, an urban-adapted malaria vector, illustrates how vector ecology can change independently of climate and why urban entomological surveillance is important. Climate-informed malaria programmes should therefore combine meteorological information with vector surveillance, resistance monitoring and case-based surveillance.

Dengue

Dengue is caused by four antigenically distinct dengue virus serotypes and is transmitted primarily by *Aedes aegypti* and *Aedes albopictus.* Aedes mosquitoes are closely associated with human settlements and breed in artificial water-holding containers. Temperature, rainfall and humidity influence development, survival, feeding and viral replication.

WHO reports that reported dengue cases increased from 505 430 in 2000 to 14.6 million in 2024, with 2024 representing the highest reported annual burden to that time. WHO identifies multiple interacting contributors, including changing vector distribution, climate change, urbanization, population movement, surveillance limitations and pressure on health systems. [5,6]

The Lancet Countdown reports that average climate-defined transmission potential for dengue increased by 17.0% for Aedes aegypti and 48.5% for Aedes albopictus between 1951–60 and 2015–24. These are modelled climate-suitability indicators, not percentage increases in clinical cases. [7]

Chikungunya

Chikungunya is an alphavirus transmitted mainly by Aedes aegypti and Aedes albopictus. Its climatic determinants overlap with dengue. Temperature influences vector and viral biology, while rainfall and water storage affect breeding habitats. Because chikungunya and dengue can present with similar acute febrile illness, laboratory confirmation is valuable when both circulate.

Zika virus disease

Zika virus is transmitted mainly by Aedes mosquitoes. Climate change can alter seasonal and geographic suitability for Aedes vectors, but outbreaks also require viral introduction, sufficient vector density and susceptible hosts. Zika is particularly important in pregnancy because congenital infection can result in severe fetal outcomes.

Japanese encephalitis

Japanese encephalitis virus is maintained in zoonotic cycles involving mosquitoes, pigs and birds. Culex mosquitoes associated with rice-growing and other aquatic environments are important vectors. Climate, irrigation, rainfall, animal husbandry and land-use change interact to determine risk; vaccination and surveillance are major control measures.

West Nile virus disease

West Nile virus is maintained mainly between Culex mosquitoes and birds, with humans and horses as incidental hosts. Temperature can influence mosquito development and viral replication within physiological limits, while rainfall, drought, vegetation and bird ecology may have contrasting local effects.

Leishmaniasis

Leishmaniasis is transmitted by phlebotomine sandflies. Temperature and humidity influence sandfly survival and seasonal activity, while land-use change, reservoir ecology, housing and socioeconomic conditions determine human exposure. Climate-driven shifts in sandfly suitability may modify the geographic distribution of transmission.

Tick-borne diseases

Ticks transmit numerous bacterial, viral and parasitic pathogens. Warmer winters can increase overwinter survival and extend seasonal activity, while vegetation and wildlife-host changes modify habitat suitability. IPCC assessments report climate-associated changes in tick activity and geographic range in several regions. [8]

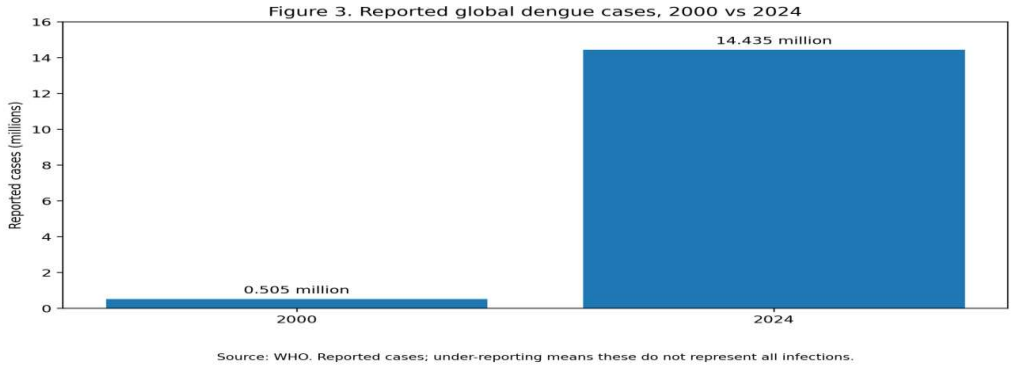


Figure 3. Reported global dengue cases, 2000 versus 2024. Source: WHO. Reported cases are not equivalent to all infections because many infections are asymptomatic or unreported. [5,6]

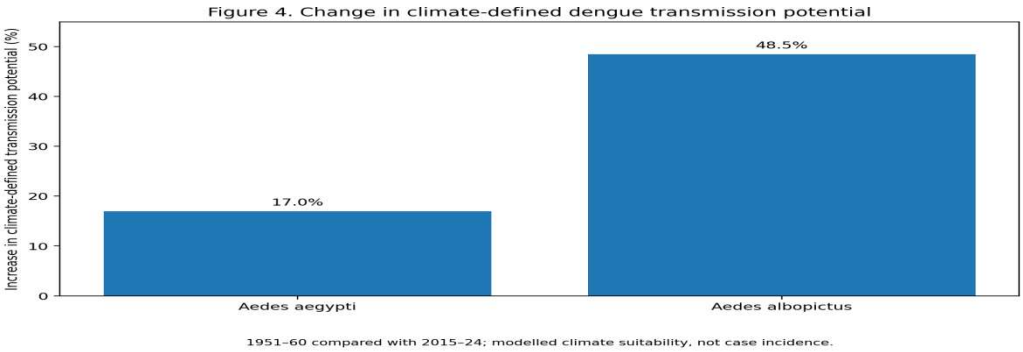


Figure 4. Change in climate-defined dengue transmission potential from 1951–60 to 2015–24. Source: Lancet Countdown 2025. [7]

Table 2. Selected VBDs and climate-sensitive pathways

Disease	Principal vector(s)	Key climate drivers	Important modifiers
Malaria	Anopheles spp.	Temperature, rainfall, humidity	Altitude, housing, vector control, immunity
Dengue	Aedes aegypti; A. albopictus	Temperature, rainfall, humidity	Urbanization, water storage, mobility, serotypes
Chikungunya	Aedes aegypti; A. albopictus	Temperature, rainfall	Population movement, immunity, vector density
Zika	Aedes aegypti; A. albopictus	Temperature, rainfall, seasonality	Pregnancy, mobility, immunity
Japanese encephalitis	Culex spp.	Temperature, rainfall, irrigation	Pigs, birds, rice cultivation, vaccination
West Nile disease	Culex spp.	Temperature, rainfall, seasonality	Bird reservoirs, wetlands
Leishmaniasis	Phlebotomine sandflies	Temperature, humidity	Reservoirs, housing, land use
Lyme disease	Ixodes spp.	Temperature, vegetation, seasonality	Wildlife hosts, land use, outdoor exposure

Urbanization, land use and human mobility

Unplanned urbanization can amplify Aedes-borne disease through inadequate drainage, intermittent water supply, uncovered storage containers, discarded tyres and solid waste. Poor housing can increase exposure to mosquitoes and sandflies. WHO identifies urbanization, travel and trade, climate change and vector adaptation as interacting contributors to VBD spread. [1]

Mobility can introduce pathogens into receptive vector populations. Displacement after floods, cyclones or conflict may increase exposure while simultaneously interrupting diagnosis, treatment and vector control. Land-use change, irrigation, deforestation and agricultural practices can alter vector and reservoir habitats.

Climate-resilient planning therefore requires health authorities to work with urban planners, water and sanitation agencies, disaster-management authorities and environmental services.

Climate change and vector-borne diseases in India

India has diverse climatic and ecological zones, with monsoon variability, rapid urbanization, irrigation, agricultural change and extensive population mobility. Important VBDs include malaria, dengue, chikungunya, Japanese encephalitis, lymphatic filariasis and visceral leishmaniasis.

India's National Action Plan for Climate Change and Human Health (NAPCCHH) identifies vector-borne and zoonotic diseases as a major climate-sensitive theme. The plan recommends climate-informed vulnerability mapping, active and passive surveillance, sentinel sites, capacity building, risk communication, adequate logistics and outbreak preparedness. [9]

The national programme also emphasizes that weather variables such as temperature, rainfall, humidity, floods, drought and wind interact with vector population dynamics, breeding sites, feeding behaviour, pathogen development and health-system access. [9]

For India, climate information should be interpreted at district and sub-district levels where possible. A monsoon anomaly may have different effects on dengue in a densely populated city, malaria in a forested area and Japanese encephalitis in a rice-growing landscape.

Table 3. Climate-sensitive VBD priorities for India

Disease	Surveillance priorities	Environmental/climate indicators	Priority interventions
Dengue	Cases, NS1/NAAT/serology, Aedes indices	Temperature, rainfall, humidity, water storage	Source reduction, larval control, community action, preparedness
Malaria	Microscopy/RDT/NAAT where indicated, species, vectors	Rainfall, temperature, humidity, altitude	Vector control, diagnosis/treatment, surveillance
Chikungunya	NAAT/serology, syndromic surveillance	Temperature, rainfall, Aedes density	Aedes control, early case detection
Japanese encephalitis	AES surveillance, IgM/NAAT where appropriate	Rainfall, irrigation, rice ecology	Vaccination, surveillance, vector/host measures
Visceral leishmaniasis	Parasitological/serological/molecular confirmation	Temperature, humidity, land use	Vector control, early diagnosis and treatment
Lymphatic filariasis	Programme surveillance, antigen/molecular tools as appropriate	Rainfall, temperature, vector habitats	MDA, morbidity management, vector control

Laboratory diagnosis and the role of microbiology

Changing VBD distributions create diagnostic challenges because clinicians may not initially suspect an infection that was previously uncommon locally. Microbiology laboratories can provide an early signal of changing epidemiology when laboratory-confirmed results are linked with geographic and temporal information.

- Maintain syndromic awareness of malaria and arboviral infections in acute undifferentiated fever.

- Select diagnostic methods according to disease stage, specimen quality and national guidance.
- Use dengue NS1 antigen, nucleic-acid amplification or serology appropriately according to illness day and validated laboratory algorithms.
- Use malaria microscopy and/or validated rapid diagnostic tests; molecular methods can support species confirmation and surveillance when indicated.
- Apply appropriate molecular or serological tests for chikungunya, Zika, Japanese encephalitis and other arboviruses according to national guidance.
- Maintain quality assurance, biosafety, proficiency testing and adequate surge capacity.
- Share unusual clusters, imported infections and geographic or seasonal shifts with public-health surveillance authorities.

Laboratory information becomes substantially more useful when integrated with entomological and meteorological data. An increase in Aedes indices followed by increasing dengue positivity may provide a stronger early-warning signal than either indicator alone. Likewise, detection of malaria in a new altitude or geographic zone should trigger investigation of travel history, local vectors, land-use change and possible local transmission.

Surveillance and climate-informed early warning

Traditional surveillance is often reactive. Climate-informed early-warning systems seek to identify periods and locations of increased transmission risk before clinical peaks occur. IPCC assessments identify enhanced surveillance and early-warning/early-response systems as important adaptation measures for climate-sensitive VBDs. [2]

Figure 5. Climate-informed vector-borne disease early-warning cycle

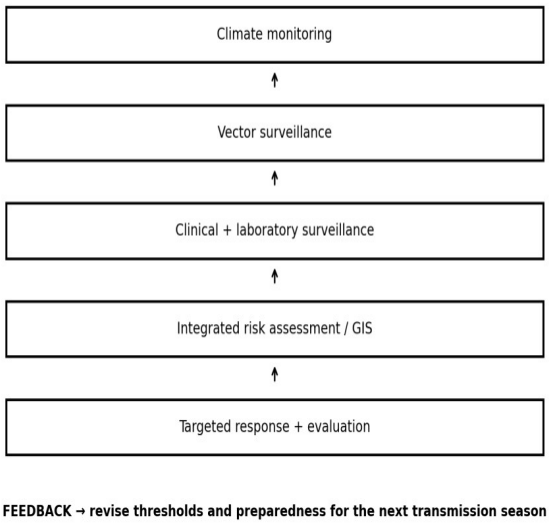


Figure 5. Climate-informed VBD early-warning cycle. Climate, vector, clinical and laboratory data should be integrated and used to trigger targeted response.

- ✓ Monitor temperature, rainfall, humidity and extreme-weather indicators.
- ✓ Measure vector abundance, distribution, breeding indices and insecticide resistance.
- ✓ Track clinical syndromes, admissions and unusual geographic or seasonal patterns.
- ✓ Monitor laboratory positivity, serotypes/species and unusual diagnostic findings.
- ✓ Integrate information using GIS and risk maps.
- ✓ Issue timely alerts to health workers, local authorities and communities.
- ✓ Intensify vector control and clinical preparedness before predicted peaks.
- ✓ Evaluate predictions against observed transmission and revise thresholds.

Integrated vector management

Integrated vector management (IVM) combines environmental, biological, chemical and personal protection measures according to local vector ecology and epidemiology. Climate change increases the value of IVM because the timing and geographical location of vector abundance may shift.

- Environmental management: eliminate standing water, improve drainage and manage solid waste.
- Larval-source management: apply appropriate larvicides or biological methods where indicated.
- Adult vector control: use indoor residual spraying or space spraying according to programme guidance and epidemiological need.
- Personal protection: insecticide-treated nets for malaria risk, repellents, protective clothing and screening.
- Community participation: household source reduction and community clean-up.
- Insecticide-resistance surveillance: monitor susceptibility and adapt tools according to evidence.
- Urban planning: incorporate drainage, reliable water supply and vector-risk assessment into infrastructure design.

One Health approach

Many VBDs involve ecological cycles connecting humans, vectors, animals and the environment. A One Health approach links human clinical surveillance, veterinary surveillance, entomology, environmental monitoring and climate information. This is particularly relevant to Japanese encephalitis and West Nile disease.

Domain	Examples of actions
Human health	Clinical surveillance, diagnosis, treatment and reporting
Animal health	Monitoring reservoir/amplifying hosts and vaccination where appropriate
Entomology	Vector identification, density, infection and resistance testing
Environment	Water bodies, vegetation, land use and waste management
Climate science	Meteorological monitoring and forecasting
Public health	Integrated risk assessment, communication and response

Climate-resilient health systems

Resilience means anticipating climate-related disease pressure rather than responding only after outbreaks occur. Essential components include trained staff, laboratory surge capacity, reliable supplies, protected infrastructure, digital surveillance and emergency preparedness.

Component	Climate-resilient action
Surveillance	Integrate climate, vector, clinical and laboratory indicators
Laboratory	Maintain diagnostics, quality assurance and surge capacity
Workforce	Train clinicians, microbiologists, entomologists and vector-control teams
Infrastructure	Protect laboratories, hospitals and cold chains from extreme weather
Supplies	Pre-position diagnostics, medicines and vector-control commodities before high-risk seasons
Data systems	Use interoperable reporting and GIS-based dashboards
Emergency preparedness	Maintain plans for floods, cyclones, heatwaves and displacement
Community engagement	Use locally appropriate risk communication and source reduction

Challenges and uncertainties

- Climate–disease relationships are non-linear and species-specific.
- Observed increases in disease incidence usually have multiple causes; attribution to climate alone is often inappropriate.
- Under-reporting and surveillance gaps can obscure changes in distribution.
- Vector-control programmes may offset increased climatic suitability.
- Vector adaptation and insecticide resistance may alter future transmission.
- Human mobility can introduce pathogens into areas with competent vectors.
- Extreme heat may reduce transmission once vector survival thresholds are exceeded.
- Local projections are uncertain and should be interpreted with surveillance data.

The IPCC emphasizes that climate effects interact with population growth, migration, urbanization, land-use change, biodiversity and public-health measures. [2,3] Operationally, the key question is whether climatic conditions are changing the probability, timing or geographical opportunity for transmission—not whether climate change alone 'caused' an outbreak.

Conclusion

Climate change is altering the ecological context in which VBDs are transmitted. Rising temperatures, changing rainfall and humidity, altered seasonality and extreme weather can modify vector survival, distribution and pathogen development. These changes can increase or shift transmission potential, but disease burden remains a product of climate interacting with urbanization, land use, mobility, immunity, socioeconomic conditions and public-health interventions.

Dengue illustrates the combination of expanding climate-defined suitability and increasing reported global burden, while malaria demonstrates how climate effects can be offset or amplified by vector control, treatment, vaccination, socioeconomic development and ecological change. [4–7] In India, climate-sensitive disease preparedness should integrate meteorological information with entomological, laboratory and clinical surveillance at local levels.

The most effective response combines climate mitigation with adaptation: strengthening surveillance, early warning, integrated vector management, laboratory capacity, community participation, One Health coordination and climate-resilient healthcare infrastructure.

Take-home messages for clinicians and microbiologists

- Consider VBDs in acute undifferentiated fever when local or travel history indicates exposure.
- Do not assume geographic exclusion because a disease was previously uncommon in the area.
- Use appropriate laboratory tests according to illness stage and national guidance.
- Recognize overlapping clinical syndromes among dengue, chikungunya, Zika and other arboviruses.
- Report unusual clusters and changes in geographic or seasonal distribution.
- Integrate laboratory findings with clinical, entomological and meteorological information.
- Support multidisciplinary outbreak investigations.
- Promote prevention and community education alongside diagnosis and treatment.

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Multidrug Resistant Tuberculosis (MDR TB) & Newer TB Regimens

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Introduction

Tuberculosis (TB) strains that are resistant to Anti tubercular drugs are more difficult to treat than drugsusceptible ones, and present a major challenge for patients, health care workers and health care services. In addition, the increase of drug-resistant TB (DR-TB) threatens global progress towards the targets set by the End TB Strategy of the World Health Organization (WHO). Thus, there is a critical need for the continual development on the treatment of patients with drug resistant TB (DR-TB), based on the most recent and comprehensive evidence available.*(1)*

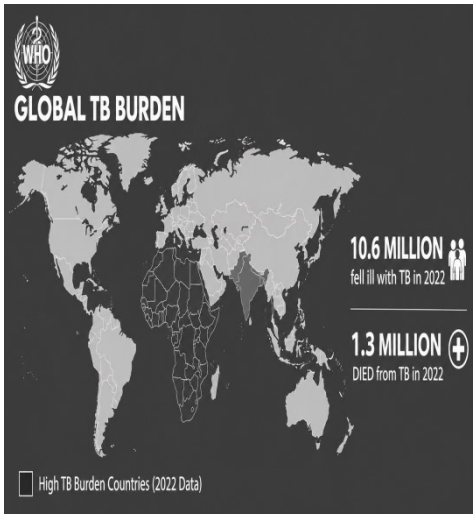


Fig 1:Global TB burden by WHO



Fig 2: End TB Strategy Targets by WHO

Classification [1,2]:

Anti-tubercular drugs can be broadly classified into two types

First line drugs: It includes Rifampicin (R), Isoniazid (H), Ethambutol (E) and Pyrazinamide (Z).They are given for drug susceptible TB cases.

Second line drugs: These drugs are reserved for drug resistant TB cases.According to WHO they are grouped as follows

Group	Drugs
Group A	Levofloxacin (Lfx) Moxifloxacin(Mfx) Bedaquiline (Bdq) Linezolid (Lzd)
Group B	Clofazimine (Cfz) Cycloserine (Cs) or terizidone (Trd)
Group C	Ethambutol (E) Delamanid (Dlm) Pyrazinamide (Z) Amikacin (Am) or Streptomycin (S) Ethionamide (Eto) or prothionamide (Pto) P-aminosalicyclic acid (PAS) Imipenem-cilastatin (Ipm-Cln) or Meropenem (Mpm)

Definitions[1,2]

Bacteriologically confirmed: when a biological specimen is positive by smear microscopy, culture or a rapid diagnostic test for tuberculosis (TB) recommended by the World Health Organization (WHO).

Clinically diagnosed: when a person who does not fulfil the criteria for bacteriological confirmation has been diagnosed with TB disease by a medical practitioner who has decided to give the person a full course of TB treatment.

Drug susceptibility testing (DST): In vitro testing using either molecular or genotypic techniques to detect resistance-conferring mutations, or phenotypic methods todetermine susceptibility to a medicine.

Drug-susceptible TB (DS-TB): A bacteriologically confirmed or clinically diagnosed case of TBwithout evidence of infection with strains resistant to rifampicin and isoniazid.

Drug-resistant TB (DR-TB): TB disease caused by a strain of *Mycobacterium tuberculosis* complex that is resistant to any TB medicines.

Mono-resistant TB (MR TB) (2): TB caused by M.tb strains that are resistant to one first- line anti-TB drug only

Poly-drug resistant TB (PDR-TB): TB caused by M.tb strains that are resistant to more than one first-line anti-TB drug other than HR and RR.

Rifampicin resistant TB (RR-TB): TB caused by M.tb strains that are resistant to rifampicin (R), detected using phenotypic or genotypic methods, with or without resistance to other anti-TB drugs. It includes any resistance to R in the form of mono-resistance, poly-resistance, MDR-TB, or XDR-TB.

Isoniazid-resistant TB (Hr-TB) (2): TB is caused by M.tb strains that are resistant to isoniazid, and resistance to rifampicin has been ruled out.

Multidrug-resistant TB (MDR-TB): TB caused by M.tb strains that are resistant to both H and R with or withoutresistance to other first-line anti-TB drugs.

MDR-TB patients also include subsets of Pre-XDR and XDR TB.

Pre-extensively drug-resistant TB (Pre-XDR-TB): TB caused by M.tb strains that fulfil thedefinition of MDR/ RR-TB and are also resistant to any fluoroquinolone .

Extensively drug-resistant TB (XDR-TB): TB caused by M.tb strains that fulfil the definition of MDR/RR-TB and are also resistant to any fluoroquinolone (levofloxacin or moxifloxacin) and at least one additional Group A drug (presently to either bedaquiline or linezolid [or both]) .

Treatment guidelines for Drug resistant TB

The treatment guidelines for drug resistant Tuberculosis by WHO and National Tuberculosis Elimination Program (NTEP) are similar.The regimens are as follows

1. Treatment of drug-resistant TB using 6-month regimens
2. Treatment of drug-resistant TB using 9-month regimens
3. Treatment of drug-resistant TB using longer regimens

4. Treatment of rifampicin-susceptible and isoniazidresistant TB (Hr-TB)

WHO &NTEP recommendations for drug resistant tuberculosis (DR-TB)[1,2,3]

1.Treatment of drug-resistant TB using 6-month regimens

❖ **BPaLM Regimen**

The 6-month bedaquiline, pretomanid, linezolid and moxifloxacin (BPaLM) regimen for MDR/RR TB patients.

Pre treatment evaluation:

Clinical evaluation	Laboratory based evaluation
●History and physical examination (including previous drug use, alcohol/ substance abuse,family planning methods etc) ● Previous history of ATT taken, especially Bdq, Pa, Dlm and Lzd (defined as more than one month exposure) ● A thorough clinical examination ● Assess nutritional status [Height (m), Weight(kg),BMI] ● Neurological evaluation, if required ● Ophthalmic evaluation, visual acuity, and color vision test	●Random blood sugar (RBS) ● HIV testing following counselling ● Complete blood count (Hb, TLC, DLC, platelet count) ● Liver function tests# ● Renal function tests ● Serum electrolytes (Na, K, Mg, Ca) ● Urine pregnancy test (in women of reproductive age group) ● Chest X-ray ● ECG

Inclusion criteria& Exclusion criteria:

Inclusion Criteria	Exclusion Criteria
Age ≥14 years with newly microbiologically confirmed MDR/RR-TB	Age <14 years
Drug exposure: • <1 month exposure to Bdq, Lzd and/or Pa • >1 month exposure with documented sensitivity	Documented resistance to Bdq, Lzd and/or Pa

• No prior failure of Bdq/Lzd regimen with confirmed sensitivity	
QTcF ≤450 ms (males) or ≤470 ms (females), or normal after correction of electrolytes	Persistent QTcF>450 ms (males) or >470 ms (females) despite correction
Non-pregnant women; pregnant <20–24 weeks willing for MTP; lactating but not breastfeeding	Significant cardiac disease (structural heart disease, long QT syndrome, AV block, arrhythmias, syncope)
	Uncontrolled cardiac arrhythmia requiring medication
	Risk factors for Torsade de Pointes (heart failure, hypokalemia, family history of long QT)
	Significant liver dysfunction (LFT >3× ULN)
	Severe extrapulmonary MDR-TB (CNS, spinal/skeletal, disseminated)

Relative contraindications:

Relative contraindications	**Notes**
Concurrent use of medications that have known interactions or overlapping toxicities with BPaLM	● Use of strong inhibitors or inducers of cytochrome P450 enzyme ● Drugs that prolong the QT interval (anti-fungals, antiarrhythmics, antipsychotics etc.) ● Drugs that increase serotonin levels and other serotoninergic ● Monoamine Oxidase Inhibitors (MAOIs) or prior use within two weeks of treatment ● Currently on serotoninergic antidepressants/ tricyclic antidepressants or prior use within three days of treatment ● Concomitant use of any drug known to induce myelosuppression ● If a patient requires an oral magnesium-containing substance e.g. magnesium trisilicate, magnesium sulphate this must be dosed two hours separate from the fluoroquinolone.

Severe anaemia, thrombocytopenia or leukopenia	●Haemoglobin (Hb) level < 8.0 g/dL ● Platelet count < 75 000/mm3 ● Absolute neutrophil count < 1000/mm3
Severe renal failure	●Serum creatinine > 3.0 × ULN ●Owing to limited experience with the use of this regimen, caution should be exercised in patients with severe renal failure
Severe neuropathy	● Peripheral neuropathy of grade 3 or grade 4

Regimen composition and dosage:

Drugs	**Dosage**
Bedaquiline	200mg once daily for 8 weeks followed by 100mg once daily (or) 400mg once daily for 2 weeks followed by 200mg 3 times per week
Pretomanid	200mg once daily
Linezolid	600mg once daily (can be reduced to 300mg in case of toxicity)
Moxifloxacin	400mg once daily

Duration: BPaLM regimen given for a duration of 6 months (26 weeks) and BPaL regimen given for 9 months (39 weeks) if sputum cultures are positive between months 4 and 6.

All medicines in the regimen are to be used throughout treatment duration, including a potential extension from 26 to 39 weeks (when BPaL is used). Ideally, missing doses of all three or four drugs in the regimen should be avoided; however, if doses are missed, any interruption of longer than 7 days should be made up by extending the treatment duration (for the number of missed doses); therefore, 26 or 39 weeks of prescribed doses should be completed within an overall period of 7 or 10 months, respectively

BPaLM follow up:

Follow-up assessments	Timeline
Clinical review, including weight and BMI, concomitant medication, adherence, igns/symptoms suggesting adverse events	Monthly
CBC (with Hb, platelets) and ECG	Day 15, 30, then monthly till month six, and more frequently if clinically indicated
Visual acuity, and color vision test	Week 09, 13, 26 and more frequently if clinically indicated
Smear microscopy	With culture at the C&DST lab
Culture	Monthly from month two onwards (i.e., at month 2, 3,4,5,6). If the culture results of month 4 or later are positive, collect one repeat specimen immediately and send it for culture to rapidly ascertain bacteriological conversion or reversion and if the repeat specimen is culture negative, then collect and send the subsequent monthly or end-of-treatment specimen.
DST	NAAT MTB/XDR or FL and SL LPA (Lfx, Mfx, Am, Eto) and LC DST (Mfx 1.0, Lzd, Z, Bdq, Pa*, Dlm*) if culture +ve at the end of month 4, end of Rx and as and when clinically indicated during treatment
Urine pregnancy test	As and when clinically indicated
Chest X-Ray and LFT#	At the end of month three, the end of treatment, as and when clinically indicated
S. Electrolytes (Na, K, Mg, Ca)	As and when clinically indicated in case of any QTcF prolongation
Specialist (Ophthalmic, Neurological) consultation	As and when clinically indicated

Surgical evaluation	After culture conversion
Long term follow-up	At 06, 12, 18, and 24 months after completion of treatment (Clinical, CXR, Smear and C&DST, if symptomatic) and whenever the patient returns to the health system

BDLLfxC Regimen (New as per WHO)

The 6-month bedaquiline, delamanid, linezolid, levofloxacin and clofazimine (BDLLfxC) regimen forMDR/RR-TB patients with or without fluoroquinolone resistance.

Recommendations:

The shorter all-oral bedaquiline-based regimen is indicated for patients with MDR/RR-TB, including pre-XDR-TB (fluoroquinolone-resistant), with less than one month of prior exposure to bedaquiline, linezolid, delamanid, or clofazimine (or longer exposure if resistance is excluded). It is suitable for pulmonary TB across all age groups, including children, adolescents, PLHIV, and pregnant or breastfeeding women. It may also be used in extrapulmonary TB except CNS, osteoarticular, or disseminated disease, and in children/adolescents with high likelihood of MDR/RR-TB without bacteriological confirmation. If fluoroquinolone resistance is unknown, start BDLLfxC and modify based on DST: continue BDLLfx if susceptible (drop clofazimine) or BDLC if resistant (drop levofloxacin).

Regimen composition and dosage :

It contains five drugs Bedaquiline(400mg given onve daily for 2 weeks & then 200mg 3 times weekly for 22 weeks or 200mg given as loading dose for first 2 months & then 100mg daily for next 4 months) ,Delamanid (given twice daily for 8 weeks & then once daily for 16 weeks), linezolid (600 mg), levofloxacin, and clofazimine (BDLLfxC) .

Duration:

Treatment duration is 6 months and may extended to 9 months if culture conversion is not sustained by week 16.

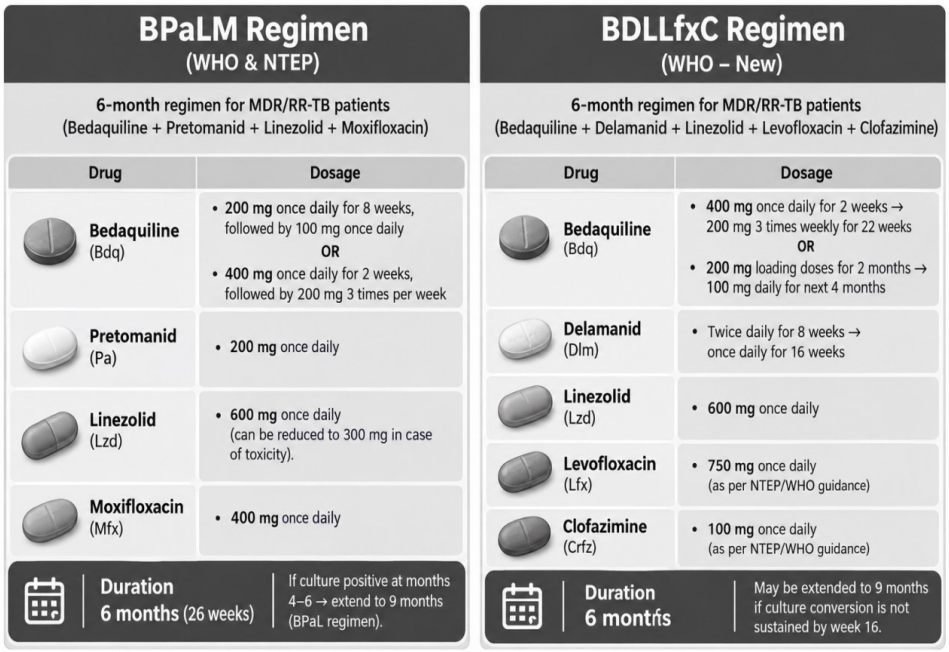


Fig 3: Summarized table of BPaLM and BDLLfxC Regimen

2. Treatment of drug-resistant TB using 9-month regimens

The 9-month all-oral regimen for MDR/RR-TB

It is recommended in patients with MDR/RR-TB and in whom resistance to fluoroquinolones has been excluded.The 9-month regimens can be used in patients not eligible for the shorter, 6-month regimens; also, they represent a preferred treatment option over the longer regimens

Eligibility Criteria :

i. RR detected/inferred

ii. MDR/RR-TB with FQ resistance not detected.

iii. No history of exposure to previous treatment with second-line medicines in the regimen (Bdq, Lfx, Cfz or Lzd as applicable) for more than one month (unless susceptibility to these medicines is confirmed)

iv. Non extensive Pulmonary TB

v. No severe forms of extra-pulmonary MDR TB like CNS TB , spinal/ skeletal TB (Miliary TB or TB with ultiorgan

involvement or disseminated TB).

vi. Lzd containing shorter oral MDR/RR-TB regimen can be given even in case of both KatG& InhA mutations are present. INHA-lower-level INH resistance that is linked to ETH cross-resistance, CATG- higher-level INH resistance

vii. Eto is contraindicated during the first 32 weeks of pregnancy due to teratogenic effects. Non-pregnant or pregnant women with < 24 weeks gestation and is willing for MTP, if Eto is considered in the regimen –LZD containing shorter oral MDR/RR-TB regimen can be given in pregnancy

Regimens composition:

The two variations of the 9-month all-oral MDR/RR-TB regimen are

Ethionamide variation:

Initial phase: 4–6 Bdq(6 m)-Lfx/Mfx-Cfz-Z-E-Hh-Eto

Continuation phase: 5 Lfx/Mfx-Cfz-Z-E

All seven drugs are administered for 4 months, extendable to 6 months if sputum remains positive at month 4. Ethionamide and high-dose isoniazid are discontinued after the initial phase. This is followed by 5 months of levofloxacin/moxifloxacin, clofazimine, ethambutol, and pyrazinamide. Bedaquiline is given for 6 months and may be extended to 9 months in cases of delayed sputum conversion.

Linezolid variation

Initial phase: 4–6 Bdq(6 m)-Lzd(2 m)-Lfx/Mfx-Cfz-Z-E-Hh

Continuation phase: 5 Lfx/Mfx-Cfz-Z-E

The linezolid variation consists of bedaquiline, linezolid, levofloxacin/moxifloxacin, clofazimine, ethambutol, high-dose isoniazid, and pyrazinamide. Linezolid is administered only for the first 2 months with close clinical and haematological monitoring for toxicity. The remaining drugs are continued for a total initial phase of 4 months, extendable to 6 months if sputum remains positive at month 4. High-dose isoniazid is discontinued after the initial phase. This is followed by 5 months of levofloxacin/moxifloxacin, clofazimine, ethambutol, and pyrazinamide. Bedaquiline is given for 6 months and may be extended to 9 months in case of delayed sputum conversion.

Regimen duration:

The regimen comprises an intensive phase of 4 months that may be extended to 6 months when no bacteriological conversion is seen at the end of the fourth month of treatment, and a continuation phase of 5 months; hence, if extended, the regimens may last 11 months.

The modified 9-month all-oral regimens for MDR/RR-TB (New as per WHO)

- ✓ WHO suggests using the 9-month all-oral regimens (BLMZ, BLLfxCZ and BDLLfxZ) over currently recommended longer (>18 months) regimens in patients with MDR/RR-TB and in whom resistance to fluoroquinolones has been excluded. Among these regimens, using BLMZ is suggested over using BLLfxCZ, and BLLfxCZ is suggested over BDLLfxZ.
- ✓ WHO suggests against using 9-month DCLLfxZ or DCMZ regimens compared with currently recommended longer (>18 months) regimens in patients with fluoroquinolone-susceptible MDR/RR-TB

Recommendations:

1. The recommended modified 9-month all-oral regimens comprise bedaquiline, linezolid and pyrazinamide in different combinations with levofloxacin/moxifloxacin, clofazimine and delamanid.

2. This recommendation applies to the following:

People with MDR/RR-TB in whom fluoroquinolone resistance has been excluded are eligible for this regimen. It is suitable for pulmonary TB across all age groups, including children, adolescents, PLHIV, and pregnant or breastfeeding women, and for most forms of extrapulmonary TB except CNS, osteoarticular, or disseminated disease. The regimen is recommended for patients with less than one month of prior exposure to its component drugs (excluding pyrazinamide and fluoroquinolones), and may still be used with longer exposure if drug resistance has been ruled out. It can also be considered in children and adolescents without bacteriological confirmation but with a high likelihood of MDR/RR-TB based on clinical features and contact history.

Regimen composition:

Regimen name	Three letter drug abbreviations	composition
BLMZ	9Bdq-Lzd-Mfx-Z	Bedaquiline,linezolid,moxifloxacin,pyrazinamide
BLLfxCZ	9Bdq-Lzd-Lfx-Cfz-Z	Bedaquiline,linezolid,levofloxacin,clofazimine, pyrazinamide
BDLLfxZ	9Bdq-Dlm-Lzd-Lfx-Z	Bedaquiline,delamanid,linezolid,levofloxacin, pyrazinamide

Regimen duration, extension & discontinuation :

Individual drugs in the modified 9-month regimen are administered for the entire duration, with all endTB trial regimens fixed at 9 months without extension. Pyrazinamide or linezolid may be discontinued in case of adverse events, with continuation of the remaining drugs; however, if more than one drug is stopped, the regimen should be abandoned and an alternative initiated. In cases of poor clinical or bacteriological response (e.g., persistent or reverting culture positivity at ≥4 months), evaluation for undiagnosed or acquired drug resistance is warranted

3. Treatment of drug-resistant TB using longer regimens

It is recommended in patients with pre-XDR TB or XDR TB and in those with MDR/RR-TB who are not responding or eligible for treatment.

Eligibility:

A longer regimen is expected to be used in the following situations:

• severe extrapulmonary TB;

• additional resistance to key medicines of the BPaLM/BPaL regimen (except moxifloxacin) or the 9-month all-oral regimen; lack of response to shorter treatment regimens (e.g. treatment failure due to no bacteriological conversion, no clinical response, emerging resistance or loss to follow-up);

• drug intolerance to the component medicines of the BPaLM/BPaL regimen (except moxifloxacin) or 9 months shorter all-oral treatment regimen; and

• pregnant and lactating women who could not benefit from the 9-month shorter all-oral regimen owing to certain clinical conditions or children

aged below 14 years who could not be treated with BpaLM/BpaL or who, for any reason, cannot opt for a 9-month regimen.

Grouping of medicines recommended for use in longer MDR-TB regimens

Group	Drugs
Group A Include all three medicines	Levofloxacin (Lfx) Moxifloxacin(Mfx) Bedaquiline (Bdq) Linezolid (Lzd)
Group B Add one or both medicines	Clofazimine (Cfz) Cycloserine (Cs) or terizidone (Trd)
Group C Add to complete the regimen and when medicines from Groups A and B cannot be used	Ethambutol (E) Delamanid (Dlm) Pyrazinamide (Z) Amikacin (Am) or Streptomycin (S) Ethionamide (Eto) or prothionamide (Pto) Q-aminosalicyclic acid (PAS) Imipenem-cilastatin (Ipm-Cln) or Meropenem (Mpm)

Regimens:

1. All three Group A agents (Lfx/Mfx,Bdq,Lzd) and atleast one Group B agents (Cfz or Cs)
2. If only One or two Group A agents are used,both Group B agents to be included
3. If the regimen cannot be composed with agents Group A and B alone,Group C agnets are added to complete it

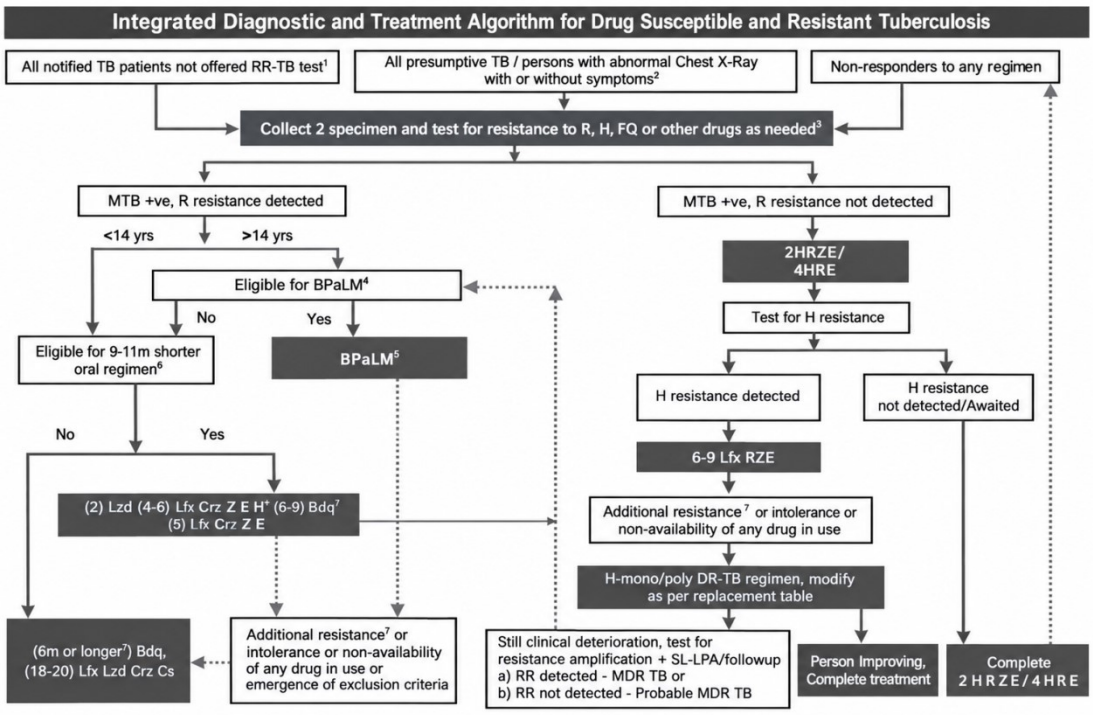
Duration:

The total duration of longer all-oral MDR-TB regimens is 18–20 months and does not include a defined intensive phase. The duration of individual drugs is guided by clinical indication, patient tolerability (e.g., continued linezolid use in the absence of significant toxicity), and treatment response (e.g., culture conversion). Overall treatment length may be individualized based on clinical condition and response.

4. Treatment of rifampicin-susceptible and isoniazidresistant TB (Hr-TB)

- In patients with confirmed rifampicin-susceptible, isoniazid-resistant tuberculosis , treatment with rifampicin, ethambutol, pyrazinamide and levofloxacin is recommended for a duration of 6 months.
- It is not recommended to add streptomycin or other injectable agents to the treatment regimen.
- It can be extended directly to 9 months in certain conditions.
  - In patients with extensive disease;
  - Uncontrolled comorbidity;
  - Extra- pulmonary TB

Fig 4: Integrated Diagnostic &Treatment Algorithm for Drug Susceptible & Resistant TB[(2)]



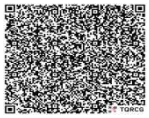
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Next Generation Vaccines and Immunotherapy

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Abstract

Emerging developments in next-generation vaccinations and immunotherapy have transformed preventative and therapeutic approachesof infections, cancer, and other immune-mediated diseases. This quick development in the field of biomedicine is a result of modern vaccine techniques that include mRNA and self-amplifying RNA, DNA vaccines, viral vectors, recombinant proteins, virus-like particles, and nanoparticle formulations, made possible due to advancements in molecular biology, genomics, nanotechnology, structural biology, and bioinformatics. Advantages offered by such vaccine types include their fast development, targeted antigens, strong immune response induction, and high protection rate. At the same time, immunotherapy, which includes immune checkpoint inhibitors, monoclonal antibodies, engineered T-cell therapy, and personalized cancer vaccines, uses the human immune system to detect and kill the diseased cells. Genomic profiling, antigen design computation, and artificial intelligence allow for the production of personalized immunotherapy. However, safety issues, adverse side effects, antigenic variation, difficulties in manufacturing process, costs, stability, regulations, and accessibility can be viewed as obstacles in this area. Further improvements in the field of immunotherapy and vaccinations will allow dealing with emerging infections faster and creating better cancer therapy techniques. When taken as a whole, these strategies mark a substantial shift from traditional, widely focused therapies to immune-based, programmable, and precision-oriented medicine.

Introduction

A significant change from traditional disease prevention to precise, individualized, and immune-based medicine is represented by the development of next-generation vaccines and immunotherapy. While more recent vaccination technologies can be created to produce particular humoral and

cellular immune responses and, in certain situations, can also be used therapeutically, traditional vaccines generally attempt to prevent infection by preparing the immune system to recognize a pathogen before to exposure. Vaccines can now be created more quickly and customized to target certain diseases, variations, or even individual patients thanks to developments in genomic sequencing, recombinant DNA technology, mRNA platforms, viral vectors, nanotechnology, and computational antigen prediction. Immunotherapy.

Vaccines can now be created more quickly and customized to target certain diseases, variations, or even individual patients thanks to developments in genomic sequencing, recombinant DNA technology, mRNA platforms, viral vectors, nanotechnology, and computational antigen prediction. By directly altering or utilizing immune responses through techniques including monoclonal antibodies, immune checkpoint inhibitors, adoptive cellular treatments, and therapeutic vaccinations, immunotherapy enhances these methods. With expanding applications in infectious illnesses, cancer, and other situations where immune system regulation might be therapeutically beneficial, these strategies work together to enhance the specificity, longevity, and efficacy of immune-mediated protection.

Next- generation vaccines

Definition

They are the vaccines which use advanced biological, structural, genetic or delivery technologies to overcome the limitations of the conventional vaccine platforms.

The main idea here is to formulate vaccines that can generate a targeted immune response, while improving antigen precision, manufacturing flexibility, stability, development speed and accessibility.

Need for Next- generation vaccines

The emergence of new infectious diseases warrants an urgent need for the development of new vaccine technologies, this was experienced with the SARS COV-2, where the development of flexible vaccine platforms proved its importance. Next challenge is the antigenic variation that happens in viruses like Influenza and Corona viruses which can in turn affect the performance or the effect of the existing vaccines. Consequently, researchers are investigating on vaccines that are capable of mounting an immune response against conserved regions or multiple variants of a pathogen. There are several

pathogens that are historically presented as major challenges for vaccine development like HIV, Tuberculosis, malaria because of their complexity, antigenic diversity, immune evasion, etc. Next generation vaccines seek to address these challenges. Moreover, there is a need for improved immune responses like neutralizing antibodies, T- cell responses, immune memory,etc.

Major types of Next- generation Vaccines

Recombinant protein and subunit vaccines

This type utilises proteins from a pathogen produced through recombinant DNA technology to stimulate an immune response.

Once the vaccine is released into the body, the protein is taken up by antigen presenting cell and is displayed on the surface as antigen. The immune helper cells then identify the antigen and trigger an immune response.

Examples: Hepatitis B vaccine (Engerix-B, Recombivax HB), Pertussis vaccine, Influenza vaccine, Novavax(Nuvaxovid) for COVID-19 and Shingrix for herpes zoster.

Virus- like particle vaccines

It uses virus- like particles that resemble viruses but lack genetic material, making them non- infectious. Their repetitive surface organization can promote strong B-cell recognition and antibody responses. VLPs can also be engineered to display selected antigens and potentially multiple antigenic targets.

Examples: Gardasil and Cervarix against Human papilloma virus

mRNA vaccines

Use of synthetic RNA to direct cells to produce a protein from a pathogen, which triggers an immune response.An mRNA vaccine contains messenger RNA encoding a selected antigen. After administration, the mRNA enters appropriate cells, where it serves as a temporary template for antigen production. The resulting antigen is processed and presented to the immune system, leading to activation of adaptive immune responses

Examples: Pfizer-BioNTech’s BNT162b2, Moderna’s mRNA-1273

These vaccines encode the spike protein of the SARS-CoV-2 virus, prompting the body to recognise and respond to the virus if exposed in the future.

DNA vaccines

Recombinant DNA, which functions as a vaccine and is injected into a person, is created by isolating the gene that codes for immunogenic protein.DNA vaccines introduce DNA encoding antigen into host cells, later, the DNA is transcribed into mRNA which is then subsequently translated into antigenic proteins.

DNA vaccines- can be administered by one of the following delivery method:

1.Nasal spray

2. Intramuscular injection

3.Intravenous injection

4.Intradermal injection

5.Gene gun or biolistic delivery

Examples: VRC01(HIV), PfSPZ vaccine(Malaria), VGX-3100(Cancer), M72/AS01E(Tuberculosis)

Recombinant vector vaccines

Uses a recombinant virus as a vector to deliver a gene encoding an antigen from the target pathogen into human cells. Once the viral vector enters the target cells, where it delivers the genetic material that leads to antigen expression, followed by antigen presentation and subsequent immune response.

Examples: Ebola Vaccine- Ervebo(rVSV-ZEBOV), COVID-19 Vaccine (VaxZevria/AstraZeneca)

Recombinant conjugate vaccines

These vaccines are formulated by combining a polysaccharide from a pathogen with a protein to enhance the immune response.Polysaccharide in conjugation to a protein improves effectiveness, particularly in young children.

Examples: Hemophilus influenzae b vaccine, Pneumococcal conjugate vaccine.

Recombinant attenuated vaccines

These vaccines are created by genetically modifying a pathogen to reduce its virulence while maintaining its ability to stimulate an immune

response. By removing or altering specific genes, the pathogen becomes less harmful but still prompts the body to build immunity against the disease.

Examples: Recombinant oral cholera vaccine (e.g., Vaxchora), Recombinant attenuated Salmonella vaccine, Modified Vaccinia Ankara (MVA)

Pros and Cons:

Vaccine type	Advantages	Disadvantages
Recombinant subunit protein	Safe; targeted immunity; scalable production	Complex and costly production; may require adjuvants or multiple doses
Virus-like particle (VLP)	Non-infectious; targeted and strong immune response; no reversion risk	May require boosters; variable immune response; storage requirements
Viral-vector	Strong cellular and humoral immunity; versatile; potential for single-dose use	Pre-existing vector immunity; complex manufacturing; safety and regulatory concerns
DNA	Highly specific and customizable; induces cellular and humoral immunity; no infection risk	Limited long-term data; possible immune tolerance; regulatory challenges
mRNA	Rapid development; highly adaptable; strong immune response; no genomic integration	Cold-chain requirements; delivery challenges; cost; limited long-term data
Conjugate	Enhanced immunogenicity; immune memory; effective in infants	Complex and costly production; pathogen-specific application; may require boosters
Recombinant attenuated	Strong and durable immunity; mucosal and systemic responses	Risk in immunocompromised individuals; reversion concern; cold-chain and manufacturing requirements

Future Prospects:

1.Advancements in mRNA technology

- Personalised vaccines:Creation of customized mRNA vaccinations based on each person's unique genetic profile.
- Multi-Pathogen vaccines: Combining multiple antigens in a single mRNA vaccine to target various diseases simultaneously.

2. Improved vaccine delivery systems

- Use of nanoparticles to enhance vaccine stability and targeted delivery.
- Needle free vaccination: Development of oral, nasal or microneedle based vaccines to improve ease of administration.

3. Enhanced adjuvant technologies

4. Universal vaccines (Broad spectrum vaccines)

5. Improving vaccine durability

6. Combating emerging infectious diseases

7.Expanding use in cancer immunotherapy

8. Global access and equity

Immunotherapy

Definition

"Therapeutic approach that involves the modulation of immune system that enhances or suppress immune responses for the prevention, control or treatment of diseases including infections, autoimmune conditions, allergies and cancers".

History of Immunotherapy

In the 19th century, Dr. William B. Coley- "Father of immunotherapy", designed an experiment, where he injected patients with mixtures of live and inactivated *Streptococcus pyogenes* and *Serratia marcescens*, leading to tumor regression in some cases, proving bacteria can be used to stimulate the immune system against some cancers. Later, in 1950s, the concept of "Immunosurveillance" was proposed, suggesting that the immune system naturally detects and eliminates cancer cells. A significant breakthrough was made in 1970s, which led to the discovery of Interleukin-2 (IL-2), which allowed scientists to culture and manipulate T- cells for therapeutic use. In the

1990s and 2000s a rise in the development and usage of monoclonal antibodies which target specific cancer cells were seen [for example: Rituximab (Anti-CD 20), Trastuzumab (HER-2 inhibitor), Bevacizumab (VEGF inhibitor)]. Immune checkpoint inhibitors like PD-1/PD-L1 inhibitors (eg: Pembrolizumab, Nivolumab, Atezolizumab, Avelumab) were developed. The most recent advances include: CAR-T cell therapy, cancer vaccines and oncolytic virus therapy.

Rationale of using immunotherapy:

The rationale for immunotherapy is based on theimmune system's capacity to identify, attack, and eradicate infections and harmful cells. The immune system is highly specific and can distinguish between healthy cells and diseased or foreign cells, such as cancer cells and virus-infected cells.

1. Precision targeting – Immunotherapeutic approaches can be designed to specifically target disease-causing cells, thereby minimizing damage to healthy tissues.
2. Memory and long-term protection – The immune system has the ability to remember previously encountered antigens and can mount a faster and more effective response upon subsequent exposure.
3. Broad range of applications – Immunotherapy can be applied in the management of various diseases, including cancer, infectious diseases, autoimmune diseases and allergies.

Thus, by harnessing the specificity and memory of the immune system, immunotherapy provides a targeted approach for the prevention, control, and treatment of disease.

Classification of Immunotherapy:

Immunotherapy can be categorized into Active and Passive immunotherapy. Active immunotherapy is where the body’s own immune system is stimulated to recognize and eliminate diseases, examples are: vaccines and immune checkpoint inhibitors. Passive immunotherapy is when there is administration of external in-vivo generated immune elements (like, antibodies and immune cells). These elements don’t stimulate host immune response like active immunotherapy. Examples are: Monoclonal antibodies (mAbs), adaptive cell transfer (ACT).

Principles of Immunotherapy:

Various approaches are followed recently in the immunotherapy, they are: Monoclonal antibodies, Adaptive cell transfer, immune checkpoint blockade, vaccination with tumor antigens, and other approaches for stimulating anti- tumor activity like: cytokine therapy, oncolytic viruses, and non- specific inflammatory stimuli.

1. Monoclonal Antibody therapy:

mAbs are identical antibodies produced by a single clone of B cells in the lab, they are designed to target a specific antigen, such as a protein found on the surface of cancer cells. The mechanism of action of mAbs can happen by 4 possible mechanisms: (1) mAb specific for cell surface tumor antigen- here the destruction of cancer cell can happen by Complement-mediated tumor killing, Fc receptor- mediated phagocytosis and tumor killing, and NK-mediated ADCC. (2) mAb specific for cell surface tumor antigen with conjugated drug, here the antibody delivers the chemotherapy drug to the cancer cell, which is then internalized by the cancer cell. (3) mAb specific for cell surface tumor signaling molecule- here the antibody blocks off the growth factor receptor function, thereby, inhibiting the further growth and progression of the cancer cell. (4) Bispecific T- cell engager (BITE) specific for cell surface tumor antigen and CD3- there is BITE mediated Cytotoxic T-lymphocyte (CTL) killing of the tumor cell.

Clinical applications:

Monoclonal antibodies have been widely employed in the treatment of various viral diseases like COVID- 19, Respiratory syncytial virus, HIV, Ebola virus, in bacterial infections of *Clostridium difficile, Bacillus anthracis, Staphylococcus aureus* (in trial), *Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* through various mechanisms like neutralization of bacterial toxins, blocking bacterial adhesion and invasion, opsonization and enhanced phagocytosis, complement activation and immune modulation. In fungal infections, the mAbs usually target fungal surface antigens, toxins or immune checkpoints to enhance host defense. In parasitic diseases, the use of mAbs is still under experimentation and the most common mechanisms studied here are neutralization of parasitic antigens or enhanced clearance.

	Organism	mAb used	Mechanism of action
Viral diseases	SARS COV-2	Casirivimab + Imdevimab	Targets SARS COV-2 spike protein Blocks interaction with ACE2 receptor
		Sotrovimab	Binds to the spike protein Prevents viral entry Immune mediated clearance
	Respiratory syncytial virus	Palivizumab	Targets RSV F(fusion) protein Prevents entry into respiratory epitheial cells
	HIV	Broadly Neutralising Antibodies(bNAbs) like VRC01	Targets gp120 or gp41 Broad neutralsiation across HIV strains
	Ebola virus	Inmazeb (Atoltivimab+ Maftivimab+ Odesivimab)	Targets the Ebola glycoprotein
Bacterial diseases	*Clostridium difficile*	Bezlotoxumab	Targets Toxin B produced by *C. difficle*
	Bacillus anthracis	Raxibacumab and Obiltoxaximab	Bind to protective antigen (PA) of anthrax toxin
	Staphylococcus aureus	Several mAbs in trial- Suvearoxumab, Clazakinumab, MEDI4893, AR-301, Pagibaximab	Target α- toxin, Protein A or surface adhesins
	Pseudomonas aeruginosa	Panobacumab, KB001-A, MEDI3902(under development)	Inhibit type III secretion system(T3SS) Neutralize exotoxins Disrupt biofilms
	Klebsiella pneumoniae	WRAIR mAb 17H12	Target capsular polysaccharides, LPS

		A1101+A1270 KB001(under research)	Prevent sepsis and promote phagocytosis
Fungal diseases	*Cryptococcus neoformans*	mAB 18B7	Targets *C. neoformans* capsule antigen(glucuronoxylomannan)
	Candida albicans	Mycograb(antibody fragment)	Targets Hsp90 Enhances activity of amphotericin B
		Efungumab (clinical trials halted due to safety concerns)	Anti- *Candida albicans* antibody

The mAbs against parasitic diseases are still at experimental stage. For *Typanosomacruzi,* mAbs against trans- sialidase proteins, for Plasmodium, mAbs targeting CSP or other surface proteins(e.g., mAb CIS43 for malaria prevention) and for *Leishmania*, Antibodies against gp63 and lipophosphoglycan (still preclinical).

1. Adoptive Cellular Therapy (ACT):

ACT refers to the introduction of immune cells with anti-tumor reactivity into a host with a tumor. After being extracted from a cancer patient's blood or solid tumor, immune cells are treated in vitro in a variety of methods to increase their quantity and improve their anticancer activity before being reinfused into the patient.

ACT can be done by 2 approaches:

1. ACT with Autologous Tumor- specific T cells
2. Chimeric Antigen Receptor (CAR) T cell therapy

➨ ACT with Autologous Tumor- specific T cells: T cells specific for tumor antigens can be harvested from a patient’s tumor tissue or blood, expanded and activated in vitro (for example, with anti- CD3, anti CD28, IL-2), and infused back into the patients, after which there is T- cell mediated killing of tumor cells.

➨ Chimeric Antigen Receptor (CAR) T cell therapy: Just like the above approach the T cells are harvested, inactivated, but additionally, a

lentiviral vector is used to introduce the gene encoding the CAR into the T cell, once integrated, the T cell now expresses Chimeric Antigen Receptor (CAR) on its surface. Now the CAR-T cells are transferred back into the patient and there will be CAR T cell- mediated killing of tumor cells.

Adoptive therapy using T cells expressing chimeric antigen receptors (CARs) has proved successful in some hematologic malignancies, and this approach is being developed for other tumors

Applications of ACT:

Infection	Disease / Pathogen	Immunotherapeutic Approach	Mechanism / Objective
Viral	HIV	CAR-T cells targeting gp120/gp41	Recognize HIV envelope proteins and target infected cells.
	Hepatitis B (HBV)	CAR-T cells targeting HBsAg/HBcAg	Direct T-cell activity against HBV targets.
	Viral infections	Virus-specific T cells (VSTs)	Recognize and kill virus-infected cells through MHC-restricted mechanisms.
Bacterial	Tuberculosis (TB)	T cells specific for ESAT-6 and Ag85B	Expanded ex vivo and reinfused to enhance host immunity.
	MRSA and Gram-positive infections	TCR-T/CAR-T cells targeting α-toxin; macrophage/neutrophil-based ACT	Target bacterial virulence factors and enhance bacterial clearance.
	Pseudomonas aeruginosa and Klebsiella pneumoniae	Engineered T cells, macrophages and γδ T cells	Recognize bacterial proteins or toxins and enhance antibacterial activity.
	Clostridioides difficile	Toxin B-specific T cells	Aim to prevent recurrence and reduce intestinal damage.
Fungal	Invasive aspergillosis	Aspergillus-specific T cells (Asp-STs)	Expanded and infused to enhance fungal clearance.
	Mucormycosis	Fungus-specific or	Improve fungal

		genetically modified T cells	recognition and clearance.
	Candida infections	Th1 CD4+ T cells targeting Candida cell-wall antigens	Enhance immune response when neutrophil or monocyte function is deficient.
	Candida and Aspergillus	Dectin-1 CAR-T cells	Target β-glucans on fungal cell walls; experimental approach.
Parasitic	Leishmaniasis	Leishmania-specific T cells targeting gp63/LACK	Promote Th1 immunity and parasite clearance.
	Trypanosomiasis (Chagas disease)	T cells targeting trans-sialidase	Reduce parasite load; CD8+ T-cell therapy is being studied experimentally.
	Malaria	Plasmodium-specific CD8+/CD4+ T cells; TCR/CAR-based T cells	Target liver-stage parasites and proteins such as CSP or AMA1.
	Toxoplasmosis	Toxoplasma-specific T cells	Used experimentally after transplantation to prevent or control reactivation.

3. Immune Checkpoint Blockade:

Immune checkpoints are regulatory molecules on T cells that help keep the immune system from attacking normal cells

Blocking T cell inhibitory molecules has become one of the most promising approaches for effectively boosting patients' immune responses against their tumors.

This system of blockade happens via 2 steps, they are:

a. Induction of anti- tumor immune response in lymph node:

Tumour antigens are presented by dendritic cells as tumour peptide–MHC complexes to the TCR on $CD8^+$ T cells. Co-stimulation occurs when B7

on the dendritic cell binds CD28 on the T cell, leading to T-cell activation. However, CTLA-4 on activated T cells binds B7 and provides an inhibitory signal, thereby limiting T-cell activation. Anti-CTLA-4 antibodies block the CTLA-4–B7 interaction, allowing CD28-mediated co-stimulation to predominate, resulting in enhanced T-cell priming and generation of tumour-specific CTLs.

b. CTL-mediated killing of tumour cells

The activated CTLs migrate to the tumour site, where the TCR recognizes tumour peptide–MHC-I on tumour cells. Tumour cells may express PD-L1, which binds to PD-1 on CTLs and inhibits their effector function. Anti-PD-1 or anti-PD-L1 antibodies block this inhibitory interaction, thereby restoring CTL activity. The activated CTLs then release cytotoxic molecules such as perforin and granzymes, resulting in tumour-cell killing.

Applications in various infections: mechanism

Infections	Mechanism
HIV	Checkpoint blockade → restores exhausted $CD8^+$ T cells → increases IFN-γ/TNF-α → enhances viral clearance.
HBV	Anti-PD-1 → reinvigorates exhausted HBV-specific $CD8^+$ T cells → restores cytotoxicity.
Tuberculosis	PD-1/CTLA-4 blockade → restores $CD4^+$/$CD8^+$ T-cell activity → improves bacterial clearance.
Invasive aspergillosis	Anti-PD-1 → restores exhausted T-cell function → enhances fungal clearance.
Candidiasis	PD-1 blockade → restores T-cell cytokine production and neutrophil function → improves fungal control.
Malaria	PD-1/LAG-3 blockade → improves T-cell activation → enhances parasite control.
Leishmaniasis	PD-1/CTLA-4 blockade → restores Th1 response and macrophage activation → reduces parasite burden.

3. Vaccination with Tumor Antigens:

Administering vaccines containing tumor antigens to individuals with tumors may lead to stronger immune reactions against the cancer.

From the patient's tumor, tumor DNA is obtained, which then undergoes Next- generation sequencing to identify tumor- specific mutations. Then by using computational algorithms mutated HLA- binding peptides are identified. In the patient T cells that are specific for putative neoantigens are identified by functional assays or by binding of MHC- peptide multimers. Once the neoantigen-specific T cell recognizes the neoantigen presented by HLA, it becomes activated and can expand and mediate tumour-cell killing, mainly through perforin–granzyme-mediated apoptosis

It has various applications in infectious diseases like Chronic Hepatitis B, HIV, Candida infections, Aspergillus, C. *neoformans, Blastomyces dermatitidis, Paracoccidiodesbrasiliensis, Plasmosidum falciparum, Leishamania*spp., Tuberculosis, *S. pneumoniae, C. diphtheriae, Clostridium tetani, Bordatella pertussis, Hemophilus influenzae type b, N, meningitidis, Salmonella typhi, V. cholerae , Bacillus anthracis,* although a lot of these are still under experimentation and trials.

3. Other approaches for stimulating anti- tumor immunity:

This includes Cytokine therapy, oncolytic viruses and nonspecific inflammatory stimuli.

a. Cytokine therapy:

Cytokines are small proteins that act like messengers in the immune system. They work by: Activating immune cells like dendritic cells (DCs) and T cells, promoting inflammation, thereby, fighting infections and cancer. Cancer patients can be treated with cytokines that stimulate the proliferation and differentiation of T lymphocytes and NK cells.

For example:

1. IL-2 in melanoma, renal cell carcinoma acts by activating T cells and NK cells
2. IFN- α in leukemia, lymphoma, Kaposi sarcoma acts by stopping tumor growth and boosts NK and CTL activity
3. TNF, INF-**γ** are still under trial in animals

4. GM- CSF, G- CSF is often used to stimulate bone marrow to make blood cells in patients recovering from chemotherapy or bone marrow transplant.

b. Oncolytic viruses:

These are lab-engineered viruses that only multiply in cancer cells, triggering their self-destruction. They trigger CTL responses focused on tumor antigens. The first oncolytic virus ever identified was talimogenelaherparepvec (T-VEC), approved for treating metastatic melanoma. TVEC is a herpes simplex virus that helps trigger cell lysis while reducing chronic nerve infection. It's delivered straight into solid tumors and helps jump-start the body's antitumor CTL response. Clinical trials are currently running to explore how TVEC works and what it does in the body.

c. Nonspecific inflammatory stimuli:

Immune responses to tumors may be stimulated by the local administration of inflammatory substances or by systemic treatment with agents that function as polyclonal activators of lymphocytes. Some examples are: Coley's toxin by William Coley, nonspecific immune stimulation by killed bacillus Calmette-Guérin (BCG) at the sites of tumor growth and intravesicular BCG is currently used to treat bladder cancer.

Pros and cons of Immunotherapy:

Immunotherapy has lots of advantages, one of it being high accuracy, specificity and targeting ability. It's very comprehensive and persistent in identifying and killing tumor cell, effectively preventing its recurrence and metastasis and most importantly the side effects are less when compared to the traditional treatment.

One of the biggest disadvantages here is that, using immune checkpoint inhibitors can sometimes cause the immune system to overreact, triggering autoimmune diseases—or even death. Some patients may experience vague toxic side effects after use, and in rare cases, the disease can worsen rapidly, and eventually cause death.The survival rate and prognosis of patients are uncertain even though the effect of the treatment is very good and the treatment costs are often high.

Conclusion

Next generation vaccines and immunotherapy are two parallel breakthroughs in modern medicine, merging novel vaccine technologies with selective manipulation of immune response. The technology has the potential to facilitate rapid vaccine development, targeted delivery of antigens, long-lasting immunity, and personalized treatment of infections and cancers. Future advances in molecular biology, genomics, immunology and biotechnology will certainly improve their efficiency. However, safety, sustainability, affordability, access, and proper regulation should be secured for effective application in practice.

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Advances in Diagnostic Microbiology

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Introduction

Diagnostic microbiology has undergone a remarkable transformation over the past few decades, evolving from conventional culture-based techniques to rapid, highly sensitive, and technology-driven methods. Traditionally, microbial identification relied on microscopy, staining, and culture, which, although reliable, were often time-consuming and limited in detecting fastidious or non-culturable organisms. The growing demand for early, accurate diagnosis—especially in the context of emerging infections, antimicrobial resistance, and global pandemics—has driven the development of innovative diagnostic platforms.

Recent advances have focused on improving speed, sensitivity, specificity, and automation in laboratory workflows. Automated systems such as advanced stool analyzers now enable rapid detection of ova, cysts, parasites, and occult blood with minimal human intervention, enhancing efficiency and standardization in routine parasitological diagnostics. Simultaneously, molecular techniques have revolutionized pathogen detection at the genetic level.

Among these, CRISPR-Cas–based diagnostics have emerged as powerful tools offering rapid, point-of-care detection with high precision by targeting specific nucleic acid sequences. Similarly, DNA sequencing technologies, including next-generation sequencing (NGS), allow comprehensive analysis of microbial genomes, enabling pathogen identification, outbreak tracking, and detection of antimicrobial resistance genes. Digital PCR (dPCR) further enhances nucleic acid quantification by providing absolute quantification with superior sensitivity, particularly useful in detecting low-level infections and monitoring viral loads.

In addition to molecular innovations, biosensor-based detection systems are gaining prominence due to their potential for real-time, rapid, and portable diagnostics. These systems integrate biological recognition elements with physicochemical detectors, enabling the detection of pathogens, toxins, and biomarkers with high specificity, even in resource-limited settings.

Recent updates in diagnostic microbiology also emphasise the integration of artificial intelligence, lab automation, and point-of-care technologies, which are reshaping the landscape of clinical laboratories. These advancements not only reduce turnaround time but also improve diagnostic accuracy and patient outcomes.

This chapter aims to provide a comprehensive overview of these emerging and advanced diagnostic modalities—including automated stool analysers, CRISPR-Cas systems, DNA sequencing, digital PCR, and biosensor-based detection—highlighting their principles, applications, advantages, and future potential in modern microbiological practice.

1.Automated analysis of Stool Specimen in Clinical laboratories

The MISPA F60 Fully Automated Fecal Analyzer represents a significant advancement in Parasitology, the automation of routine stool microscopy, particularly for the detection of ova and cysts in parasitology. It is designed to improve diagnostic efficiency, standardization, and reproducibility in clinical microbiology laboratories.

Principle and Technology

The MISPA F60 operates on the principle of automated digital microscopy combined with image recognition algorithms. Stool samples are processed using standardized preparation methods, followed by high-resolution image acquisition. The system utilizes artificial intelligence (AI)-assisted pattern recognition to identify parasitic elements such as helminth ova and protozoal cysts based on their morphology, size, and internal structures.

Microscopic Examination: Visible Formed Elements in feces such as cells, eggs, fat globules, are detected and identified**.**

Physical Examination : Physical parameters like color, character, etc. can be evaluated by automatic Image captured**.**

Fecal Occult Blood Detection: Automatic analysis of fecal occult blood and transferrin through colloidal gold method test parameters.

Virological Test: Test parameters, like rotavirus, adenovirus, coxsackie virus, etc. can be evaluated through Colloidal gold method**.**

Bacterlological Test: Colloidal gold method test parameters like helicobacter pylori can be evaluated automatically**.**

The equipment employs patented rail-type sampling method it automatically filters negative samples. Sample collection cup uses double-chamber design with double-filtering technique helps mixing and better filtering, specially designed for normal and watery specimen. Bullet-cartridge design and moisture-proof card collection device helps test maximum 200 testswithout manual operation. High-precision quartz -based counting chamber and imported pinch valveprevents pipeline blocking and cross contamination**.**

Sample Processing :

This analyzer employs a closed, automated workflow incorporates Saline and Iodine mount for better identification. A defined aliquot is loaded into a disposable slide or cartridge. The system scans multiple microscopic fields systematically. This reduces operator variability and ensures uniform sample examination, which is crucial for detecting low parasite loads.

Formed elements Detection:

The MISPA F60 is particularly useful in identifyingLeucocytes, erythrocyte, Pus cells, yeast cells, fat globules, macrophages, lucidum spores,Starch granules,Epithelial cells, Charcotleyden crystal, calcium oxalate crystal, blood, egg of clonorchis, eggs of *Ascaris lumbricoides* eggsof *Trichirus trichuris*, egg of tapeworm, egg of hookworm, egg of *Enterobius vermicularis*, *Strongyloides stercoralis*, egg of S*chistosoma* , egg of *Fasciolopsis buski*, *Trichomonas*, egg of *Paragonimus westermani, amoeba, cryptosporidium, Giardia lamblia*

The AI system flags suspected ova/cysts, which are then reviewed and validated by a microbiologist, maintaining diagnostic accuracy , reducing workload. It also allows archival of images, facilitating teaching, audit, and documents quality control.

Advantages

- Standardization: Eliminates inter-observer variation seen in manual microscopy.

- High throughput: Processes 200 test at the rate of 10 -60 samples per hour is ideal for busy laboratories.
- Enhanced sensitivity: Systematic scanning increases the probability of detecting rare ova/cysts.
- Reduced biohazard exposure: Minimal manual handling of infectious stool samples.



MISPA -F60



2. CRISPR -Cas System for Rapid detection of Infectious Diseases:

CRISPR-based detection methods represent a next-generation diagnostic tool in microbiology, combining molecular specificity with rapid and user-friendly detection systems. CRISPR-based detection methods have emerged as a powerful, rapid, and highly specific approach for identifying pathogens in clinical microbiology. Originally known for their role in genome editing, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and associated proteins (Cas enzymes) are now widely applied in molecular diagnostics, especially for detecting nucleic acids of bacteria, viruses, and parasites.

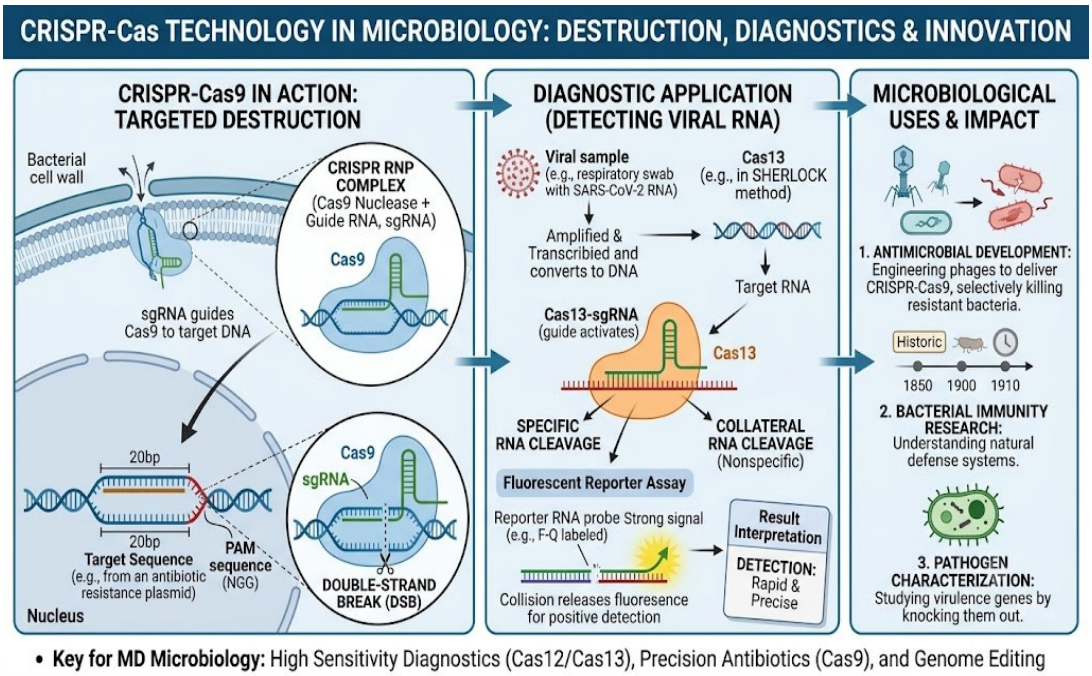
Principle of CRISPR-Based Detection (Li et al)

CRISPR diagnostic systems rely on the sequence-specific recognition of nucleic acids by a guide RNA (gRNA) that directs a Cas enzyme (such as Cas12 or Cas13) to a target DNA or RNA sequence. Once the target is recognized, certain Cas proteins exhibit collateral cleavage activity, meaning they nonspecifically cut nearby reporter molecules, thereby repair or edit the genome, generating a detectable signal either fluorescent or colorimetric.

Workflow of CRISPR Diagnostics:

- Sample collection and nucleic acid extraction.
- Target amplification.
- CRISPR-Cas reaction using specific guideRNA.
- DNA cleavage.
- Repair / Editing
- Signal detection via fluorescence or lateral flow assay.

This workflow enables detection even in low-copy-number infections.



Major CRISPR-Based Diagnostic Platforms

A. SHERLOCK (Specific High-sensitivity Enzymatic Reporter UnLOCKing)

- Uses Cas13 enzyme (targets RNA)
- Highly sensitive; can detect viral RNA (e.g., dengue, Zika, SARS-CoV-2)
- Suitable for RNA viruses

B. DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter)

- Uses Cas12 enzyme (targets DNA)
- Applied for DNA viruses and bacterial pathogens
- Can be adapted for rapid point-of-care testing

C. Other Systems

- Cas14 and newer CRISPR variants for ultra-sensitive detection.
- Multiplex CRISPR assays for detecting multiple pathogens simultaneously.

Applications in Microbiology

- Viral infections: SARS-CoV-2, HIV, HPV
- Bacterial infections: Tuberculosis (*Mycobacterium tuberculosis*), antimicrobial resistance genes (e.g., mecA, bla genes)
- Parasitology: Detection of malaria parasites and other protozoa.

Advantages

- High specificity due to sequence-guided targeting.
- Rapid turnaround time (within 1 hour).
- Point-of-care potential with minimal equipment.
- Cost-effective compared to conventional PCR in some settings.

Limitations

- Requires initial nucleic acid amplification in most protocols.
- Risk of contamination during amplification steps.
- Limited availability in routine laboratories.

- Requires optimization for each pathogen.

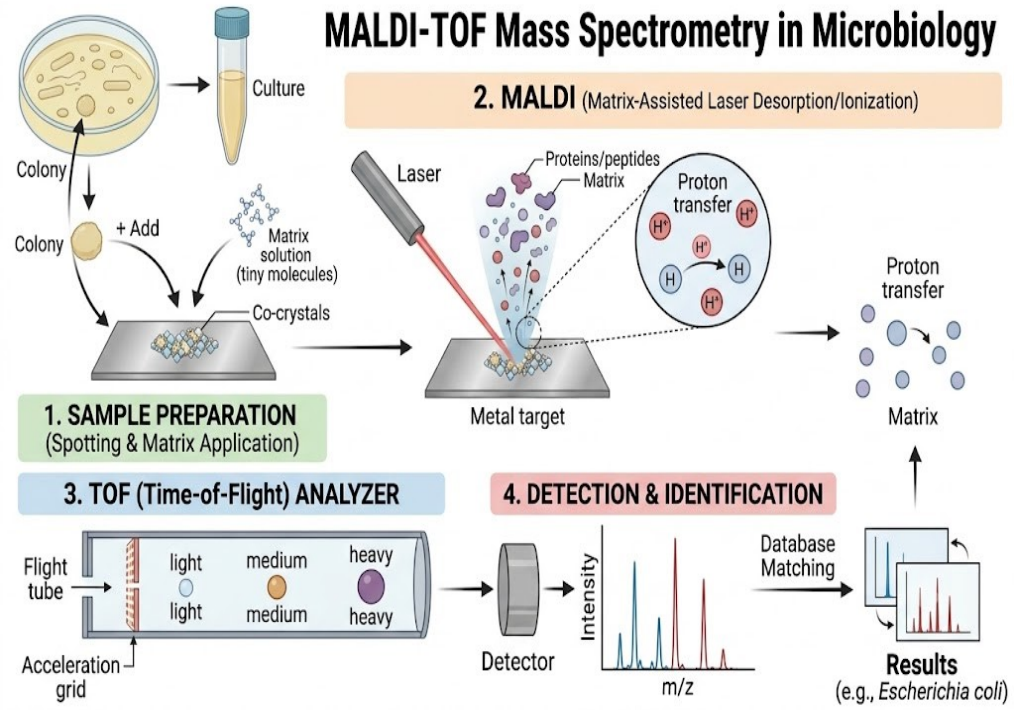
3.MALDI-TOFMS : Advances in MicrobialIdentification and Antimicrobial Susceptibility testing.

MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization–Time of Flight) is a rapid diagnostic technique used in microbiology to identify microorganisms.
It works by ionizing microbial proteins with a laser and measuring their mass-to-charge ratio.The generated spectral "fingerprint" is compared with a database for organism identification.It provides accurate results within minutes, significantly reducing turnaround time compared to conventionalmethods.Widely used in clinical labs for bacteria, fungi, and some mycobacteria identification.

MALDI Biotyper-Antibiotic Susceptibility Test Rapid Assay.(Florio et al., 2020; Goraya et al., 2026)

The MBT-ASTRA is a rapid method for detection of antibiotic-resistance based on a MALDI-TOF MS software tool that calculates and compares the area under the curves (AUCs) of spectra of bacteria either exposed or not to an antibiotic. The AUCs of the tested bacterial strain are compared to assess bacterial growth. If the microbial strain is susceptible, the AUC of the bacterial suspension with the antibiotic will be reduced compared to that without antibiotic, whereas with a resistant strain the AUCs with or without antibiotic will be comparable.

The MBT-ASTRA can be used for all classes of antibiotics and microbial species. Overall, this technology , MBT-ASTRA could be a promising approach for rapid AST of bacterias directly recovered from monomicrobial Blood Cultures.



4.Advanced PCR platforms in molecular diagnostics : Multiplx To Digital Pcr.

Multiplex PCR is a molecular biology technique that uses multiple sets of primers within a single reaction tube. This allows the simultaneous amplification and detection of several target DNA or RNA sequences at once, making it cost-effective and highly efficient for detecting multiple pathogens or gene targets from one sample.

Nested PCR involves two sequential rounds of amplification using two different sets of primers (outer and inner primers). The second set amplifies a specific sequence within the product generated by the first set, which significantly enhances the overall sensitivity and specificity of the detection while reducing non-specific binding.

BIOFIRE® Technology (BioFire FilmArray) is a fully automated diagnostic platform that combines sample extraction, purification, and nested multiplex PCR inside a sealed reagent pouch.

- It performs a broad, multiplexed first-stage PCR to amplify multiple potential targets simultaneously, followed by specific, individual second-stage nested PCR reactions.
- It uses high-resolution melting curve analysis to deliver comprehensive diagnostic results (identifying bacteria, viruses, fungi, or parasites) in about an hour with minimal hands-on time.

Digital PCR (dPCR)

Digital PCR is an advanced nucleic acid amplification technique that enables absolute quantification of DNA/RNA without the need for standard curves. It is widely used in clinical microbiology, oncology, and molecular diagnostics.

Principle

- Sample is partitioned into thousands–millions of tiny reactions (droplets or micro-wells).
- Each partition contains 0 or 1 (rarely more) target molecule.
- PCR amplification occurs in each partition independently.
- End-point detection: positive (fluorescent) vs negative partitions.
- Absolute copy number calculated using Poisson distribution.

Types of dPCR

- Droplet Digital PCR (ddPCR) – water-oil emulsion droplets.
- Chip-based dPCR – microfluidic partitions on a chip.

Workflow

The assay can be performed using the QX100 droplet digital PCR system (Bio-Rad Laboratories) according to the manufacturer's instructions. The reaction mixture was partitioned into droplets using an automated droplet generator (Bio-Rad). The generated droplet emulsion to be transferred to a 96-well PCR plate and subjected to amplification using a ProFlex PCR system (Thermo Fisher Scientific, Waltham, MA, USA) under the following cycling conditions, Initial denaturation: 95 °C for 10 minutes , 40 cycles of Denaturation: 94 °C for 30 seconds , Annealing/extension: 57 °C for 1 minute & Final enzyme deactivation: 98 °C for 10 minutes

Following amplification, droplets will be analyzed using the QX100 droplet reader (Bio-Rad). Data acquisition and analysis were performed using QuantaSoft software (Bio-Rad), which determines the number of positive droplets and calculates the absolute concentration of target DNA (copies/µL).

Limit of Detection (LOD) Determination(Goraya et al., 2026; Kitagawa et al., 2025)

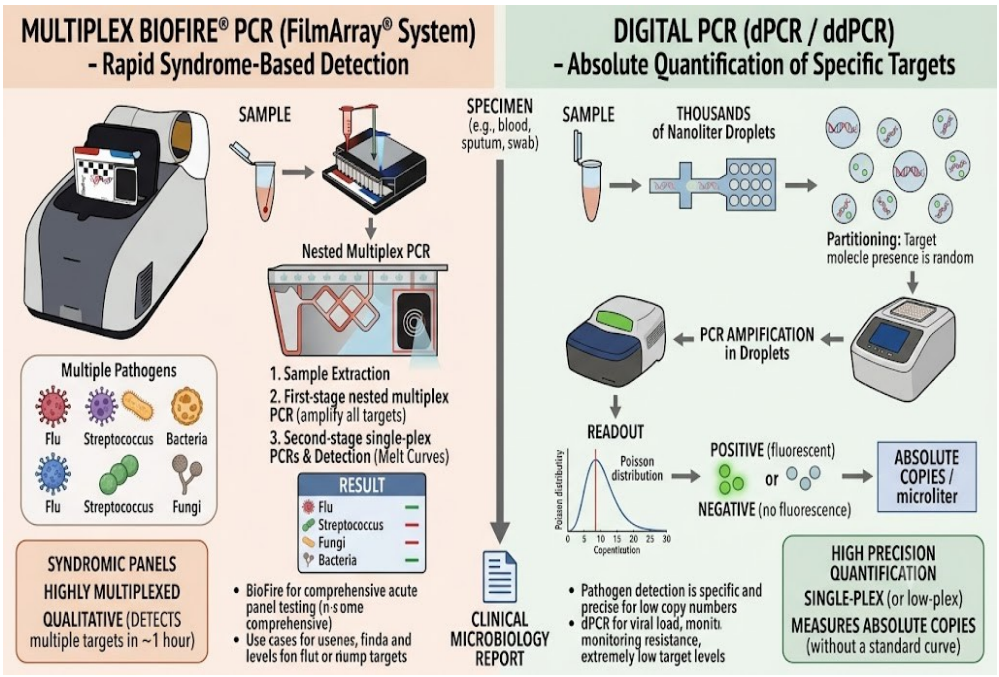
The concentration of target DNA in the samples will be expressed as copies/mL. The LOD was defined as the minimum detectable concentration corresponding to three positive droplets, which was calculated to be approximately 75 copies/mL of whole blood.

Advantages

- Absolute quantification (no standard curve)
- High sensitivity – detects low copy number targets
- High precision & reproducibility
- Less affected by PCR inhibitors
- Excellent for detecting rare mutations

Limitations

- Higher cost than conventional PCR
- Lower throughput compared to real-time PCR in some setups
- Requires specialized equipment
- Limited multiplexing (compared to qPCR)



Clinical Applications:

1. Infectious Diseases

- Quantification of viral load (e.g., HIV infection, Hepatitis B, COVID-19)
- Detection of low-level or latent infections
- Monitoring treatment response

2. Oncology

- Detection of minimal residual disease.
- Identification of rare mutations.

3. Antimicrobial Resistance

- Detection of resistance genes (e.g., carbapenemase genes)

5. Sequencing: Principles, Generations, and Applications

Sequencing refers to the process of determining the exact order of nucleotides in DNA or RNA. Over the past few decades, sequencing technologies have evolved significantly, leading to major advancements in molecular biology, genomics, and clinical diagnostics. Modern sequencing methods allow detailed analysis of genetic material, enabling insights into

genome structure, gene expression, and disease mechanisms.Evolution of Sequencing Technologies**(Goraya et al., 2026; Li et al., 2026; Satam et al., 2023)**

Sequencing technologies are broadly classified into three generations based on their methodology, throughput, and capabilities.

1. First-Generation Sequencing (Sanger Sequencing)

The earliest sequencing approaches involved chemical degradation or enzymatic cleavage of nucleic acids. The first successful sequencing of a nucleic acid (alanine tRNA) was achieved in 1964. Later, the development of the chain termination method by Frederick Sanger marked a major breakthrough.

- Principle: Uses dideoxynucleotides (ddNTPs) to terminate DNA chain elongation
- Read length: Up to a few hundred base pairs
- Advancement: Automation with fluorescent labeling and capillary electrophoresis (e.g., ABI 370)
- Significance: Highly accurate was considered the gold standard.

However, this method is low throughput and time-consuming, limiting its use in large-scale genomic studies.

2. Second-Generation Sequencing (Next-Generation Sequencing, NGS)

Second-generation sequencing revolutionized genomics by enabling massively parallel sequencing, allowing thousands to millions of DNA fragments to be sequenced simultaneously.

Key Platforms & Principles:

- Pyrosequencing (Roche 454): Detects pyrophosphate release
- Ion Torrent: Detects hydrogen ion release
- Illumina: Sequencing-by-synthesis using reversible dye terminators
- SOLiD: Sequencing by ligation

Advantages:

- High throughput
- Faster and cost-effective
- Suitable for whole-genome, exome, and transcriptome analysis.

NGS has enabled breakthroughs in genetic variation studies, diagnostics, research and personalized medicine**.**

3. Third-Generation Sequencing

Third-generation sequencing technologies overcome limitations of earlier methods by providing long-read and single-molecule sequencing.

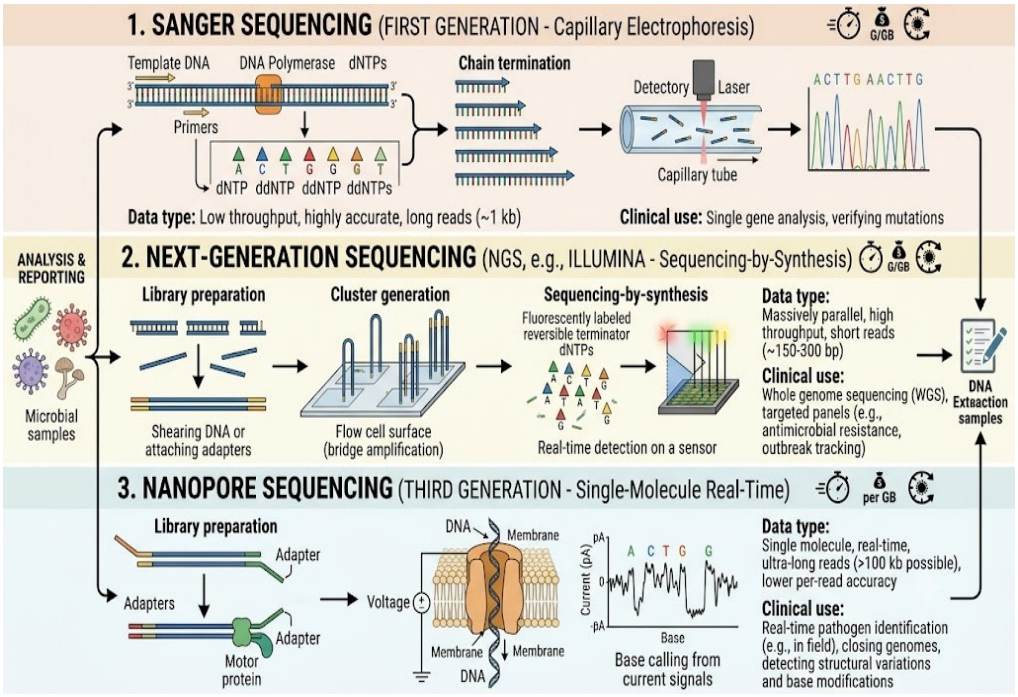
Examples:

- PacBio (SMRT sequencing): Real-time detection using fluorescent signals
- Oxford Nanopore: Measures electrical current changes as DNA passes through nanopores

Advantages:

- Long read lengths (up to tens kilobases)
- Real-time sequencing
- Detection of structural variations and epigenetic changes**.**

These technologies are particularly useful for studying complex genomes and structural variations.



Applications of Sequencing

Sequencing technologies have wide-ranging applications in biomedical sciences:

- Genomics: Whole-genome and exome sequencing to identify genetic variations..
- Transcriptomics: RNA sequencing to study gene expression.
- Epigenomics: Analysis of DNA methylation and chromatin modifications.
- Metagenomics: Identification of microbial communities without culture.
- Clinical diagnostics: Detection of genetic diseases, cancers, and infectious agents.

NGS, in particular, has transformed research by enabling simultaneous sequencing of millions of DNA fragments, providing comprehensive genomic insights.

6. CY -Tb an intradermal Skin Test for screening Tuberculosis.

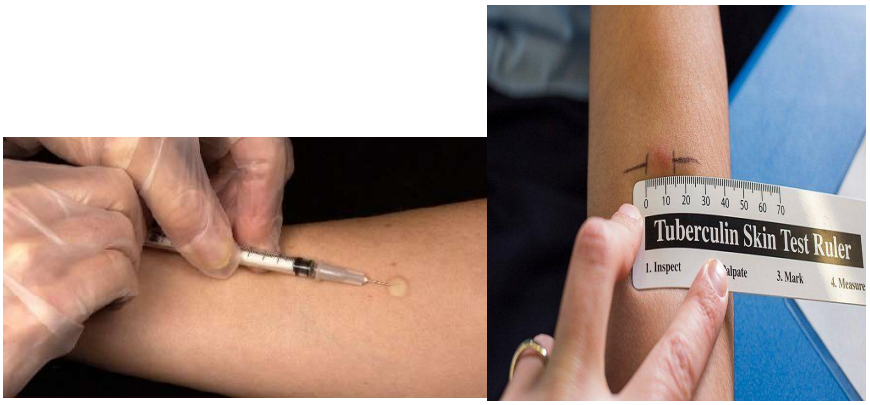
The C-Tb skin test is a newer intradermal diagnostic test for detecting infection with *Mycobacterium tuberculosis*. Unlike the traditional Mantoux (PPD), it uses specific TB antigens (ESAT-6 and CFP-10) to improve specificity.(Aditi Bhartiet al., 2025)

Principle

- Based on Type IV (delayed-type) hypersensitivity
- Uses recombinant antigens ESAT-6 & CFP-10
- These antigens are:

- Present in *M. tuberculosis*
- Absent in BCG strains and most NTM

- Sensitizes T-cells and produces localized induration at 48–72 hours



Procedure

- 0.1 mL intradermal injection on forearm (similar to Mantoux)
- Formation of a wheal confirms correct technique
- Interpret after 48–72 hours
- Measure induration in millimeters, not the erythema.

Interpretation

- ≥5 mm induration indicates positive Positive
- Indicates TB infection either latent or active.
- Same cut-off for most groups (simpler than Mantoux risk-based cutoffs)

Advantages over Mantoux (TST)

- Higher specificity not affected by BCG vaccination.
- Less cross-reactivity with non-tuberculous mycobacteria .
- Comparable sensitivity to Mantoux /IGRA
- Simple, field-friendly, no laboratory infrastructure needed like IGRA.

Limitations

- Cannot differentiate latent vs active TB
- False negatives may occur inImmunocompromised patients and early infection.
- Limited availability in some settings

7. Biosensors-based detection of Infectious Diseases:

Biosensor-based diagnostics represent a major advancement in clinical microbiology. They enable early detection of infectious diseases, rapid clinical decision-making, and improved patient outcomes. With ongoing technological improvements, biosensors are expected to become a cornerstone of modern diagnostic medicine, especially in point-of-care settings.Biosensors are analytical devices designed for rapid, accurate, and point-of-care (POC) diagnosis of diseases. They consist of three main components:

1. **Biorecognition element (enzyme, antibody, nucleic acid).**
2. **Transducer (converts biological interaction into signal).**
3. **Signal processor (interprets and displays results).**

They convert biological reactions into measurable signals such as electrical, optical, or mechanical outputs. Their growing importance lies in providing rapid, sensitive, and accessible diagnostics, especially in resource-limited settings .

Types of Biosensors in Diagnostics(Chen, n.d.)

1. Electrochemical Biosensors

These are widely used for infectious disease diagnosis due to their high sensitivity, low cost, and rapid response. It works with the principle of converting biochemical reactions into electrical signals such as current, voltage and impedance.

Key Features:

- Portable and suitable for POC testing
- Require small sample volume
- Can detect analytes quantitatively

These biosensors allow rapid detection of infections using clinical samples such as blood, serum, or swabs, improving early diagnosis and treatment decisions**.**

2. Optical Biosensors

These biosensors detect disease by measuring changes in light properties such as absorbance, fluorescence, or refractive index. It works with the principle that interaction between analyte and sensor surface causes optical changes, which are measured.

Advantages:

- High sensitivity and specificity
- Real-time detection
- Label-free detection possible

Optical biosensors provide rapid and precise diagnosis, though they may require advanced instrumentation**.**

3. Piezoelectric Biosensors

These sensors detect mass changes due to biomolecular interactions.

It works with the principle that the binding of analyte increases mass on sensor surface .This causes a change in vibration frequency, which is measured

Advantages:

- Label-free detection
- High sensitivity
- Real-time analysis

These biosensors are useful for rapid and highly sensitive detection of pathogens, especially in liquid biological samples.Role of Biosensors in Point-of-Care Diagnostics.Biosensors play a crucial role in POC testing, which allows diagnosis near the patient site.

Key benefits include:

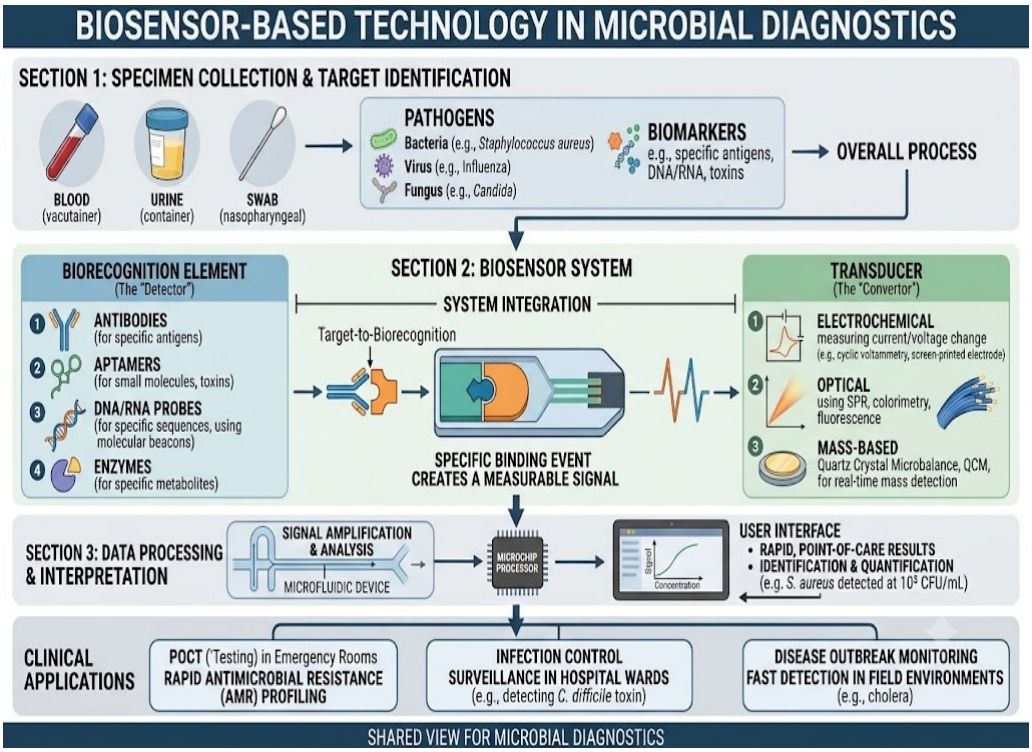
- Rapid results for clinical decision-making
- Minimal infrastructure requirements
- Ease of use
- Suitable for remote and low-resource settings

POC biosensors are designed to meet REASSURED criteria (Real-time connectivity, Ease of use, Affordable, Sensitive, Specific, User-friendly, Rapid, Equipment-free, Deliverable).

Advantages of Biosensor-Based Diagnostics(Parihar et al., 2025)

- Rapid detection of infectious agents
- High sensitivity and specificity
- Minimal sample preparation

- Potential for miniaturization and portability
- Real-time monitoring



8. Artificial Intelligence and Machine Learning in Diagnostic Microbiology:

Artificial Intelligence (AI) and Machine Learning (ML) are transforming diagnostic microbiology by enabling **rapid, accurate, and automated detection of pathogens**. These technologies analyze large datasets, recognize patterns, and support clinical decision-making, thereby improving laboratory efficiency and patient outcomes.

Basic Concepts

- **Artificial Intelligence (AI):** Simulation of human intelligence by machines to perform tasks such as decision-making and pattern recognition.
- **Machine Learning (ML):** A subset of AI where algorithms learn from data and improve performance without explicit programming.

- **Deep Learning (DL):** Advanced ML using neural networks for complex image and signal analysis.

Applications in Diagnostic Microbiology(Gurajala, 2024)

1. Automated Microbial Identification

AI-based systems can rapidly identify microorganisms using:

- Colony morphology (digital plate reading)
- Microscopy images (Gram stain, AFB smear)
- MALDI-TOF spectral data

These tools reduce human error and improve turnaround time.

2. Interpretation of Laboratory Tests(Khangarot et al., 2026)

ML algorithms assist in interpreting:

- Culture results
- Antimicrobial susceptibility testing (AST)
- Serological and molecular assays

They help in detecting subtle patterns that may be missed by conventional analysis.

3. Antimicrobial Resistance Prediction

AI models analyze genomic and phenotypic data to:

- Predict resistance genes
- Forecast antibiotic susceptibility
- Support antimicrobial stewardship programs

This helps clinicians choose appropriate therapy early.

4. Molecular Diagnostics and Genomics

AI enhances:

- PCR and real-time PCR data interpretation.
- Next-generation sequencing (NGS) analysis.
- Detection of mutations and outbreak strains.

It enables **rapid pathogen detection and genomic surveillance**.

5. Digital Pathology and Image Analysis

Deep learning algorithms are used for:

- Automated reading of microscopy slides
- Detection of parasites, bacteria, and fungi
- Quantification of microbial load

Example: Detection of malaria parasites or TB bacilli from digital images.

6. Biosensors and Point-of-Care Testing

AI integration with biosensors improves:

- Signal interpretation
- Real-time diagnostics
- Accuracy of point-of-care devices

This is especially useful in **resource-limited settings**.

7. Outbreak Detection and Epidemiology

AI helps in:

- Tracking infectious disease outbreaks
- Predicting disease spread
- Analyzing surveillance data

This supports public health interventions and infection control.

Advantages

- Rapid and automated diagnostics
- High accuracy and reproducibility
- Ability to process large datasets
- Early detection of outbreaks and resistance patterns
- Reduced workload for laboratory personnel

Limitations

- Requirement of large, high-quality datasets
- High initial cost and infrastructure needs

- Lack of standardization
- Ethical and data privacy concerns

9. Recent WHO and CDC recommendations for specimen collection and Microbiological investigations :

a.Near point-of-care tests, tongue swabs, and sputum pooling for TB.

- The new guidelines on TB diagnosis recommend:
- A new class of near-point-of-care nucleic acid amplification tests (NPOC-NAATs) for the initial detection of TB without rifampicin resistance at peripheral levels of the health system (i.e., peripheral laboratories, primary healthcare centers and communities) and at lower unit costs than other molecular test and instrument types.
- Tongue swabs as new, readily available and easy-to-collect specimens for use with NPOC-NAATs and low-complexity automated NAATs (LC-aNAATs) for the initial detection of TB with and without rifampicin resistance among adults and adolescents that are unable to produce sputum; and
- Pooling of sputa as a diagnostic strategy for the initial detection of TB and rifampicin resistance using LC-aNAATs with the potential to improve turnaround times and costs when resources are constrained.

Principles of pulmonary TB detection on pooled sputum:

- In practice, testing pooled sputum for TB is a two-step process. For LC-aNAAT testing, up to four sputum samples that are identified for pooling are brought together, labeled with a unique identifier for their pool, and individually treated with sample processing reagents according to manufacturer instructions. A portion of each liquefied sputum sample is then added, one at a time to the pool, and the pooled sample is tested. All the individual and pooled sputum samples are stored under manufacturer-recommended conditions until test results are available.
- If a pooled sample tests negative, then no further testing is performed as it is presumed that all of the individual samples that contributed to the pool are negative for TB detection. If a pooled sample tests positive, then the individual samples that contributed to the pool must be re-accessed and tested individually to identify which of them is positive. One or more of the individual samples in the pool may be positive for TB.

Recommendation

- When resource constraints do not allow for the testing of individual samples: In adults and adolescents with signs and symptoms for pulmonary TB or who screen positive for pulmonary TB, LC-aNAATs on (up to 4) pooled sputa may be used as the initial diagnostic strategy for diagnosing TB rather than LC-aNAATs on individual samples (conditional recommendation, high certainty of evidence).

b. WHO updates policy for the use of lateral flow urine lipoarabinomannan assay (LF-LAM) for diagnosing active tuberculosis in people living with HIV

Tests based on the detection of mycobacterial LAM antigen in urine have emerged as potential point-of-care tests for TB. The WHO Global TB Programme recommended the use of LF-LAM. Urinary LAM assays have shown greater sensitivity (when used for the diagnosis of TB in people living with HIV. The sensitivity increases significantly in patients with lower CD4 cell counts. Following the new evidence, WHO is now recommending LF-LAM use to assist in diagnosis in a broader group of people, both in inpatient and outpatient settings.

c.WHO prequalifies the first triple diagnostic test for HIV, hepatitis B and syphilis, a milestone toward global disease elimination goals

Designed for use by trained health-care providers at the point of care, the *Determine™ Antenatal Care Panel* is currently intended specifically for pregnant women aged 12 years and older. It enables simultaneous testing for HIV-1/2 antibodies and HIV-1 p24 antigen (Determine™ HIV Early Detect), hepatitis B surface antigen (Determine™ HBsAg 2), and syphilis antibodies to Treponema pallidum (Determine™ Syphilis TP). Each test is qualitative, visually read, and uses capillary whole blood from a finger-prick – providing a practical, efficient tool to aid in the diagnosis of HIV, HBV, and syphilis during pregnancy.

d.The Centers for Disease Control and Prevention (CDC)recommends this three-test panel for universal adult screening:

- The hepatitis B triple panel blood test checks for three specific viral markers to screen for, diagnose, or determine immunity to the hepatitis B virus.
- HBsAg (Hepatitis B Surface Antigen): Detects proteins on the surface of the virus; a positive result indicates an active acute or chronic infection.
- Anti-HBs (Hepatitis B Surface Antibody): Measures antibodies produced after a past infection or successful vaccination; a positive result indicates immunity.
- Total Anti-HBc (Total Antibody to Hepatitis B Core Antigen): Detects antibodies related to the core of the virus; a positive result shows past or ongoing exposure to the virus.

Why and When It Is Done:

- Universal Screening: The CDC recommends that all adults aged 18 and older get screened with a triple panel at least once in their lifetime.
- Pregnancy: Routine screening is advised during each pregnancy (preferably in the first trimester) to protect the newborn.
- Risk Factors: Recommended for people with higher exposure risks or specific symptoms like jaundice, dark urine, or fatigue.

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Current Advances in Medical Virology: Emerging Viral Infections, Vaccines, and Antiviral Therapies

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Introduction

Viral disease continues to reshape global public health through the interplay of zoonotic spillover, ecological disruption, and viral evolution. This chapter surveys three converging fronts in contemporary medical virology:

1. the emergence and re-emergence of viral pathogens of pandemic and epidemic concern,
2. the diversification of vaccine platforms beyond conventional inactivated and live-attenuated designs, and
3. the expansion of antiviral pharmacotherapy from single-target small molecules toward monoclonal antibodies, long-acting agents, and virus-based therapeutics.

Particular attention is given to recent developments in avian and zoonotic influenza, mpox clade diversification, filoviral and henipaviral outbreaks, next-generation ribonucleic acid (RNA) vaccine engineering, and monoclonal antibody prophylaxis against respiratory viruses. The chapter closes with a discussion of persistent gaps in diagnostics, surveillance, and equitable access that continue to constrain the translation of scientific advances into global health security.

Human populations remain in continuous ecological and evolutionary contact with a vast reservoir of viral pathogens circulating in wildlife, livestock, and vector species. Land-use change, intensified animal husbandry, climate-driven shifts in vector range, and dense global travel networks have shortened the interval between spillover events and their detection in human populations. The COVID-19 pandemic demonstrated both the catastrophic potential of a novel respiratory virus and the capacity of modern virology to respond with unprecedented speed, most visibly through the rapid clinical deployment of messenger RNA (mRNA) vaccine platforms (Baden et al., 2021; Polack et al., 2020).

In the years since, medical virology has moved along three parallel and mutually reinforcing tracks. First, global surveillance networks have identified a widening set of viruses with epidemic or pandemic potential, including highly pathogenic avian influenza strains that have crossed into mammalian hosts, recombinant mpox virus lineages, and recurrent outbreaks of filoviruses and henipaviruses. Second, the vaccine platforms proven during the pandemic are now being generalized to pathogens long considered intractable, including human immunodeficiency virus (HIV), while delivery science is addressing the thermostability and access barriers that limited earlier RNA vaccines. Third, antiviral pharmacology has diversified beyond small-molecule inhibitors to include long-acting monoclonal antibodies, oral post-exposure prophylactic agents, and, in oncology, genetically engineered oncolytic viruses that exploit tumor-selective replication. This chapter reviews these developments and situates them within the broader challenges of diagnostics, equitable distribution, and pandemic preparedness.

Did you know? *The gap between identifying a novel pathogen's genetic sequence and testing a matching vaccine candidate in humans has collapsed from years to weeks. The original mRNA-1273 COVID-19 vaccine candidate entered a phase 1 clinical trial only 63 days after the SARS-CoV-2 genome was first published, a timeline that helped inspire the global "100 Days Mission" for future pandemic response.*

Emerging and Re-emerging Viral Infections

Most viral pathogens of epidemic concern do not arise de novo; they cross into human populations along a recognizable ecological pathway from an animal reservoir, sometimes through an intermediate amplifying host, before acquiring the capacity for sustained human-to-human transmission (Figure 1). Understanding where a given pathogen currently sits along this pathway is central to risk assessment and to prioritizing surveillance and countermeasure investment.

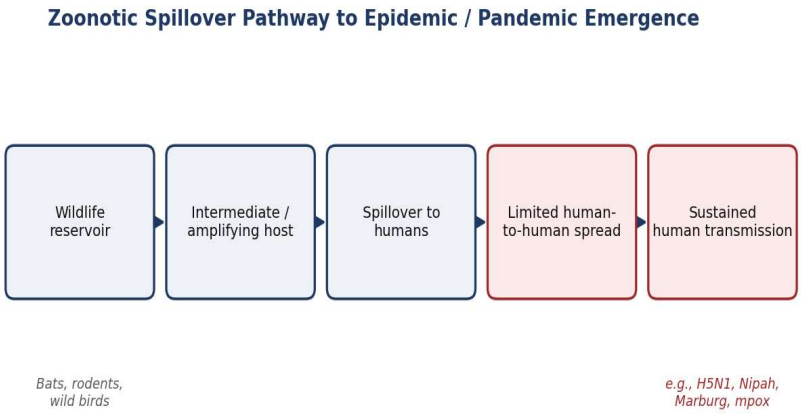


Figure 1. Generalized zoonotic spillover pathway from an animal reservoir to sustained human transmission.

Zoonotic and Avian Influenza

Highly pathogenic avian influenza A(H5N1) has become geographically and taxonomically more widespread than at any point in its documented history, sustaining outbreaks in wild bird populations, poultry, and, since 2024, dairy cattle herds in the United States. Serological and epidemiological data indicate that cattle-to-human transmission has occurred on multiple occasions among agricultural workers, and public health agencies continue close surveillance for any genetic change that would permit sustained human-to-human transmission, which remains the critical, and so far unmet, threshold for pandemic emergence (CDC, 2026; The Conversation, 2026). Beyond H5N1, sporadic human infections with other avian influenza subtypes, including A(H10N3) and A(H5N5), have been reported in 2025 and 2026, generally linked to direct poultry exposure and without documented onward human transmission (ECDC, 2026).

Current seasonal influenza vaccines are not expected to provide meaningful cross-protection against H5 subtypes, and candidate H5-specific vaccines remain in earlier stages of development, underscoring a persistent gap between surveillance capability and deployable countermeasures. This gap echoes earlier pandemic influenza episodes, where genomic and phylogeographic surveillance proved decisive in tracking spread and informing containment strategy (Ferguson et al., 2005; Rambaut et al., 2008). Ferret and human household transmission studies from earlier H5N1 waves remain

instructive: the virus binds preferentially to avian-type receptors deep in the human lower respiratory tract rather than the upper airway receptors favoured by seasonal influenza, a structural detail that helps explain why human cases have historically been severe but transmission chains short.

Did you know? *Since the World Health Organization began tallying laboratory-confirmed human H5N1 cases in 2003, roughly half of all reported cases have been fatal, one of the highest case fatality proportions recorded for any influenza virus, though the true denominator of mild or unrecognized infections is almost certainly larger than official counts capture.*

Mpox: Clade Diversification and Recombination

Mpox virus (MPXV) transmission has continued to evolve since the multi-country clade IIb outbreak of 2022. Clade Ib MPXV, associated with more severe disease and sustained community transmission in parts of Central and East Africa, was declared a broader global concern by the World Health Organization in late 2025, and by early 2026 recombinant viruses carrying genomic elements of both clade Ib and clade IIb had been identified, raising questions about the phenotypic consequences of inter-clade recombination for transmissibility and clinical severity (WHO, 2026a, 2026b). Case counts in parts of Europe have continued at low but persistent levels through early 2026, indicating that mpox has transitioned from an acute outbreak to an endemic, if geographically uneven, feature of the global infectious disease landscape (ECDC, 2026).

Unlike the sexual-network-dominated transmission that characterized much of the 2022 clade IIb outbreak in non-endemic countries, clade Ib transmission in the Democratic Republic of the Congo and neighbouring states has involved substantial household and close-contact spread, including among children, complicating case definitions and contact-tracing strategies built around earlier epidemiological assumptions. Diagnostic polymerase chain reaction assays validated for clade IIb have required re-evaluation to ensure equivalent sensitivity for clade Ib and recombinant lineages, illustrating how viral evolution can outpace previously validated laboratory tools.

Filoviruses: Marburg and Ebola

Filoviral haemorrhagic fevers illustrate the recurring, geographically shifting nature of high-consequence viral outbreaks. Ethiopia reported its first-ever Marburg virus disease outbreak in November 2025, with fourteen laboratory-confirmed cases and a case fatality proportion exceeding sixty percent before the outbreak was declared over in January 2026 following a period without new cases (CDC, 2026; Partners In Health, 2026). A separate Marburg outbreak was subsequently reported in Equatorial Guinea. Neighbouring the Marburg outbreaks, the Democratic Republic of the Congo continued to report intermittent Ebola virus disease activity through late 2025 (WHO, 2026a). These events reinforce that filoviruses, though individually rare, now appear with sufficient regularity to warrant standing outbreak response, rather than reactive outbreak response capacity in at-risk regions.

No filovirus-specific antiviral is licensed for Marburg virus disease, in contrast to Ebola virus disease, for which two monoclonal antibody products received regulatory approval following the 2018–2020 outbreak in the Democratic Republic of the Congo. This asymmetry in countermeasure availability across closely related filoviruses is a recurring theme in outbreak response: therapeutic development tends to follow the largest or most recent outbreak of a given species, leaving related but distinct viruses within the same family comparatively less prepared for.

Did you know? *Marburg virus disease case fatality rates have ranged from roughly 24 percent to as high as 88 percent across different outbreaks, a spread that reflects differences in viral strain, healthcare access, and how early in the epidemic curve supportive care, particularly fluid and electrolyte management, begins.*

Case in point: Ethiopia, 2025 *Ethiopia's first-ever Marburg outbreak began in the Jimma zone in November 2025. Within roughly ten weeks, health authorities had traced contacts, confirmed the causative agent, and declared the outbreak over without it spreading beyond the initial cluster, an outcome credited to prior Ebola-response infrastructure and community engagement built during earlier regional filovirus scares. The episode illustrates both how quickly a filovirus outbreak can be contained with prepared systems and how narrow the margin for error remains when no licensed vaccine or antiviral exists as a backstop.*

Henipaviruses: Nipah Virus

Nipah virus continues to cause near-annual outbreaks in South Asia, with cases reported in India and Bangladesh across 2025 and into early 2026 (WHO, 2026a). Transmission occurs through consumption of contaminated date palm sap, contact with infected bats or pigs, and, less commonly, human-to-human spread in healthcare settings. The high case fatality rate of Nipah virus infection, combined with the absence of a licensed vaccine or specific antiviral therapy, has made it a priority pathogen for vaccine platform testing, including candidate approaches derived from viral vector and mRNA technologies developed originally for other pathogens. Bangladesh's recurring outbreaks follow a distinctive seasonal pattern tied to the date palm sap harvesting season, when raw sap collection pots left overnight can be contaminated by foraging Pteropus fruit bats, a transmission route that has made simple sap-collection covers an unusually low-cost, high-yield public health intervention.

Arboviral and Coronavirus Surveillance

Chikungunya virus disease activity was reported across multiple countries through 2025, consistent with the broader expansion of Aedes-borne arboviruses into regions with newly suitable climatic conditions. Middle East respiratory syndrome coronavirus (MERS-CoV), first genomically characterized in 2012 (van Boheemen et al., 2012), continues to cause sporadic zoonotic infections linked to dromedary camel exposure, with global case updates issued as recently as December 2025 (WHO, 2026a). Continued genomic surveillance of SARS-CoV-2 remains a standing priority, with periodic variant updates tracking immune and antibody escape mutations that inform vaccine strain composition (Chakraborty et al., 2022). The same surveillance imperative applies to mosquito-borne viruses: genomic epidemiology previously reconstructed the introduction and spread of Zika virus across the Americas (Kindhauser et al., 2016), a template now applied to tracking chikungunya and dengue expansion.

Collectively, these ongoing threats illustrate that pandemic preparedness cannot be organized around any single pathogen; expert assessments for 2026 consistently list coronaviruses, filoviruses, and mosquito-borne viruses alongside influenza as priorities where diagnostic and therapeutic development continues to lag behind vaccine research (Gavi, 2026b).

One Health and Environmental Drivers of Emergence

The recurring pattern across avian influenza, mpox, filoviruses, henipaviruses, and arboviruses is that emergence is rarely a purely virological event; it is an ecological one. Deforestation and agricultural expansion increase contact frequency between wildlife reservoirs and humans or livestock; climate change is shifting the geographic range of mosquito and bat vector species poleward and to higher elevations; and intensified, high-density animal husbandry, as seen in the dairy cattle H5N1 situation, creates conditions favourable for viral amplification and adaptation. The One Health framework, which integrates human, animal, and environmental health surveillance under a single coordinated structure, has consequently moved from an academic concept to an operational priority for national and international health agencies (Figure 2).

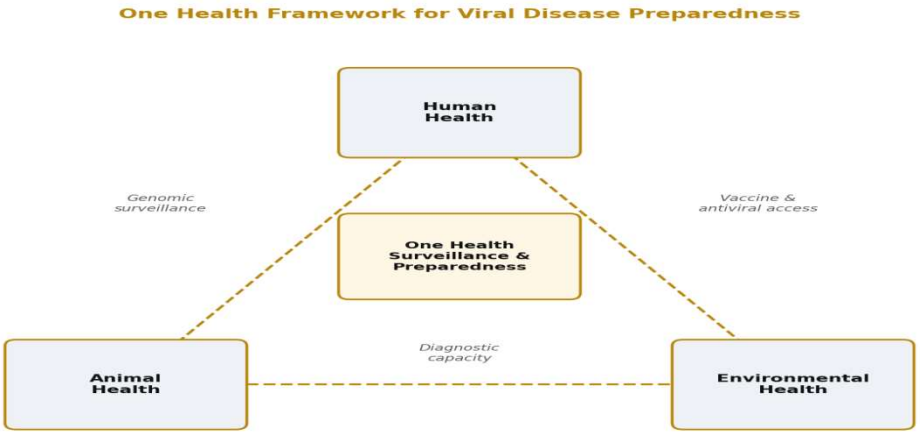


Figure 2. The One Health triad linking human, animal, and environmental health surveillance.

Table 1 draws together the pathogens discussed in this section for quick comparison. Case fatality figures are approximate and drawn from the World Health Organization and Centres for Disease Control and Prevention situation reports cited throughout this chapter; true rates vary by outbreak, care setting, and how systematically mild or subclinical infections are captured.

Virus	Reservoir	Transmission Route	Approx. CFR	Licensed Vaccine	Licensed Antiviral
H5N1 avian influenza	Wild birds, poultry, dairy cattle	Direct animal contact; no sustained human spread	~50%*	None widely deployed	Oseltamivir (variable activity)
Mpox (clade Ib)	Rodents (presumed), NHPs	Close/household contact; some sexual	1–10%	MVA-BN (yes)	Tecovirimat (limited data)
Marburg virus	Egyptian fruit bat	Bat exposure; human-to-human via fluids	24–88%	None licensed	None licensed
Nipah virus	Pteropus fruit bats	Contaminated sap, pigs; rare human spread	40–75%	None licensed	None licensed
MERS-CoV	Dromedary camels	Camel contact; limited human spread	~35%	None licensed	None specific
SARS-CoV-2	Bats (proposed origin)	Respiratory/airborne; human-to-human	<1% (current)	Multiple (mRNA, protein, vector)	Remdesivir, nirmatrelvir/ritonavir
Zika virus	Non-human primates	Aedes mosquito; sexual; vertical	Rare fatality†	None licensed	None specific
Chikungunya virus	Non-human primates	Aedes mosquito	<1%	Ixchiq (licensed 2023, US)	None specific

*Table 1. Selected emerging and re-emerging viral pathogens discussed in this chapter. *Reflects laboratory-confirmed cases reported to WHO since 2003, not all infections. †Primary clinical concern is congenital Zika syndrome rather than acute fatality. CFR = case fatality rate; NHP = non-human primate.*

Advances in Viral Vaccinology

mRNA and Self-Amplifying RNA Platforms

The clinical success of lipid nanoparticle (LNP)-formulated mRNA vaccines against COVID-19, first established in the pivotal BNT162b2 and mRNA-1273 efficacy trials (Baden et al., 2021; Polack et al., 2020), has catalysed a broader platform shift in vaccinology. By 2026, mRNA-based vaccines had received regulatory approval not only for COVID-19 but also for respiratory syncytial virus (RSV) and seasonal influenza, with the approval of Moderna's mFLUSIVA in August 2026 marking the first mRNA-based influenza vaccine to reach the market (Biopharma PEG, 2026). Beyond first-generation designs, self-amplifying RNA (saRNA) and circular RNA (circRNA) constructs are in active development; these approaches use smaller mRNA payloads or enhanced RNA stability to achieve comparable or superior immunogenicity at lower doses, a property with direct implications for manufacturing cost and dose-sparing in low-resource settings.

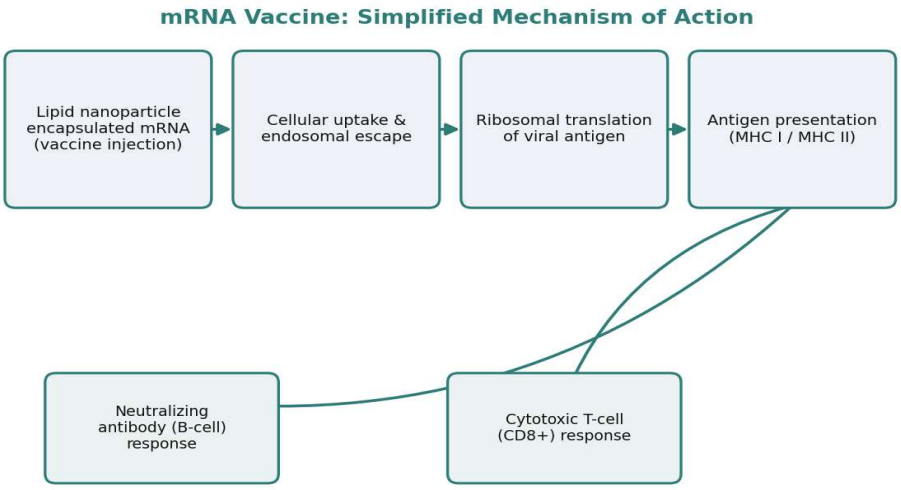


Figure 3. Simplified mechanism of action of a lipid-nanoparticle mRNA vaccine, from cellular uptake to adaptive immune priming.

The core biological logic of the platform, shown schematically in Figure 3, is that the vaccine itself carries only genetic instructions rather than viral protein or attenuated virus: host ribosomal machinery transiently manufactures the target antigen inside the vaccinated person's own cells, which then present it to the immune system through both major histocompatibility

complex class I and class II pathways, priming a combined antibody and cytotoxic T-lymphocyte response. This dual humoral and cellular activation is one reason mRNA platforms have generalized so readily across unrelated viral targets: the delivery chemistry and manufacturing process remain largely constant, while only the encoded antigen sequence changes between products.

Delivery Innovations

Lipid nanoparticles remain the most clinically validated RNA delivery vehicle, but formulation science continues to address cold-chain dependency, a major barrier to distribution in low- and middle-income countries. Emerging approaches include pKa-tuned ionizable lipids intended to improve endosomal escape and antigen expression, biomimetic and exosome-based carriers for improved tissue targeting, and stimuli-responsive nanocarriers designed to release payload selectively within disease-relevant microenvironments (PMC, 2026a). In parallel, needle-free delivery systems, including inhalable LNP powders and microneedle array patches, are being developed to improve thermostability and reduce the logistical burden of intramuscular injection, with particular relevance to vaccination campaigns in remote or resource-constrained settings (PMC, 2026a; Gavi, 2026a). Microneedle patches carry the additional advantage of being self-administrable and generating minimal biohazardous sharps waste, both practically important in mass vaccination campaigns outside formal clinical settings.

Vaccines Against Historically Intractable Viruses

HIV has resisted five decades of vaccine development, owing to extreme viral diversity, rapid immune escape, and the absence of a natural immune correlate of protection. The mRNA platform is now being applied to this problem: candidate mRNA-1644 is designed to prime B-cell lineages toward broadly neutralizing antibody development, and a 2026 phase 1 trial (IAVI G004) is testing a booster intended to deepen this response, including protocols in which participants pause antiretroviral therapy under close medical supervision to assess whether vaccination can help maintain virologic control (Gavi, 2026a; Biopharma PEG, 2026). No HIV vaccine candidate has yet advanced to phase 3 testing, and the recent demonstration that long-acting antiretroviral pre-exposure prophylaxis can achieve near-complete protection in trial settings has raised the evidentiary bar that any future vaccine will need to clear to justify its added public health value (Gavi, 2026a).

Universal and Broadly Protective Vaccine Strategies

A parallel research direction seeks to move away from strain-specific vaccine design toward broadly protective, or universal, approaches. One framework organizes viral families around conserved 'prototype pathogens,' reasoning that protective immunogens identified in a well-studied member of a viral family are likely to generalize to related, less-studied members, thereby building preparedness against future, as-yet-unidentified outbreak strains (PMC, 2026b). This logic underlies ongoing efforts to design influenza vaccines targeting conserved internal viral proteins rather than the rapidly mutating surface hemagglutinin, building on the earlier discovery of broadly neutralizing antibodies against a conserved hemagglutinin anchor epitope (Guthmiller et al., 2022) and decades of work on M2e-based universal influenza vaccine candidates (Mezhenskaya et al., 2019). Analogous broadly protective strategies are being explored for coronaviruses and henipaviruses.

Vaccine Equity and Global Manufacturing

Scientific platform advances do not automatically translate into equitable global access. The COVID-19 pandemic demonstrated that manufacturing capacity, intellectual property arrangements, and cold-chain infrastructure can bottleneck vaccine distribution even after a product is proven safe and effective. Regional messenger RNA manufacturing hubs, technology-transfer partnerships, and thermostable formulations compatible with existing national immunization cold chains are now recognized as being as important to pandemic preparedness as the underlying antigen science itself. This is a direct practical motivation behind the delivery innovations discussed in Section 3.2, since a vaccine that cannot survive transport to a rural clinic without continuous deep-freeze storage offers limited real-world protection, however strong its efficacy data.

Table 2 situates the mRNA advances discussed above within the broader landscape of viral vaccine platforms currently in clinical or licensed use, illustrating how conventional and next-generation approaches now coexist across different target viruses.

Platform	Example Vaccine	Target Virus	Status
mRNA-LNP	BNT162b2 / mRNA-1273	SARS-CoV-2	Approved (2020–2021)
mRNA-LNP	mFLUSIVA (mRNA-1010)	Seasonal influenza	Approved (Aug. 2026)
Viral vector (non-replicating)	Ebola vaccine (rVSV-ZEBOV type)	Ebola virus	Approved (2019–2020)
Modified vaccinia Ankara	MVA-BN	Mpox virus	Licensed
Protein subunit + adjuvant	Arexvy / Abrysvo	RSV	Approved (2023)
Live-attenuated / inactivated	Oral polio; inactivated influenza	Poliovirus; influenza	Long-licensed
Investigational mRNA	mRNA-1644	HIV	Phase 1 (in progress)

Table 2. Selected licensed or late-stage viral vaccines by platform technology, illustrating the coexistence of conventional and next-generation approaches.

Advances in Antiviral Therapeutics

Antiviral drug classes are conventionally organized around the stage of the viral replication cycle they interrupt, from initial receptor attachment through genome replication, protein synthesis, particle assembly, and final budding or release (Figure 4). This organizing framework, developed over decades of antiviral pharmacology research, remains useful for understanding where genuinely new drug classes are emerging versus where recent approvals represent refinements of established mechanisms.

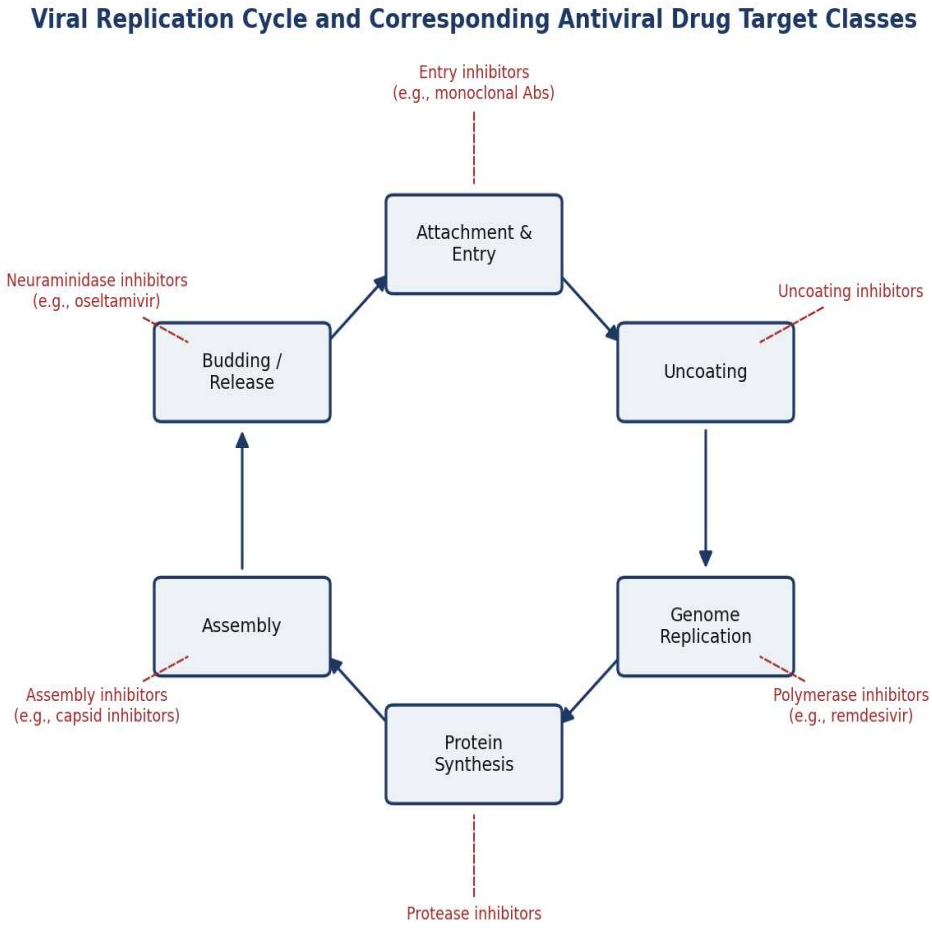


Figure 4. The viral replication cycle and the corresponding classes of antiviral drug targets.

Target Stage	Drug Class	Representative Agent(s)	Example Virus(es)
Attachment / entry	Monoclonal antibodies	Clesrovimab, palivizumab	RSV
Uncoating	M2 ion-channel blockers	Amantadine, rimantadine	Influenza A (largely resistant now)
Genome replication	Polymerase (RdRp) inhibitors	Remdesivir, favipiravir	SARS-CoV-2, Ebola, influenza
Protein synthesis	Protease inhibitors	Nirmatrelvir, ritonavir	SARS-CoV-2, HIV
Assembly	Capsid inhibitors	Lenacapavir	HIV
Budding / release	Neuraminidase inhibitors	Oseltamivir, zanamivir, peramivir	Influenza

Table 3. Representative antiviral drug classes mapped to the replication-cycle stage they target, corresponding to Figure 4.

Influenza Antivirals

The 2025–2026 influenza season treatment armamentarium includes three neuraminidase inhibitors, oral oseltamivir (Hayden et al., 1999), intravenous peramivir (Kohno et al., 2011), and inhaled zanamivir (Hayden et al., 1997), alongside the cap-dependent endonuclease inhibitor baloxavir marboxil, which offers single-dose oral dosing rather than the multi-day regimens required by neuraminidase inhibitors (Hayden et al., 2018; The Medical Letter, 2025). All influenza antivirals are most effective when initiated within 48 hours of symptom onset, and clinicians are advised to monitor regional resistance patterns, since emergence of resistant variants can materially reduce drug effectiveness (The Medical Letter, 2025). Combination regimens pairing baloxavir with a standard neuraminidase inhibitor have been tested in hospitalized patients with severe influenza, on the reasoning that targeting two distinct steps of the replication cycle simultaneously, endonuclease activity and neuraminidase-mediated release, might reduce the risk of resistance emergence relative to monotherapy.

Monoclonal Antibodies for Respiratory Virus Prevention

Long-acting monoclonal antibodies have emerged as an important prophylactic complement to active vaccination, particularly in infants and other populations that respond poorly to vaccines. The U.S. Food and Drug Administration approved clesrovimab (marketed as Enflonsia) in June 2025 for prevention of RSV lower respiratory tract disease in infants entering their first RSV season, extending the monoclonal antibody strategy pioneered by earlier RSV-specific antibodies to a longer-acting molecule suitable for single-dose seasonal administration (ScienceDirect, 2026). Because monoclonal antibodies confer passive immunity directly, without requiring the recipient's own immune system to mount a response, they are particularly valuable in populations, such as neonates and immunocompromised patients, in whom active vaccination is either not yet possible or unlikely to generate adequate protection in time.

Coronavirus Antivirals and Post-Exposure Prophylaxis

Oral antiviral development for coronaviruses has extended from acute treatment toward post-exposure prophylaxis. Ensitrelvir, an oral 3C-like protease inhibitor, is under FDA review as a candidate for prevention of COVID-19 following known exposure; if approved, it would represent the first oral agent specifically indicated for post-exposure prevention of COVID-19, complementing existing treatment-focused antivirals such as remdesivir, whose survival and recovery benefit in hospitalized patients was established earlier in the pandemic (Beigel et al., 2020; Shionogi, 2025). The distinction between treatment and prophylaxis matters clinically: a prophylactic agent must clear a substantially higher safety bar, since it will be given to exposed but not-yet-infected, and often otherwise healthy, individuals.

Long-Acting Antiretroviral Therapy

In HIV medicine, lenacapavir, a long-acting capsid inhibitor of the class whose structural and mechanistic basis was first elucidated in HIV-1 capsid-targeting compounds (Bester et al., 2020), is administered as infrequently as twice yearly and has reported close to complete efficacy for pre-exposure prophylaxis in African trial populations, a result that has reset expectations for what pre-exposure biomedical prevention can achieve without daily oral adherence (Gavi, 2026a). This performance bar now shapes the design and ethical framing of concurrent HIV vaccine trials, which must demonstrate additive benefit over an already highly effective pharmacological alternative.

Did you know? *A twice-yearly injection regimen means a person can now receive complete annual HIV pre-exposure prophylaxis coverage in as few as two clinical visits, compared with the daily adherence historically required by oral pre-exposure prophylaxis, a difference expected to matter enormously in settings where clinic access or pill-taking privacy is limited.*

Oncolytic Viruses at the Interface of Virology and Oncology

Tumor virology and antiviral pharmacology increasingly intersect in the development of oncolytic viral therapies, which are viruses genetically engineered to replicate selectively within tumor cells while stimulating local and systemic antitumor immunity. The approach has a longer history than many clinicians realize: the first oncolytic virus therapy approved by the FDA, talimogene laherparepvec, a modified herpes simplex virus engineered to replicate in tumor tissue and express an immune-stimulating cytokine, reached the market in 2015 for advanced melanoma (Andtbacka et al., 2015). Building on that precedent, the FDA granted approval in August 2026 to vusolimogene oderparepvec, a further genetically modified oncolytic viral therapy, for use in combination with an immune checkpoint inhibitor in advanced cutaneous melanoma (Drugs.com, 2026). This approval reflects a broader trend of applying core virological principles, tropism engineering, immune evasion gene deletion, and controlled replication competence, to therapeutic ends far removed from classical antiviral treatment of infection.

Antiviral Resistance and Stewardship

Every antiviral class discussed above is shadowed by the possibility of resistance, since RNA viruses in particular replicate with error-prone polymerases that generate substantial genetic diversity within a single infected host. Oseltamivir-resistant seasonal and pandemic influenza strains have been documented since shortly after the drug's introduction, and remdesivir-resistant SARS-CoV-2 variants have emerged, albeit rarely, during prolonged treatment of immunocompromised patients. This underscores that antiviral stewardship, using the right drug, at the right time, and avoiding unnecessary monotherapy in high-viral-burden settings, is as important to sustaining long-term drug effectiveness as the discovery of new agents themselves.

The Antiviral Development Pipeline

Beyond agents that have already reached approval, a substantial pipeline of investigational antivirals is moving through preclinical and clinical development across more than a dozen viral disease indications, tracked through periodically updated landscape reviews that classify candidates by

clinical phase, molecular target, and developer (Intrepid Alliance, 2026). Such landscaping exercises are valuable precisely because individual drug approvals are episodic and hard to anticipate from any single data source; a standing, regularly updated view of the entire pipeline gives clinicians and policymakers a more realistic sense of which viral diseases are likely to gain new treatment options in the near term, and which remain comparatively neglected.

Diagnostic and Surveillance Advances

Genomic and Metagenomic Surveillance

Genomic and metagenomic sequencing has become the backbone of modern viral surveillance (Greninger, 2018; Miller et al., 2019), enabling near real-time detection of the recombinant mpox lineages and influenza reassortants discussed above. Untargeted metagenomic approaches, which sequence all nucleic acid in a clinical sample rather than amplifying a single suspected pathogen, are particularly valuable for detecting genuinely novel or unexpected viruses, since they do not require prior knowledge of what to look for. Complementing individual clinical testing, wastewater-based surveillance, which quantifies viral genetic material shed into sewage by an entire community, has matured from a research tool into a routine public health surveillance stream for SARS-CoV-2, influenza, and poliovirus, offering an early warning signal that does not depend on individuals seeking clinical care or testing.

The Diagnostics Gap

Despite this progress, independent assessments in 2024 and 2025 identified diagnostics, rather than vaccines, as the weakest link in global pandemic preparedness, citing slow translation from research-grade sequencing platforms to field-deployable, point-of-care assays, and inconsistent regulatory harmonization across regions (Gavi, 2026b). A research-grade next-generation sequencing assay that takes days to return a result and requires specialized laboratory infrastructure offers little practical value during an active outbreak in a remote clinic; strengthening adaptable, field-deployable testing platforms and regional evaluation capacity has therefore been identified as a priority alongside continued vaccine and therapeutic innovation.

Challenges and Future Perspectives

Three structural challenges recur across the developments reviewed in this chapter. First, surveillance and diagnostic capacity remain unevenly distributed globally, with high-income countries retaining disproportionate

sequencing and monitoring infrastructure relative to the regions where many spillover events originate. Second, novel vaccine and antiviral platforms, however promising, must still resolve manufacturing scalability, cold-chain independence, and cost barriers before they can achieve equitable global access, particularly for low- and middle-income countries carrying much of the burden of filoviral, henipaviral, and arboviral disease. Third, the increasing use of a single platform technology, mRNA, across an expanding range of pathogens concentrates both opportunity and risk: it accelerates development timelines but also creates common-mode vulnerabilities in manufacturing capacity and regulatory pathways that pandemic preparedness planning must explicitly address.

A further, less frequently discussed challenge is workforce and infrastructure continuity between outbreaks. Much of the genomic surveillance, contact tracing, and laboratory capacity mobilized during an active filovirus or Nipah virus outbreak is temporary, assembled under emergency funding and dismantled once case counts fall. Sustaining baseline surveillance capacity in outbreak-prone regions between epidemics, rather than only during them, is increasingly recognized as more cost-effective than repeatedly rebuilding response capacity from a low baseline each time a new outbreak begins.

Regulatory and ethical questions have also grown more complex as countermeasure development has accelerated. Emergency use authorization pathways, which allowed COVID-19 vaccines and antivirals to reach patients months or years faster than conventional approval timelines, remain a double-edged tool: they save lives during active emergencies but can complicate public trust if the evidentiary basis for authorization is not clearly communicated. Trial designs for interventions such as HIV vaccine candidates tested against a backdrop of highly effective long-acting pre-exposure prophylaxis also raise genuine ethical questions about what constitutes an adequate comparator and how to counsel trial participants about relative risk. Resolving these regulatory and ethical questions in advance of the next emergency, rather than improvising them under outbreak pressure, is itself a preparedness task as important as any single vaccine or antiviral in the pipeline.

Continued investment in broadly protective vaccine design, field-deployable diagnostics, and technology transfer to regional manufacturing hubs will likely determine how effectively these scientific advances translate into reduced global mortality from viral disease.

Conclusion

Medical virology in 2026 sits at an unusually productive juncture, with surveillance systems detecting novel and recombinant viral threats with increasing speed, vaccine platforms diversifying and generalizing to historically intractable targets such as HIV, and antiviral pharmacology broadening from small molecules to long-acting antibodies, oral prophylactics, and engineered oncolytic viruses. Sustaining this momentum will depend less on further scientific breakthroughs in isolation and more on closing persistent gaps in diagnostics, manufacturing equity, and global surveillance coordination, so that the next emerging viral threat is met with countermeasures that are not only scientifically advanced but also globally deployable.

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Antimicrobial Resistance: Molecular Mechanisms and Global surveillance

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1. Introduction

Antimicrobial resistance (AMR) has evolved into one of the most profound public health, socio-economic, and clinical crises of the 21st century. The widespread implementation of antimicrobial therapy during the mid-20th century revolutionized modern medicine, vastly extending life expectancy and paving the way for sophisticated medical procedures such as solid organ transplantation, advanced cancer chemotherapy, and complex surgeries. However, the relentless evolutionary pressure exerted by the widespread and often indiscriminate use of antibiotics in human medicine, veterinary practices, and agriculture has accelerated the emergence of resistant bacterial strains. This 'survival of the fittest' dynamic highlights the remarkable genetic plasticity of bacterial pathogens, enabling them to adapt to environmental stressors and evade the lethal effects of antimicrobial agents.

The current trajectory of AMR poses an existential threat to global healthcare systems, threatening to return humanity to a pre-antibiotic era where minor injuries and routine infections could prove fatal. The 2025 Global Antimicrobial Resistance and Use Surveillance System (GLASS) report released by the World Health Organization (WHO) presents a stark reality: approximately one in six laboratory-confirmed bacterial infections worldwide is resistant to standard antibiotic regimens. Alarmingly, in settings with weaker health infrastructures, the burden is even more pronounced, forming a syndemic of escalating resistance, fragile healthcare delivery, and limited diagnostic capabilities. The projections suggest that without immediate, coordinated interventions, AMR could be responsible for up to 10 million deaths annually by 2050. Thus, understanding the intricate molecular mechanisms of AMR, deploying cutting-edge diagnostic technologies, and establishing robust global surveillance networks are paramount imperatives for contemporary microbiology and public health.

2. Types of Antibacterial Drug Resistance

The presence of bacteriostatic or bactericidal antimicrobial agents can enable the growth of resistant microorganisms at concentrations that normally inhibit their growth. This adaptation is a classical example of Darwinian selection in microbial populations. Drug resistance can be broadly classified into natural (intrinsic/induced), acquired, and adaptive categories based on how the resistance trait is originally developed or manifested.

2.1 Natural Resistance

Natural resistance can be categorized as either intrinsic (always present in the species) or induced (where genes naturally found in the bacteria are expressed to resistance levels only after antibacterial exposure). Intrinsic resistance is a universal characteristic shared within a bacterial species, independent of prior antibacterial exposure, and unrelated to horizontal gene transfer (HGT). It represents a structural or functional trait that naturally limits the efficacy of a drug.

For example, cell wall-less bacteria like *Mycoplasma* species are naturally and universally resistant to beta-lactam antibacterials (penicillins, cephalosporins), which specifically target peptidoglycan synthesis. Similarly, Gram-negative bacteria possess an outer membrane largely composed of lipopolysaccharides (LPS) that inherently limits the penetration of large, hydrophobic glycopeptides like vancomycin, rendering them intrinsically resistant. Anaerobic bacteria are intrinsically resistant to aminoglycosides because the uptake of these drugs requires an oxygen-dependent electron transport chain, which anaerobes lack.

2.2 Acquired Resistance

Acquired resistance occurs when a bacterium, previously sensitive to an antibacterial agent, develops resistance through a novel genetic mechanism. This adaptation arises primarily through two pathways: chromosomal mutation or the acquisition of new genetic material from an external source via HGT.

Chromosomal resistance originates from spontaneous mutations in the bacterial DNA during replication. These mutations may alter the structural conformation of drug targets (e.g., DNA gyrase mutations conferring fluoroquinolone resistance) or decrease cell envelope permeability (e.g., porin loss). Although spontaneous chromosomal mutations occur at a low frequency, the massive scale of bacterial replication and the intense selective pressure of antibiotic exposure ensure these mutants rapidly dominate the population.

Extrachromosomal resistance relies on mobile genetic elements (MGEs) transferred through horizontal gene transfer. Plasmids, transposons, and integrons serve as critical vehicles for disseminating resistance traits. These elements frequently carry genes encoding drug-inactivating enzymes (e.g., extended-spectrum beta-lactamases) or target-protection proteins. Extrachromosomal resistance is particularly pernicious because it can spread across different bacterial species and genera, rapidly converting previously susceptible microbiomes into reservoirs of multidrug resistance.

2.3 Adaptive Drug Resistance

Adaptive resistance is characterized by the capacity of microorganisms to adapt reversibly to specific environmental signals. Unlike acquired resistance, adaptive resistance is not driven by genetic mutations or acquired DNA. Instead, it is an epigenetic or transcriptional response to environmental stressors such as pH changes, nutrient limitation, varying ion concentrations, or exposure to sub-inhibitory antibiotic levels.

This form of resistance is transient. It enables bacteria to respond swiftly to antibacterial challenges, but once the inducing signal is no longer present, the bacteria typically revert to their original state of susceptibility. The formation of persister cells within biofilms—cells that enter a state of metabolic dormancy to survive bactericidal antibiotic concentrations—is a prime clinical example of adaptive resistance.

2.4 Cross Resistance, Multi-Drug Resistance, and Pan-Resistance

Cross-resistance occurs when resistance to one specific drug simultaneously confers resistance to other drugs with similar mechanisms of action or structural similarities. For example, a single enzymatic modification or target mutation might render an entire class of antibiotics (e.g., all macrolides) ineffective.

The emergence of Multidrug-Resistant (MDR) organisms is a growing public health catastrophe. MDR bacteria are defined as non-susceptible to at least one agent in three or more distinct antimicrobial categories. The ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp.) are notorious for their MDR profiles.

Extensively drug-resistant (XDR) strains remain susceptible to only one or two antimicrobial categories. Ultimately, pan-drug-resistant (PDR) strains are defined by their resistance to all available antimicrobial agents. PDR

infections carry exceedingly high mortality rates, as clinicians are left with no effective pharmacological interventions.

3. Molecular Mechanisms of Antibiotic Drug Resistance

To survive and thrive in the presence of lethal antimicrobial agents, bacterial strains have evolved highly sophisticated biochemical and genetic mechanisms to interfere with critical stages of drug action. These mechanisms are profoundly complex and often act synergistically to confer high-level resistance.

Table 1: Examples of Antibacterial Drug Resistance Mechanisms

Mechanism of Action	Antibiotic Class / Drug	Primary Resistance Strategy	Molecular Mechanism of Resistance
Cell Wall Synthesis Inhibitors	**Beta-lactams** (Penicillins, Cephalosporins, Carbapenems)	Enzymatic Inactivation	Hydrolysis of the beta-lactam ring by beta-lactamases, including Extended-Spectrum Beta-Lactamases (ESBL), serine carbapenemases (KPC, OXA), and metallo-beta-lactamases (NDM, VIM, IMP).
Cell Wall Synthesis Inhibitors	**Methicillin** (Anti-staphylococcal Penicillin)	Target Alteration	Acquisition of the *mecA* or *mecC* gene on the SCCmec cassette, encoding Penicillin-Binding Protein 2a (PBP2a), which has a markedly reduced affinity for beta-lactam antibiotics (characteristic of MRSA).
Cell Wall Synthesis Inhibitors	**Glycopeptides** (Vancomycin, Teicoplanin)	Target Alteration (Reprogramming)	Acquisition of *van* gene clusters (e.g., *vanA*, *vanB* in enterococci) that alter the peptidoglycan precursor terminus from D-Alanyl-D-Alanine to D-Alanyl-D-Lactate or D-Alanyl-D-Serine, eliminating the drug's binding site.
Cell Membrane Disruptors	**Polymyxins** (Colistin)	Target Alteration	Covalent modification of lipopolysaccharide (LPS) lipid A with molecules like L-Ara4N or

			phosphoethanolamine. Driven by mobilized colistin resistance (*mcr*) plasmid genes or chromosomal mutations in the PmrAB/PhoPQ two-component regulatory systems.
Cell Membrane Disruptors	**Daptomycin** (Lipopeptide)	Target Alteration (Charge Repulsion)	Mutations in *mprF* (synthesizes positively charged lysyl-phosphatidylglycerol), as well as LiaFSR and WalKR regulatory systems. This increases the positive charge of the cell membrane, repelling the calcium-daptomycin complex.
Protein Synthesis Inhibitors (30S Subunit)	**Aminoglycosides**	Enzymatic Inactivation & Target Alteration	Covalent modification of the drug by Aminoglycoside Modifying Enzymes (AMEs: acetyltransferases, adenyltransferases, phosphotransferases). Target alteration occurs via 16S rRNA methylation (e.g., *armA*, *rmt* genes) conferring high-level resistance.
Protein Synthesis Inhibitors (50S Subunit)	**Macrolides**	Target Alteration	Methylation of the adenine residue at position 2058 of the 23S rRNA by Erythromycin resistance methyltransferase (*erm* genes). This often confers the overlapping MLSB (Macrolide-Lincosamide-Streptogramin B) resistance phenotype.
Protein Synthesis Inhibitors (50S Subunit)	**Linezolid** (Oxazolidinones) **&Chloramphenicol**	Target Alteration	Methylation of the 23S rRNA (specifically at nucleotide A2503) mediated by the plasmid-borne *cfr* gene product. This confers cross-resistance to phenicols, lincosamides,

			oxazolidinones, pleuromutilins, and streptogramin A (PhLOPSA phenotype).
Nucleic Acid Synthesis Inhibitors	**Quinolones & Fluoroquinolones**	Target Alteration & Target Protection	Chromosomal point mutations in the Quinolone-Resistance-Determining Region (QRDR) of *gyrA*/*gyrB* (DNA gyrase) and *parC*/*parE* (Topoisomerase IV). Plasmid-mediated resistance includes *qnr* proteins that physically protect the target enzymes.
Nucleic Acid Synthesis Inhibitors	**Rifampicin**	Target Alteration	Chromosomal point mutations specifically localized in the *rpoB* gene, which encodes the beta-subunit of the DNA-dependent RNA polymerase, preventing the drug from binding and blocking RNA elongation.
Antimetabolites (Folate Pathway)	**Trimethoprim**	Target Bypass & Target Alteration	Plasmid-mediated acquisition of variant *dfr* genes encoding alternative Dihydrofolate Reductase (DHFR) enzymes with intrinsically low affinity for the drug. Can also occur via chromosomal overproduction or structural modification of native DHFR.

3.1 Modification (Alteration) of the Drug Target

Modifying or altering the antibacterial target is a profoundly effective evolutionary strategy that reduces the drug's binding affinity while preserving the essential physiological function of the target protein. This can occur via target site mutation, enzymatic alteration of the target, or complete target replacement.

Target Site Mutation: Point mutations in chromosomal genes frequently result in amino acid substitutions that alter the steric conformation or electrostatic properties of the antibiotic binding pocket. The most typical

example is fluoroquinolone resistance. Fluoroquinolones exert their bactericidal effect by stabilizing the DNA cleavage complexes formed by DNA gyrase (encoded by gyrA and gyrB) and Topoisomerase IV (encoded by parC and parE). Spontaneous point mutations within the highly conserved Quinolone-Resistance-Determining Region (QRDR) of these genes significantly decrease fluoroquinolone binding affinity, conferring high-level resistance across both Gram-positive and Gram-negative pathogens.

Similarly, rifampicin, a cornerstone of anti-tuberculosis therapy, targets the bacterial DNA-dependent RNA polymerase. Mutations in the rpoB gene, particularly within an 81-base pair core region, alter the rifampicin-binding pocket on the beta-subunit of the polymerase, abrogating drug binding while maintaining transcriptional viability.

Enzymatic Alteration of the Target: Bacteria can also deploy enzymes to chemically modify their own cellular structures to prevent drug binding. Macrolide, lincosamide, and streptogramin B (MLSB) resistance is largely mediated by erythromycin ribosomal methylase (Erm) enzymes. Erm enzymes dimethylate a specific adenine residue (A2058) in domain V of the 23S rRNA. This subtle methylation causes significant steric hindrance in the ribosomal exit tunnel, simultaneously blocking the binding of three distinct antibiotic classes. In a similar vein, the chloramphenicol-florfenicol resistance (cfr) gene encodes an S-adenosylmethionine (SAM)-dependent methyltransferase that methylates position A2503 of the 23S rRNA. This modification confers cross-resistance to linezolid, phenicols, lincosamides, pleuromutilins, and streptogramin A.

Target Replacement or Bypass: In this strategy, the bacterium acquires an exogenous gene encoding a functionally equivalent enzyme that exhibits insensitivity to the antibiotic. The most infamous example is methicillin-resistant Staphylococcus aureus (MRSA). The acquisition of the staphylococcal cassette chromosome mec (SCCmec) introduces the mecA or mecC gene, which encodes an alternative transpeptidase, Penicillin-Binding Protein 2a (PBP2a). Unlike native PBPs, PBP2a possesses an altered active site cleft with exceptionally low affinity for virtually all beta-lactam antibiotics, allowing cell wall peptidoglycan cross-linking to proceed uninterrupted.

Vancomycin resistance in enterococci (VRE) represents a highly sophisticated target bypass mechanism. Vancomycin normally binds tightly to the terminal D-alanyl-D-alanine (D-Ala-D-Ala) dipeptide of nascent peptidoglycan precursors, inhibiting cell wall synthesis. Bacteria possessing van gene clusters (e.g., the vanA operon) undergo a complex metabolic reprogramming orchestrated by a two-component regulatory system

(VanS/VanR). The operon produces enzymes (VanH, VanA) that synthesize a modified precursor terminating in D-alanyl-D-lactate (D-Ala-D-Lac), and simultaneously produces peptidases (VanX, VanY) that destroy the wild-type D-Ala-D-Ala precursors. The substitution of a single oxygen atom for an NH group removes a crucial hydrogen bond, decreasing vancomycin binding affinity by a factor of 1000.

Target Site Shielding: Certain resistance determinants encode proteins that physically protect the target without altering it. Tetracycline resistance is often mediated by ribosomal protection proteins (RPPs) such as Tet(M) and Tet(O). These proteins mimic elongation factors (EF-G), bind to the ribosome, and dislodge the bound tetracycline molecule through a GTP-dependent mechanism. Plasmid-mediated quinolone resistance (PMQR) can involve Qnr proteins, which are pentapeptide repeat proteins that physically shield DNA gyrase and topoisomerase IV from fluoroquinolone interaction.

3.2 Decreased Accumulation: Permeability Barriers and Active Efflux

Decreased Influx (Permeability Barriers): For an antibiotic to reach its intracellular or periplasmic target, it must successfully navigate the bacterial cell envelope. Gram-negative bacteria possess a double-membrane architecture that serves as a formidable barrier. The outer membrane's outer leaflet is composed of highly densely packed lipopolysaccharides (LPS), rendering it impermeable to large, lipophilic molecules. Consequently, hydrophilic antibiotics (e.g., beta-lactams, fluoroquinolones) must pass through water-filled channel proteins known as porins.

Bacteria can acquire resistance by mutating these porin genes, resulting in porin loss, decreased expression, or structural narrowing of the channel. The loss of the OprD porin in Pseudomonas aeruginosa is a primary driver of imipenem resistance. In Klebsiella pneumoniae, the downregulation of major porins OmpK35 and OmpK36 severely restricts the entry of advanced cephalosporins and carbapenems, drastically elevating the Minimum Inhibitory Concentration (MIC) when coupled with beta-lactamase production.

Furthermore, bacteria can structurally alter the cell envelope itself to repel drugs. Colistin (Polymyxin E), a last-resort drug for multi-drug resistant Gram-negative infections, acts by binding to the negatively charged lipid A moiety of LPS, causing membrane disruption. Resistance arises either through chromosomal two-component systems (PmrAB and PhoPQ) or the highly alarming plasmid-borne mcr-1 gene. These mechanisms lead to the addition of a phosphoethanolamine or 4-amino-4-deoxy-L-arabinose group to lipid A. This

modification neutralizes the negative charge of the cell surface, electrostatically repelling the cationic colistin molecule.

Increased Expression of Efflux Pumps: Efflux pumps are ubiquitous, energy-dependent transporter proteins located in the cytoplasmic membrane that actively extrude toxic compounds, including a vast array of antibiotics, out of the cell. They act as molecular 'vacuum cleaners' and are major contributors to multidrug resistance. These pumps are classified into five major superfamilies: the ATP-binding cassette (ABC) superfamily, the major facilitator superfamily (MFS), the multidrug and toxic compound extrusion (MATE) family, the small multidrug resistance (SMR) family, and the resistance-nodulation-division (RND) family.

The RND family is particularly problematic in Gram-negative ESKAPE pathogens. RND pumps, such as the AcrAB-TolC system in Escherichia coli and the MexAB-OprM system in Pseudomonas aeruginosa, assemble into complex tripartite superstructures. The inner membrane transporter (AcrB/MexB) captures substrates from the cytoplasm or periplasm and utilizes the proton motive force to pump the drug through a periplasmic adaptor protein (AcrA/MexA) directly out through an outer membrane channel (TolC/OprM). This bypasses the periplasmic space entirely, rendering beta-lactamases more effective since the drug concentration is kept exceedingly low. The overexpression of RND pumps is typically governed by mutations in local repressors (e.g., acrR) or the constitutive activation of global transcriptional activators like MarA, SoxS, and RamA.

3.3 Enhanced Secretion of Antibacterial Inactivating Enzymes

The enzymatic destruction or structural modification of the antibiotic molecule before it can reach its target is arguably the most prevalent and clinically impactful resistance mechanism. The production of beta-lactamases is the cardinal example of this strategy.

Beta-Lactamases: These enzymes catalyze the hydrolysis of the amide bond within the beta-lactam ring, rendering penicillins, cephalosporins, monobactams, and carbapenems utterly ineffective. Based on Ambler's structural classification, they are divided into four classes. Class A, C, and D utilize an active-site serine for hydrolysis, while Class B enzymes are zinc-dependent metalloenzymes.

Ambler Classification of β-Lactamases

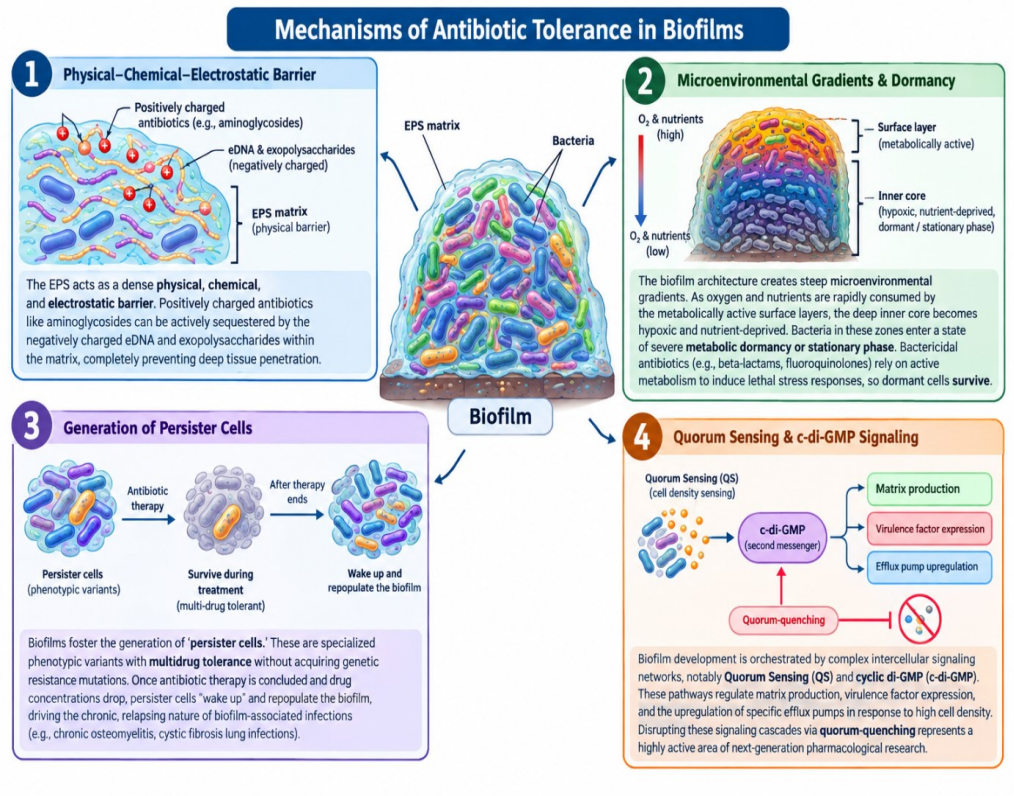
Ambler Class	Catalytic Mechanism	Key Enzyme Examples	Primary Resistance Profile	Inhibitor Susceptibility	Notable Pathogens & Characteristics
Class A	• Active-site Serine	• Penicillinases (TEM-1, SHV-1) • ESBLs (CTX-M) • Carbapenemases (KPC)	• 3rd-generation cephalosporins (cefotaxime, ceftriaxone) • Carbapenems	• KPCs are resistant to many classical beta-lactamase inhibitors.	• *E. coli, K. pneumoniae.* • Globally disseminated.
Class B (MBLs)	• Zinc-dependent Metalloenzyme	• NDM, VIM, IMP	• Virtually all beta-lactams **except** monobactams.	• Cannot be inhibited by clavulanate, tazobactam, or avibactam.	• Highly feared due to extensive resistance profile.
Class C (AmpC)	• Active-site Serine	• AmpC	• Broad-spectrum cephalosporins.	• Generally resistant to clavulanic acid.	• *Enterobacter cloacae, Pseudomonas aeruginosa.* • Typically chromosomally encoded and inducible; plasmid-mediated increasing.
Class D (OXA)	• Active-site Serine	• OXA-48-like • OXA-23, OXA-58	• Oxacillins, Carbapenems (significant carbapenem-hydrolyzing capabilities).	• Variable.	• *Enterobacterales, Acinetobacter baumannii.* • Primary drivers of carbapenem resistance in these pathogens.

Aminoglycoside-Modifying Enzymes (AMEs): Aminoglycosides exert their bactericidal effect by binding to the 30S ribosomal subunit. Bacteria neutralize them by secreting enzymes that covalently attach various chemical groups to the antibiotic's structure. These include Aminoglycoside N-Acetyltransferases (AAC), Aminoglycoside O-Phosphotransferases (APH), and Aminoglycoside O-Nucleotidyltransferases (ANT). The addition of acetyl, phosphate, or adenyl groups alters the drug's shape and charge, creating steric hindrance that precludes ribosomal binding. Furthermore, bifunctional enzymes such as AAC(6')-Ie-APH(2")-Ia have emerged in staphylococci and enterococci, conferring broad-spectrum high-level resistance to almost all clinically relevant aminoglycosides (except streptomycin).

4. Biofilm Formation and Global Cellular Adaptations

Moving beyond individual cellular mechanisms, bacterial populations deploy sophisticated multicellular strategies to survive antibiotic onslaught. Biofilm formation is a highly structured, community-based lifestyle that exponentially increases antimicrobial tolerance. A biofilm consists of a community of microorganisms irreversibly attached to a biotic or abiotic surface (such as indwelling medical devices, catheters, or necrotic host tissue), encased within a self-produced extracellular polymeric substance (EPS) matrix. The EPS is a complex hydrogel composed of exopolysaccharides, proteins, lipids, and extracellular DNA (eDNA).

The Minimum Inhibitory Concentration (MIC) for bacteria within a biofilm can escalate up to 1,000 times compared to genetically identical planktonic (free-floating) cells.]This extreme tolerance is multifactorial, arising from several complementary mechanisms within the biofilm architecture as shown in the Figure below.



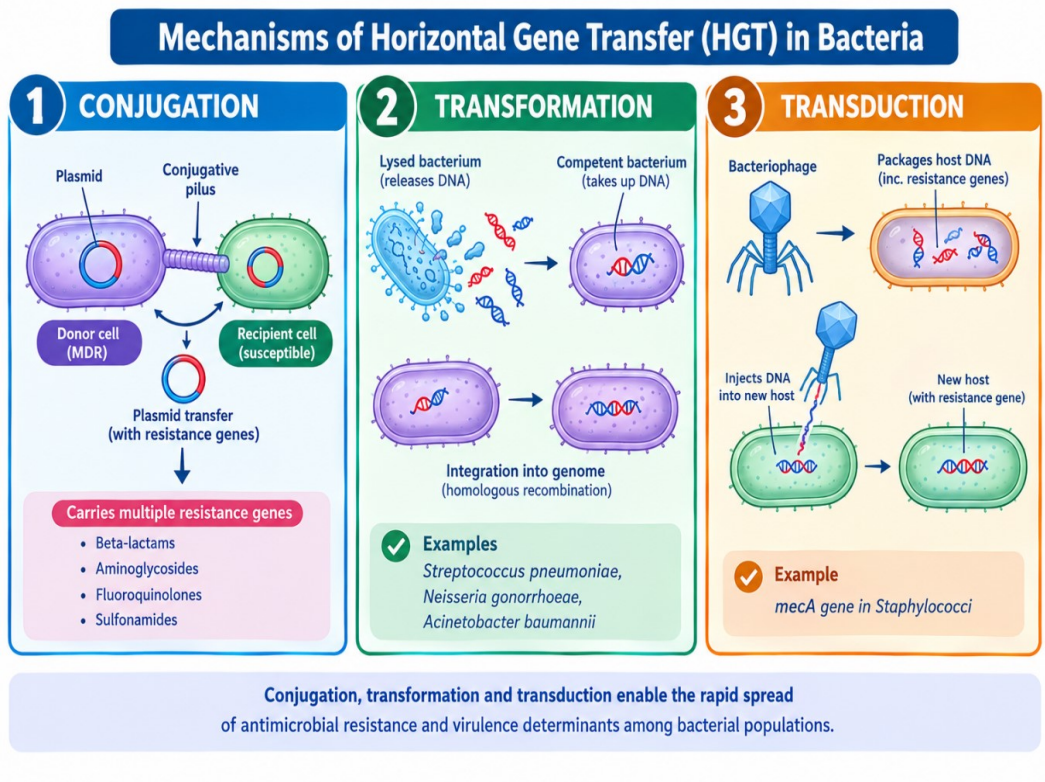
5. Horizontal Gene Transfer (HGT) and the Mobilome

The explosive global spread of AMR is largely fueled not by slow, spontaneous mutations, but by the rapid sharing of resistance genes across bacterial populations via Horizontal Gene Transfer (HGT). The 'mobilome' encompasses all mobile genetic elements in a genome, including plasmids, transposons, integrons, and bacteriophages, which serve as the vehicles for this genetic exchange.

Conjugation is the most efficient and clinically disastrous mechanism of HGT. It requires direct physical contact between a donor and a recipient cell, mediated by a conjugative pilus. Plasmids, which are extrachromosomal circular DNA molecules, replicate independently and are transferred through this pilus. Plasmids frequently carry integrons—genetic elements that capture and express multiple gene cassettes. A single conjugative event can therefore transfer resistance to beta-lactams, aminoglycosides, fluoroquinolones, and sulfonamides simultaneously, instantaneously converting a susceptible bacterium into an MDR superbug.

Transformation involves the uptake and genomic integration of naked, extracellular DNA released into the environment from lysed bacterial cells. Some species, such as Streptococcus pneumoniae, Neisseria gonorrhoeae, and Acinetobacter baumannii, are naturally 'competent' to import this DNA. Transformation plays a critical role in the generation of mosaic genes, such as the altered penicillin-binding protein (pbp) genes in pneumococci, which are created through homologous recombination with DNA from related commensal streptococci.

Transduction is mediated by bacteriophages (bacterial viruses). During the lytic cycle, as the phage packages its newly synthesized viral DNA into capsids, it can mistakenly package fragments of the host bacterium's DNA—including antimicrobial resistance genes. When these phages subsequently infect a new bacterial host, they inject the resistance genes instead of viral DNA. Transduction is highly specific but plays a crucial role in disseminating virulence factors and resistance genes, such as the mecA cassette in Staphylococci.



6. Detection and Identification of Molecular Resistance

The traditional paradigm of antimicrobial susceptibility testing (AST) relies heavily on phenotypic culture-based methods, such as disk diffusion (Kirby-Bauer), broth microdilution, and automated systems (VITEK 2, BD Phoenix). While these methods remain the definitive gold standard for determining Minimum Inhibitory Concentrations (MIC), they are inherently slow, typically requiring 24 to 72 hours for bacterial cultivation and growth analysis. In cases of severe sepsis, this delay leads to prolonged empirical therapy with broad-spectrum antibiotics, exacerbating resistance and increasing patient mortality. To overcome these limitations, spectacular advancements have been made in rapid molecular, genomic, and spectroscopic diagnostics.

Molecular Diagnostics (Genotypic AST): Polymerase Chain Reaction (PCR) and multiplex nucleic acid amplification tests have revolutionized clinical microbiology. Modern platforms (e.g., GeneXpert, BioFire FilmArray) can detect species-specific DNA and key resistance genes directly from positive blood cultures or clinical swabs within hours. For example, rapidly identifying

the mecA gene in S. aureus or the blaKPC, blaNDM, and blaOXA-48 genes in Gram-negative bacteremia allows clinicians to instantly optimize therapy and implement critical isolation protocols. Furthermore, isothermal amplification techniques, such as Loop-Mediated Isothermal Amplification (LAMP), offer highly sensitive DNA amplification without the need for expensive thermal cyclers, enabling rapid Point-of-Care (POC) testing in resource-limited settings.

Next-Generation Sequencing (NGS) and Metagenomics: Whole-Genome Sequencing (WGS) has transitioned from a pure research tool to a critical component of public health surveillance. WGS provides ultimate resolution, identifying the complete chromosomal 'resistome' and mapping the architecture of plasmids. It allows for the precise tracking of clonal outbreaks within hospitals and uncovers novel or uncharacterized resistance mechanisms. Clinical metagenomics takes this a step further by sequencing all microbial DNA directly from a clinical sample (like cerebrospinal fluid or bronchoalveolar lavage) without the need for prior cultivation, identifying fastidious pathogens and their resistance profiles simultaneously.

Spectroscopic and Proteomic Methods: Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is now the standard for rapid bacterial species identification based on ribosomal protein profiling. Recently, its utility has been expanded to AMR detection by monitoring the mass shift of beta-lactam antibiotics after they are cleaved by bacterial beta-lactamases. Fourier-Transform Infrared (FTIR) spectroscopy is another emerging tool that measures the absorption of infrared light by bacterial biomolecules (lipids, proteins, carbohydrates), generating a highly specific biochemical 'fingerprint.' Advanced algorithms can use these spectra to distinguish between ESBL-producing and non-producing strains in minutes.

Microfluidics, Biosensors, and CRISPR: Lab-on-a-chip technologies and microfluidic AST rapidly measure bacterial growth dynamics at the single-cell level using microscopic imaging or electrochemical sensors, slashing AST turnaround time to less than 4 hours. CRISPR-Cas biology has also been leveraged for diagnostics; systems like SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) use Cas12 or Cas13 enzymes to detect specific resistance genes with attomolar sensitivity, triggering a fluorescent collateral cleavage reaction that yields results on a simple paper strip.

Artificial Intelligence (AI) and Machine Learning (ML): The sheer volume of genomic and phenotypic data has necessitated the integration of AI. Machine learning algorithms are currently being trained on massive WGS datasets to predict phenotypic MICs directly from the genome with high accuracy. AI is also critical in analyzing FTIR spectral data and optimizing rapid diagnostic workflows.

Table 2: Comparison of various AMR Diagnostic Methods

Diagnostic approach	Scientific Principle	Primary Advantages	Key Limitations
Disk Diffusion / Broth Dilution	Measurement of visible bacterial growth inhibition in presence of antibiotics	Low cost; standardized worldwide; directly measures phenotypic MIC	Extremely slow turnaround time (24-72 hours); labor-intensive
Automated Phenotypic Systems (e.g. VITEK 2)	Automated turbidimetric/colorimetric growth monitoring	Reduced manual labor; standardized automated interpretation	Still requires pure culture (18-24 hours); limited predefined panels
Multiplex PCR	Targeted amplification of specific resistance genes (e.g., mecA, blaKPC)	Highly rapid (hours); high sensitivity; direct from clinical sample	Cannot detect unknown or novel resistance genes; does not provide MIC
Whole-Genome Sequencing (WGS)	Comprehensive sequencing of entire bacterial genome and mobilome	Ultimate resolution; detects all known ARGs; vital for outbreak epidemiology	High cost; requires advanced bioinformatics expertise; slow data processing
MALDI-TOF Mass Spectrometry	Protein profiling (mass-to-charge ratio) and detection of antibiotic hydrolysis	Extremely rapid species ID (minutes);	Primarily for ID; resistance detection is

		minimal sample prep	limited to specific enzymatic pathways
Microfluidics & Single-Cell AST	Microscopic/electrochemical monitoring of single-cell growth dynamics	Ultra-rapid phenotypic AST (<4 hours); high throughput	Complex instrumentation required; currently lacks widespread clinical validation
CRISPR-Based Resistance Detection	Sequence-specific recognition or cleavage of resistance-associated nucleic acids using CRISPR-based molecular systems	High specificity; potentially rapid and portable; adaptable to selected resistance determinants	Usually target-specific; requires appropriate assay design; does not directly measure phenotypic MIC

7. Next-Generation Antimicrobial Therapies and Interventions

As the ESKAPE pathogens evolve into Pan-Drug Resistant (PDR) entities, the traditional antibiotic discovery pipeline—largely exhausted since the 1980s—must be supplemented with innovative therapeutic strategies. One major advance involves mining novel environmental sources or employing AI. Lariocidin, a recently characterized ribosome-targeting lasso peptide, is a prime example. Lariocidin binds to an entirely novel, previously unexploited binding site on the bacterial 30S ribosomal subunit. Because this site has never been subject to evolutionary pressure from existing drugs, lariocidin completely circumvents ubiquitous resistance mechanisms like Erm-mediated ribosomal methylation and AME activity. It has shown profound efficacy against multidrug-resistant Acinetobacter baumannii in vivo without demonstrating toxicity to mammalian cells.

Artificial Intelligence is drastically shortening the drug discovery timeline. Deep neural networks recently screened libraries containing millions of chemical structures to identify 'Abaucin,' a novel narrow-spectrum compound highly effective against A. baumannii. By narrowing the spectrum, such drugs preserve the host microbiome and reduce collateral resistance selection.

Antibiotic Adjuvants represent a strategy to revive 'broken' legacy antibiotics. Next-generation beta-lactamase inhibitors (e.g., avibactam, vaborbactam, relebactam) possess unique non-beta-lactam structures, such as diazabicyclooctane (DBO) or cyclic boronic acid cores. These compounds form highly stable, reversible or irreversible covalent bonds with serine carbapenemases (like KPC), effectively neutralizing the enzyme and restoring the bactericidal efficacy of partner cephalosporins or carbapenems (e.g., ceftazidime-avibactam).

Phage Therapy—the application of highly specific lytic bacteriophages—is experiencing a global renaissance. Phages can penetrate biofilms, replicate at the site of infection, and kill MDR bacteria independent of conventional antibiotic resistance mechanisms. Engineered 'phage cocktails' are increasingly used in compassionate care for untreatable systemic infections.

Nanomedicine leverages nanoparticles (silver, zinc oxide) and targeted liposomal delivery systems. Nanoparticles physically disrupt bacterial membranes, generate lethal reactive oxygen species (ROS), and protect encapsulated antibiotics from enzymatic degradation, thereby improving drug pharmacokinetics and localized accumulation at infection sites.

8. Global and Regional Antimicrobial Resistance Surveillance

Vigilant and continuous AMR surveillance constitutes the backbone of the global response to the antibiotic resistance crisis. Surveillance programs are indispensable for monitoring the prevalence, incidence, and dynamic trends of resistance across diverse pathogens and geographic landscapes. These epidemiological insights are the primary drivers for formulating evidence-based national and international treatment guidelines, ensuring that empirical antibiotic prescribing remains effective against locally circulating strains. Furthermore, surveillance networks function as early-warning systems, rapidly identifying the emergence of highly virulent or pan-drug-resistant clones, allowing for immediate public health intervention.

The World Health Organization established the Global Antimicrobial Resistance and Use Surveillance System (GLASS) to standardize global AMR data. The comprehensive 2025 GLASS report highlights alarming trends. With participation surging to over 104 countries, the data reveals that approximately one in six laboratory-confirmed infections globally is resistant to standard regimens. The resistance rates among critical Gram-negative pathogens are staggering. Global resistance to ciprofloxacin in *E. coli* exceeds 60%, while resistance to third-generation cephalosporins approaches 40%. Klebsiella pneumoniae displays a formidable ~20% resistance rate to carbapenems—the designated drugs of last resort. The report unequivocally highlights that the burden is highly asymmetrical, concentrated heavily in Low- and Middle-Income Countries (LMICs) with weaker health systems.

In India, AMR poses a catastrophic threat exacerbated by high population density, heavy infectious disease burden, and unregulated antibiotic access. The Indian Council of Medical Research (ICMR) established the Antimicrobial Resistance Surveillance Network (AMRSN), which currently aggregates data from nodal tertiary care centers nationwide. Recent ICMR data indicates that nearly one in three bacterial infections in India exhibits multidrug resistance. There are extraordinarily high levels of resistance among Enterobacterales against cephalosporins, fluoroquinolones, and carbapenems. The mortality associated with carbapenem-resistant bloodstream infections frequently exceeds 40%. Despite these challenges, progressive models are emerging. The state of Kerala, operating under the National Action Plan on AMR (NAP-AMR), has instituted a pioneering stewardship model with a goal to achieve universal 'antibiotic literacy' by December 2025, heavily regulating antibiotic dispensing and integrating environmental monitoring.

9. The One Health Approach

The sheer complexity and interconnectivity of AMR necessitate a paradigm shift from anthropocentric strategies to the 'One Health' approach. This holistic framework recognizes the inextricable linkages between human health, animal health, and the broader ecological environment. A vast proportion of global antimicrobial consumption occurs in the agricultural and livestock sectors, where antibiotics are extensively used not just for disease treatment, but as growth promoters and for mass prophylaxis. This intense selective pressure fosters the emergence of resistant strains and mobilizable resistance genes in animal microbiomes.

Through direct occupational contact, the food supply chain, and the dissemination of animal waste into water systems and soils, these agricultural resistomes inevitably intersect with human populations. A quintessential example of this cross-sectoral threat is the mcr-1 gene, which confers resistance to colistin—a crucial last-resort antibiotic for human medicine. Initially identified in swine populations in China, mcr-1 rapidly permeated human clinical isolates globally via plasmid transfer. In response, countries like India enforced a strict ban on the use of colistin as an agricultural growth promoter in 2019, reflecting a critical One Health legislative intervention.

Comprehensive AMR mitigation requires synchronized action: optimizing human clinical Antimicrobial Stewardship (AMS), enforcing rigorous veterinary regulations, improving wastewater sanitation to prevent the discharge of pharmaceutical effluents into ecosystems, and accelerating the development of novel diagnostics and non-traditional therapeutics.

10. Conclusion

Antimicrobial resistance is an intricately woven biological, clinical, and socio-economic challenge that stands to reverse a century of medical progress. Bacterial pathogens exhibit breathtaking adaptability, employing molecular mechanisms—target modification, enzymatic degradation, permeability loss, and active efflux—often simultaneously, to thwart our most advanced pharmacological interventions. Biofilm formation further entrenches these pathogens, requiring transformative therapeutic approaches.

While global and national surveillance frameworks, such as WHO GLASS and India's ICMR AMRSN, have illuminated the terrifying scope of resistance, they also highlight the urgent need for expanded, representative data collection and structural investments in healthcare. The discovery of novel agents like lariocidin, advancements in AI-driven drug discovery, and the implementation of rapid molecular and spectroscopic diagnostics offer tangible hope. However, the endurance of modern medicine ultimately relies on our collective commitment to antimicrobial stewardship, sustained innovation in diagnostic engineering, and the rigorous global adoption of the One Health paradigm to secure the efficacy of antimicrobials for future generations.

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Current Research in Human Parasitic Diseases: Innovations in Diagnosis, Treatment, and Control

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1. Introduction

Human parasitic diseases remain a major global health challenge, particularly in tropical and subtropical regions affected by poverty, inadequate sanitation, limited healthcare access, environmental exposure, and vector transmission. Neglected tropical diseases (NTDs) affect approximately 1 billion people, while about 1.4 billion people require preventive or curative interventions (World Health Organization [WHO], 2025a). These diseases contribute substantially to morbidity, disability, malnutrition, developmental impairment, and socioeconomic loss.

Malaria demonstrates both the continuing scale of the problem and the potential of scientific innovation. In 2024, an estimated 282 million malaria cases and 610,000 malaria-related deaths occurred worldwide. The WHO African Region accounted for approximately 94% of cases and 95% of deaths (WHO, 2025b). Despite this burden, progress has been made through the WHO recommendation of two vaccines—RTS,S/AS01 and R21/Matrix-M—for the prevention of *Plasmodium falciparum* malaria in children living in endemic areas (WHO, 2024, 2026a).

Although conventional microscopy and serology remain essential, advances in molecular biology, genomics, nanotechnology, biosensing, artificial intelligence, computational drug discovery, and vaccine technology are enabling more sensitive diagnosis, targeted treatment, and improved prevention. Consequently, the research agenda is shifting toward an integrated model that combines early diagnosis, targeted therapy, drug-resistance surveillance, vaccination, vector and transmission control, and One Health surveillance.

This chapter reviews current developments in these areas, emphasizing innovations with potential for translation into clinical and public-health practice.

2. Changing Landscape of Human Parasitic Diseases

Parasitic diseases are biologically diverse and include protozoan infections such as malaria, leishmaniasis, amoebiasis, giardiasis, toxoplasmosis, and trypanosomiasis, as well as helminthic infections such as schistosomiasis, lymphatic filariasis, soil-transmitted helminthiases, taeniasis, echinococcosis, and hookworm infection.

The epidemiology of these diseases is shaped by multiple interacting factors. Climate variability, urbanization, migration, international travel, agricultural development, environmental modification, and changes in vector distribution can alter transmission patterns and expand or shift endemic areas. Zoonotic reservoirs further complicate disease control because successful elimination may require interventions that extend beyond human populations and address infected animal hosts, vectors, and environmental sources.

Evidence from vaccine-development research indicates that many parasitic diseases are characterized by persistent or progressive infections, transmission through vectors or animal reservoirs, inadequate sanitation, and socioeconomic disadvantage. These features make integrated prevention strategies essential. Such strategies may combine vaccination, vector control, improved sanitation, health education, early diagnosis, effective treatment, and strengthened disease surveillance.

The biological complexity of parasites presents an additional challenge. Unlike many viruses and bacteria, parasites often have complex life cycles involving multiple developmental stages and, in some cases, more than one host. Antigen expression may change substantially during these stages, limiting the effectiveness of immune responses directed against a single parasitic form. In addition, parasites may occupy intracellular or anatomically protected niches and possess sophisticated mechanisms for immune evasion and modulation.

These characteristics help explain why the development of vaccines, diagnostic tools, and antiparasitic drugs has generally progressed more slowly than the development of comparable interventions for many viral and bacterial infections. They also underscore the need for multidisciplinary research that integrates parasitology, immunology, genomics, epidemiology, ecology, and public health.

From Conventional Control to Precision Parasitology

Historically, parasite control has relied on chemotherapy, vector control, sanitation, and behavioral interventions, all of which remain essential.

However, prolonged use of a limited range of antiparasitic drugs can select for resistant parasites, while inappropriate or excessive treatment may cause adverse effects and environmental contamination. The emerging model of precision parasitology integrates molecular diagnosis,genomic surveillance, novel drug targets, advanced drug-delivery systems, rational combination therapy, vaccines, artificial intelligence–assisted surveillance, improved vector-control technologies, and One Health approaches. Its goal is to detect infections earlier, guide treatment more accurately, limit transmission, and anticipate drug resistance rather than simply treat established disease.

These characteristics also underscore the need for multidisciplinary research integrating parasitology, immunology, genomics, epidemiology, ecology, and public health.

3. Innovations in Diagnosis

3.1 Conventional diagnosis: continuing role and limitations

Microscopy remains a cornerstone of diagnostic parasitology; it is relatively inexpensive, widely accessible, and allows for the direct visualization of parasites. While peripheral blood microscopy remains essential for malaria diagnosis, stool microscopy continues to be the standard for identifying intestinal protozoa and helminths (Pistone et al., 2026).

Despite its utility, microscopy's effectiveness is heavily reliant on specimen quality, parasite burden, staining techniques, and the observer's expertise. Consequently, low-level infections, morphologically similar organisms, and intermittent parasite shedding may result in false negatives. Serology offers useful evidence of exposure or immune response, particularly for tissue parasites; however, antibodies often persist long after an infection has cleared, complicating the distinction between active and resolved infections.

These inherent limitations underscore the critical need for diagnostic platforms offering higher analytical sensitivity and specificity. The 2026 biosensor review highlights that conventional methods often struggle to differentiate between latent, recurrent, and acute infections, further justifying the transition toward more sensitive diagnostic platforms (Pistone et al., 2026).

3.2 Molecular diagnostics

The advent of molecular diagnostics has fundamentally transformed parasitology, enabling the detection of parasite-specific nucleic acids—DNA or RNA—rather than relying solely on morphological identification (Pistone et al., 2026). Techniques such as polymerase chain reaction (PCR), quantitative

PCR, and multiplex PCR can detect low parasite burdens while providing precise species-level identification. These methods are particularly advantageous when microscopy is insensitive or when closely related parasite species cannot be reliably differentiated via morphology.

Furthermore, molecular approaches facilitate the identification of genetic markers associated with drug resistance. In malaria, molecular surveillance of resistance-associated variants complements therapeutic-efficacy studies, providing early indicators of changing parasite susceptibility.

LAMP and RPA

Loop-mediated isothermal amplification (LAMP) is particularly promising for resource-limited settings because amplification occurs under isothermal conditions, eliminating the need for a conventional thermocycler. LAMP has been investigated for a range of parasitic infections, including malaria, leishmaniasis, schistosomiasis, and trypanosomiasis (Pistone et al., 2026). Similarly, recombinase polymerase amplification (RPA) provides an alternative isothermal strategy capable of operating at lower temperatures. Both LAMP and RPA can be integrated with lateral-flow detection, fluorescence assays, or smartphone-based readouts. The primary advantage of these technologies lies in the potential to decentralize molecular diagnosis, moving it closer to the patient via portable, rapid, and simplified platforms.

Digital PCR

Digital PCR partitions samples into multiple reaction chambers, allowing for the absolute quantification of nucleic acid targets without relying on conventional calibration curves. Its high analytical sensitivity makes it a powerful tool for detecting low parasite burdens and quantifying pathogen DNA (Pistone et al., 2026). While particularly promising for research applications—including treatment monitoring, low-level parasitaemia analysis, and the validation of other molecular assays—the widespread adoption of digital PCR in field settings remains constrained by high costs and complex instrumentation requirements.

3.3 Next-Generation Sequencing and Metagenomics

Next-generation sequencing (NGS) offers an opportunity to move beyond targeted detection toward the comprehensive characterization of parasite genomes and mixed infections (Pistone et al., 2026).

Metagenomic sequencing can potentially identify unexpected or multiple pathogens in a specimen without requiring a preselected diagnostic target. Sequencing can also contribute to:

parasite species identification;

population-genetic analysis;

transmission mapping;

detection of mixed infections;

identification of drug-resistance markers; and

surveillance of parasite evolution.

For research and surveillance, genomic approaches are particularly valuable because they can reveal parasite diversity that may not be apparent through routine diagnostic methods. However, NGS remains relatively expensive and computationally demanding. Sample preparation, bioinformatic analysis, contamination control, and the interpretation of low-abundance sequences continue to present important practical challenges.

3.4 CRISPR-Based Diagnostics

CRISPR-associated systems have emerged as promising diagnostic platforms because programmable guide RNA sequences can direct nucleases toward highly specific parasite nucleic-acid targets (Li et al., 2024; Pistone et al., 2026).

Cas12- and Cas13-based approaches can generate detectable signals following recognition of their target sequences. When combined with isothermal amplification, CRISPR-based diagnostics may enable sensitive pathogen detection without the need for conventional laboratory infrastructure.

CRISPR-based diagnostic approaches are being investigated for several parasitic infections and represent an important convergence of molecular biology, point-of-care diagnostics, and programmable biosensing. The distinction between CRISPR-based diagnostics and CRISPR-mediated genome editing is important. At present, CRISPR-based detection has more immediate diagnostic relevance, whereas the direct therapeutic genome editing of human parasites remains experimental.

3.5 Biosensors and Nanotechnology

Biosensors are among the most rapidly developing diagnostic technologies in parasitology. A conventional biosensor consists of a biological

recognition element, a transducer, and a signal-processing system (Aminizadeh et al., 2026).

A 2026 scoping review identified 136 relevant articles, including 110 focused on protozoa and 26 on helminths. The technologies examined included immunosensors, DNA biosensors, electrochemical sensors, surface-plasmon-resonance sensors, nanostructured biosensors, optical biosensors, aptasensors, lateral-flow biosensors, and other platforms.

Immunosensors

Immunosensors were the most frequently represented category in the review, with 49 studies involving protozoa and 14 involving helminths. These platforms can detect parasite antigens or host antibodies and offer rapid analysis, high sensitivity, and potential portability (Aminizadeh et al., 2026).

Genosensors and Nanomaterials

Nanomaterials such as gold nanoparticles, carbon nanofibers, and carbon nanotubes can amplify detection signals and improve analytical sensitivity. The review reported that nanomaterial-based platforms achieved detection at picogram and, in some cases, femtogram levels (Aminizadeh et al., 2026).

Examples include gold-nanoparticle-based systems for detecting malaria biomarkers and DNA biosensors for Leishmania species. A cadmium sulfide nanosheet genosensor for Leishmania infantum was reported to detect genomic DNA with a detection limit in the femtogram range, illustrating the analytical potential of nanostructured platforms.

Aptasensors

Aptamers are synthetic nucleic-acid ligands capable of recognizing specific molecular targets. Their stability, ease of modification, and potential for high specificity make them attractive alternatives or complements to antibodies.

Point-of-Care Biosensors

The integration of biosensors with microfluidics, miniaturization, and smartphone-based readouts could facilitate diagnostic testing outside conventional laboratories. This development is particularly relevant in endemic regions where centralized laboratory infrastructure is limited (Aminizadeh et al., 2026).

However, the 2026 review emphasized that many biosensors remain at the proof-of-concept stage. Reproducibility, matrix effects, false-positive results, limited testing with authentic clinical specimens, manufacturing costs, and regulatory requirements remain major barriers to their translation into routine clinical practice.

3.6 Cell-Free DNA, miRNAs, and Extracellular Vesicles

Host- and parasite-derived biomarkers represent an emerging diagnostic frontier (Pistone et al., 2026). Cell-free DNA (cfDNA) in blood and other biological fluids may indicate infection without requiring the recovery of intact parasites. Circulating microRNAs (miRNAs) are also being investigated as markers of host–parasite interactions and disease progression. In addition, extracellular vesicles and exosomes can transport nucleic acids, proteins, and other molecular signals between parasite and host cells. These biomarkers may eventually support minimally invasive diagnosis, disease staging, and treatment monitoring. However, most remain investigational and require standardized validation across diverse populations and clinical settings.

3.7 Multiplex and Point-of-Care Diagnostics

Future diagnostic platforms are likely to combine multiple analytes and detection methods rather than rely on a single target. Multiplex assays could simultaneously identify several parasites or multiple targets from the same parasite. Portable platforms that integrate nucleic-acid amplification, biosensing, and digital interpretation may enable sample-to-answer testing in peripheral healthcare settings. Key priorities include device miniaturization, multiplexing, smartphone-based readouts, affordability, accessibility, and validation under real-world conditions.

Table 1. Examples of point-of-care diagnostic modalities for malaria.

POCT modality	Primary biomarker(s)	Sample matrix	Reported performance*	Major strengths & limitations	Reference
Blood-based RDTs (HRP2/pLDH)	HRP2 (P. falciparum) and/or pLDH	Finger-stick whole blood	Example study: sensitivity 99.8%; specificity 87.6%	Rapid, portable and widely used; performance varies by setting and antigen persistence/HRP2 deletion can complicate interpretation.	Bwire et al. (2019)
Urine malaria test (UMT)	Urinary HRP2 fragments	Urine	Overall trial: sensitivity 79%; specificity 89%	Non-invasive; performance decreases at low parasite density and varies with fever/parasitaemia.	Oyibo et al. (2017)
Molecular POCT (LAMP)	Parasite DNA/RNA	Whole blood	Meta-analysis: pooled sensitivity 96–98%; specificity about 95%	High analytical performance and useful for symptomatic/asymptomatic infection; field deployment still requires equipment and quality assurance.	Picot et al. (2020)

**Reported performance is context-dependent and should not be interpreted as universal test characteristics.*

4. Advances in Antiparasitic Treatment

The therapeutic landscape is evolving beyond conventional compounds toward target-based drug discovery, computational screening, drug repurposing, and advanced drug-delivery systems. Although traditional antiparasitic agents remain indispensable, their clinical utility is frequently

constrained by toxicity, unfavorable pharmacokinetic properties, complex dosing schedules, and the emergence of drug resistance.

4.1 Novel Drug Discovery

High-throughput screening enables the rapid evaluation of large chemical libraries against parasite-specific targets or phenotypic assays. Computational chemistry and machine-learning approaches are increasingly utilized to prioritize promising compounds for laboratory testing. Artificial intelligence (AI) can support multiple stages of the drug-discovery process, includingtarget identification, virtual screening, prediction of compound activity, structure–activity relationship analysis, prediction of drug–target interactionsandoptimization of lead molecules.

Natural products remain a vital source of chemical diversity. Combining natural-product libraries with high-throughput screening and computational prioritization facilitates the identification of novel chemical entities with potent antiparasitic activity (Ebiloma et al., 2025).

4.2 Targeted Molecular Scaffolds

Medicinal chemistry is increasingly focused on molecular scaffolds with parasite-selective mechanisms of action. Triazole derivatives and hybrid molecules are being investigated for their ability to disrupt sterol biosynthesis and other essential metabolic pathways (Isern et al., 2025; Vaishnani et al., 2024).

The optimization of candidate compounds typically evaluates the following properties:

- potency
- parasite selectivity
- metabolic stability
- pharmacokinetic profile
- tissue penetration; and
- potential for limiting resistance.

These strategies are particularly relevant to kinetoplastid parasites, in which sterol metabolism represents a validated therapeutic vulnerability (Fairlamb & Wyllie, 2023; Tauheed et al., 2025).

4.3 Advanced Drug-Delivery Systems

Poor aqueous solubility, rapid metabolism, and inadequate tissue penetration often reduce the effectiveness of traditional antiparasitic drugs. Advanced delivery systems—including liposomes, polymeric nanoparticles, ethosomes, and solid lipid nanoparticles—can modify drug distribution and improve solubility, stability, bioavailability, and controlled release (Tiwari et al., 2023; Zhao et al., 2025).

These systems are particularly relevant to intracellular parasites such as Leishmania, for which therapeutic success depends on achieving adequate intracellular drug concentrations. While nanotechnology enhances the pharmacological properties of antiparasitic agents, clinical translation remains constrained by manufacturing requirements, formulation stability, toxicity, scalability, and regulatory considerations (Nwagwu et al., 2026; Tiwari et al., 2023).

Liposomal Delivery

Liposomal formulations encapsulate active compounds to modify their biodistribution. Lipid-based delivery systems are well-established in the enhanced delivery of antileishmanial agents, increasing therapeutic exposure while minimizing systemic toxicity.

Polymeric Nanoparticles

Polymeric nanoparticles provide controlled drug release and may improve tissue penetration and bioavailability. These systems have been investigated for the delivery of agents such as albendazole and various antimalarial compounds, though clinical application requires further validation regarding safety and manufacturing reproducibility (Tiwari et al., 2023).

Microneedle and Transdermal Systems

Microneedle (MN) arrays represent an emerging approach to minimally invasive transdermal drug delivery. By creating microscopic channels through the stratum corneum, these systems facilitate controlled drug administration while avoiding conventional injections (Gattu et al., 2026; Maia et al., 2025). Although MN platforms remain largely experimental in antiparasitic therapy, they represent a potential innovation in administration and patient compliance.

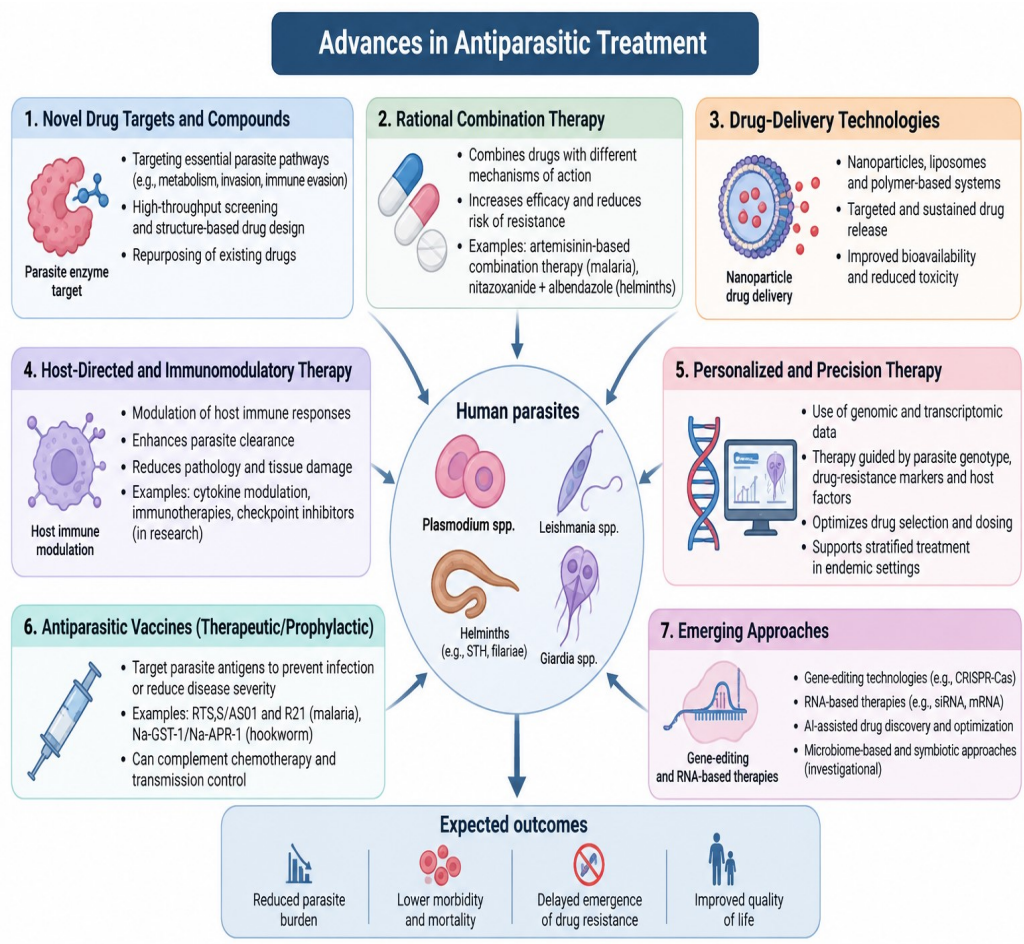


Figure 1. Advances in antiparasitic treatment: emerging therapeutic strategies and their expected outcomes.

5. Drug Resistance and New Therapeutic Strategies

Drug resistance is a critical threat to sustained parasite control, functioning as a dynamic evolutionary process rather than a single genetic event.

5.1 Mechanisms of Resistance

Parasites adapt through several key mechanisms to evade the lethal effects of antiparasitic agents:

Genetic Mutations: Genomic alterations can inhibit drug binding. For example, in Plasmodium falciparum, mutations in the K13 gene are strongly

associated with delayed parasite clearance following artemisinin exposure (Arya et al., 2021).

Enhanced Drug Efflux: Parasites reduce intracellular drug concentrations by up-regulating transporter-mediated efflux systems, a mechanism observed in both malaria and leishmaniasis.

Target Modification: Changes in the structure of the drug target (e.g., enzyme) or increased target expression can reduce drug effectiveness.

Metabolic Adaptation: Parasites reprogram their metabolic and cellular stress pathways to survive drug exposure.

5.2 Combination Therapy

Combination therapy is essential for resistance management. In malaria, artemisinin-based combination therapies (ACTs) pair rapidly acting artemisinin derivatives with longer-acting partner drugs to expose parasites to multiple distinct mechanisms of action simultaneously (Arya et al., 2021). However, resistance to partner drugs can compromise the efficacy of the entire combination, underscoring the need for continuous therapeutic efficacy monitoring and molecular surveillance.

5.3 Drug Repurposing

Drug repurposing evaluates existing, approved medicines for antiparasitic activity. Because these compounds already possess established pharmacokinetic and safety profiles, repurposing significantly reduces the time and cost required for drug development (Andrews et al., 2014). This strategy is particularly valuable for neglected tropical diseases (NTDs), where commercial incentives for de novo drug discovery are limited.

5.4 Nanotechnology against Therapeutic Barriers

While nanocarriers can overcome pharmacokinetic barriers and improve intracellular drug exposure, they are not a universal solution for genetic resistance. Their primary value lies in optimizing drug concentrations at infection sites, reducing toxicity, and improving patient adherence while novel compounds are in development.

5.5 Integrated Resistance Management

Modern strategies emphasize a proactive, multidimensional approach to resistance management. The framework below illustrates the integration of surveillance, testing, and treatment:

Surveillance & early detection

Routine monitoring of treatment outcomes, resistance markers, and parasite populations

↓

Evidence-based therapy

Selection of effective first-line regimens based on current efficacy and resistance data

↓

Combination & targeted therapy

Synergistic drug combinations, optimized dosing, and pathogen-specific strategies

↓

Pharmacokinetic & formulation optimization

Improved absorption, bioavailability, distribution, and adherence through novel formulations

↓

Integrated vector & transmission control

Chemotherapy combined with vector control, reservoir management, vaccination, and prevention

↓

Continuous evaluation & adaptation

Regular reassessment of efficacy, resistance, safety, and programmatic outcomes

Resistance management therefore requires the combined use of therapeutic efficacy testing, molecular surveillance, genomic analysis, and epidemiological monitoring. In malaria, surveillance of K13 and partner-drug resistance markers can provide early warning of declining treatment efficacy (Arya et al., 2021).

6. Innovations in Prevention and Control

6.1 Vaccines

Vaccination represents a critical long-term strategy for reducing the global burden of parasitic diseases. Development efforts focus on inducing robust humoral and cellular immunity while overcoming the challenges posed by parasite immune evasion and complex life cycles.

Malaria vaccination has progressed from experimental development to public-health implementation. The World Health Organization (WHO) currently recommends the RTS,S/AS01 and R21/Matrix-M vaccines for the prevention of P. falciparum malaria in children living in endemic areas, particularly those with moderate-to-high transmission (WHO, 2024, 2026). Vaccination is implemented as part of an integrated malaria-control strategy rather than as a stand-alone intervention. Other approaches currently under investigation include recombinant and DNA-based vaccines designed to enhance cellular immunity and reduce parasite burden.

Finally, hookworm vaccine development has advanced to clinical evaluation of recombinant Necator americanus glutathione S-transferase-1 (Na-GST-1) and aspartic protease-1 [Na-APR-1(M74)] candidates. These antigens participate in the hookworm blood-feeding and haemoglobin-digestion pathways, and the vaccine-development strategy is intended to reduce hookworm infection and its associated morbidity, particularly iron-deficiency anaemia (Hotez et al., 2016; Zinsou et al., 2024).

6.2 Vector Control

Vector control remains fundamental to the management of malaria and other vector-borne parasitic diseases. Conventional interventions include the use of insecticide-treated nets, indoor residual spraying, larval-source management, environmental modification, and the application of repellents and spatial protection. Increasingly, WHO malaria guidelines incorporate newer insecticide classes and spatial emanators within integrated vector-control programs (WHO, 2026b).

In addition to traditional methods, gene-drive technology is being explored as a sophisticated tool for vector control. These technologies employ inheritance-biasing mechanisms to spread selected traits through vector populations. Two primary approaches are under investigation: population suppression, which aims to reduce the total vector population size, and population modification, which seeks to reduce the vector’s competence for

parasite transmission. While CRISPR-based gene drives in Anopheles mosquitoes remain experimental, their potential field deployment necessitates rigorous consideration of ecological effects, resistance evolution, governance frameworks, and community acceptance (Koike & Brindley, 2025).

6.3 Integrated Parasite Management

Effective parasite control requires multimodal interventions, as no single strategy is sufficient for the majority of these diseases. Integrated management strategies combine diagnosis, effective treatment, preventive chemotherapy, and vaccination—where available—with vector control, improved sanitation, health education, and ongoing surveillance. Malaria continues to exemplify this multifaceted approach, with the WHO recommending vaccination alongside diagnostic testing, treatment, and vector-control interventions as components of a comprehensive malaria-control program

6.4 One Health Approaches

Many parasites have animal reservoirs or depend on environmental conditions that cannot be controlled through human medical interventions alone.

A One Health approach always integrateshuman health, veterinary health, environmental surveillance and vector ecology.

7. Emerging Technologies and Future Directions

The next phase of parasitology is likely to be characterized by convergence between technologies rather than isolated innovations.

CRISPR and functional genomics

CRISPR enables targeted investigation of parasite genes involved in survival, development, virulence and drug susceptibility. It can help validate candidate drug targets and clarify mechanisms of resistance. (Koike & Brindley, 2025).

Artificial intelligence

AI can support both diagnosis and drug discovery. In microscopy, deep-learning algorithms can identify parasite morphology from blood or stool images. In drug discovery, machine learning can prioritize compounds from very large libraries. (Parija & Poddar, 2024).

AI can also integrate environmental, demographic and epidemiological information to predict transmission and identify high-risk areas.

Nanotechnology

Nanotechnology has applications across the entire parasitic disease spectrum:

diagnosis → drug delivery → imaging → vaccine delivery.

The 2026 biosensor review illustrates how nanomaterials can improve analytical sensitivity, while nanomedicine approaches can improve therapeutic delivery. (Aminizadeh et al., 2026).

mRNA vaccine platforms

The rapid development of mRNA technology has generated interest in applying the platform to malaria and other complex parasitic diseases. (Hayashi et al., 2022; Scaria et al., 2024).

Potential advantages include rapid redesign, multivalent antigen expression and the ability to combine multiple parasite targets.

However, parasite antigen selection, durability of protection and the complexity of parasite life cycles remain substantial challenges.

8. Challenges and Translational Gaps

Technological innovation does not automatically translate into clinical or public-health impact.

8.1 Biological complexity

Parasites undergo complex developmental transitions and can alter antigen expression during different stages. They may also reside within cells or protected tissues.

These features complicate vaccine design, diagnostic target selection and drug development.

8.2 Drug resistance

Resistance can emerge during widespread use of existing drugs. New therapies must therefore be evaluated not only for immediate efficacy but also for their resistance potential and genetic barrier to resistance.

8.3 Cost

Many advanced technologies require expensive equipment, specialized manufacturing and highly trained personnel.

This creates a paradox: technologies designed to address diseases concentrated in resource-limited settings may initially be least accessible in those same settings.

8.4 Regulatory barriers

Novel biosensors, nanomedicines, gene-editing systems, artificial intelligence-based diagnostics, and RNA-based vaccines require appropriate validation and regulatory pathways before they can be translated into routine clinical or public-health use. Laboratory performance alone is insufficient; candidate technologies must demonstrate analytical and clinical reproducibility, appropriate sensitivity and specificity, safety, manufacturing consistency, stability, cost-effectiveness, and usability under real-world conditions. For biosensors in particular, reproducibility, biological-matrix effects, false-positive results, regulatory approval, manufacturing cost, and scalability remain important barriers to translation (Aminizadeh et al., 2026).

8.5 Field logistics

Field implementation is also constrained by infrastructure and workforce limitations. Rural and remote healthcare facilities may have unreliable electricity, limited refrigeration, inadequate laboratory infrastructure, poor internet connectivity, shortages of trained personnel, and fragile supply chains. Consequently, simplicity, robustness, portability, and operational resilience should be considered core design requirements rather than optional features when technologies are developed for endemic and resource-limited settings.

8.6 Translational gap

Many promising interventions remain at the proof-of-concept or preclinical stage.

For biosensors, the 2026 scoping review emphasizes that relatively few platforms have been tested under real-world conditions. The authors recommend standardized protocols, reliable clinical validation and collaboration among engineers, clinicians and policymakers.

The same principle applies to vaccines, nanomedicine, AI and CRISPR-based technologies.

9. Conclusion

Human parasitic disease control is increasingly shaped by the integration of established interventions with molecular diagnostics, biosensors, advanced drug discovery, nanotechnology, genomic surveillance, artificial intelligence, and next-generation vaccines.

Key advances include more sensitive and decentralized diagnostics; rational, targeted, and formulation-based therapeutic strategies; continuous surveillance for emerging drug resistance; and expanding vaccine and vector-control platforms.

Translation to patient care remains still remains a challenge because of modern age evolving ecology. Technologies must demonstrate clinical validity, safety, affordability, scalability, field robustness, regulatory readiness, and real-world utility in endemic settings.

The most effective future model is therefore integrated rather than technology-specific: precision diagnosis involving both conventional and automated technology, effective treatment, resistance surveillance, vaccination, vector and transmission control, and One Health surveillance should function as complementary components of parasitic disease control.

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Novel Therapeutic Approaches in Infectious Diseases: Phage Therapy, CRISPR and Nanomedicine

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Abstract

Antimicrobial resistance (AMR) is increasingly characterized not only by the loss of individual antibiotics but by the emergence of organisms for which conventional antibacterial strategies provide limited, toxic, or unsustainable options. The supplied reference chapter frames AMR as an evolutionary and ecological problem driven by antibiotic exposure, horizontal gene transfer, biofilm-associated tolerance, inadequate infection prevention, and One Health transmission. It also identifies bacteriophage therapy, CRISPR-Cas-based antimicrobials and nanotechnology-assisted drug delivery among the most important emerging therapeutic directions. This chapter expands that framework into a consultant-oriented review focused on clinical translation.

Phage therapy uses bacteriophages, usually strictly lytic viruses, to selectively infect and destroy bacteria. Its major attractions are species- or strain-level specificity, activity against selected drug-resistant organisms, potential synergy with antibiotics, and the possibility of individualized treatment. Its limitations include narrow host range, bacterial phage resistance, neutralizing antibodies, pharmacokinetic barriers, manufacturing and purification requirements, and a regulatory framework that is still evolving. Recent clinical experience supports feasibility, but the evidence base remains heterogeneous and includes observational studies, early-phase trials and compassionate-use cases.

CRISPR-Cas systems offer a second precision strategy. By directing Cas nucleases or other effectors against bacterial chromosomes, resistance plasmids, virulence determinants or essential genes, they can kill bacteria, reverse resistance phenotypes or selectively edit microbial populations. The principal translational problem is not target design but delivery: the CRISPR payload must reach the relevant bacterial population in sufficient quantity

while avoiding off-target effects, immunological barriers and unintended ecological consequences. Phage vectors, conjugative systems and nanocarriers are therefore central to the field.

Nanomedicine encompasses metallic nanoparticles, polymeric and lipid nanoparticles, liposomes, dendrimers, nanoemulsions, nanocomposites and biologically derived vesicles. These systems can act directly as antimicrobials or as delivery vehicles that alter antibiotic distribution, intracellular penetration, biofilm access, stability and local concentration. For consultants, the important distinction is between an intrinsically antimicrobial nanomaterial and a nanocarrier whose principal function is pharmacological delivery. Most current evidence remains preclinical; toxicity, biodistribution, manufacturing consistency, particle characterization and regulatory classification remain critical barriers.

The practical future is unlikely to be a replacement of antibiotics by a single new platform. Rather, phages, CRISPR systems and nanomedicine are best considered as components of precision anti-infective therapy integrated with microbiological diagnosis, source control, antimicrobial stewardship, pharmacokinetic/pharmacodynamic optimization, infection prevention and One Health surveillance.

Keywords: antimicrobial resistance; bacteriophage therapy; engineered phages; CRISPR-Cas; precision antimicrobials; nanomedicine; nanoparticle drug delivery; biofilm; multidrug-resistant organisms; personalized infectious disease therapy; One Health.

Learning objectives

- Explain why phage therapy, CRISPR-Cas antimicrobials and nanomedicine are being developed in response to specific biological limitations of conventional antibiotics.
- Select clinically relevant situations in which phage therapy could be considered as an adjunct or investigational rescue strategy, while recognizing the current limitations of evidence and regulation.
- Distinguish CRISPR-mediated bacterial killing, resistance-gene targeting, virulence-gene targeting and CRISPR interference, and understand why delivery is the major translational bottleneck.
- Interpret the major nanomedicine platforms used against resistant bacteria and biofilms, including their intended pharmacological advantages and toxicity concerns.

- Evaluate these approaches using consultant-level criteria: microbiological target, mechanism, tissue access, source control, PK/PD, host immunity, resistance evolution, manufacturing quality, regulatory pathway and clinical evidence.
- Recognize where current evidence is mature enough for clinical consideration and where claims remain primarily preclinical or hypothesis-generating.

1. The clinical problem that novel therapeutics are trying to solve

The supplied reference chapter begins from the central premise that the antibiotic era has transformed modern medicine but that the gains are being eroded by AMR. It describes enzymatic antibiotic degradation, target modification, efflux, reduced permeability, biofilm-associated tolerance and horizontal gene transfer as interacting mechanisms rather than isolated phenomena. It also emphasizes the ESKAPE pathogens and the One Health movement of resistance determinants.

That framework remains clinically relevant, but the therapeutic problem is now more granular. A consultant treating a patient with a difficult-to-treat infection is not simply asking whether an organism is 'MDR'. The relevant questions are: Which resistance mechanism is present? Is the phenotype stable? Is the organism in planktonic growth or a biofilm? Is the infection localized or disseminated? Can the source be controlled? Does the drug reach the infected compartment? Is the organism intracellular? Is there a safe active antibiotic combination? And, if conventional options are exhausted, is there a biologically rational non-traditional therapy that can be accessed under a clinical trial, expanded-access or compassionate-use framework?

The contemporary global burden reinforces the need for such options. The GBD 2021 AMR analysis estimated 4.71 million deaths associated with bacterial AMR and 1.14 million deaths attributable to AMR in 2021, with substantial heterogeneity by age, pathogen and geography. The same analysis projected 1.91 million attributable deaths and 8.22 million associated deaths annually in 2050 under its reference scenario. These are modelled estimates rather than observed future counts, and they should be interpreted as scenario-based planning data rather than a fixed forecast. [1]

The WHO 2024 Bacterial Priority Pathogens List identifies 24 pathogens across 15 families and prioritizes organisms according to resistance, burden, transmissibility, treatability and prevention considerations. Critical-

priority organisms include carbapenem-resistant Gram-negative bacteria and rifampicin-resistant Mycobacterium tuberculosis, while high-priority groups include resistant Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecium and multidrug-resistant Neisseria gonorrhoeae. [2] These priorities map directly onto the populations in which precision antibacterial technologies are most likely to be evaluated.

2. Therapeutic logic: from broad-spectrum killing to precision anti-infective medicine

Conventional antibiotics exert selective pressure across large bacterial populations and, depending on spectrum and exposure, can perturb commensal organisms. Precision antimicrobials attempt to narrow the biological target. Phages recognize bacterial surface structures; CRISPR systems recognize nucleic-acid sequences; and nanocarriers can be engineered to alter where, when and how an antimicrobial is delivered. These strategies therefore address different layers of the infection problem.

Platform	Primary biological target	Potential clinical advantage	Key translational limitation
Lytic bacteriophage	Susceptible bacterial cells	Specific bacterial killing; potential activity against MDR isolates; microbiome sparing	Host range, phage resistance, neutralization, formulation and regulation
Engineered phage	Bacterial cells plus engineered genetic function	Expanded host range or delivery of antimicrobial/CRISPR cargo	Genetic stability, biosafety, manufacturing and regulatory complexity
CRISPR-Cas antimicrobial	Defined DNA/RNA sequence	Resistance-gene removal, selective killing or virulence attenuation	Delivery to bacteria in vivo; off-target effects; bacterial anti-CRISPR mechanisms
Polymeric/lipid nanocarrier	Infected tissue or	Improved penetration, stability, controlled	Toxicity, biodistribution,

	intracellular bacterial compartment	release and targeting	scale-up and characterization
Metal/metal-oxide nanoparticle	Bacterial envelope, proteins and redox pathways	Intrinsic antimicrobial activity and multimodal mechanisms	Host-cell toxicity, environmental persistence and dose optimization
Combination platform	Bacterium plus drug/biologic	Can exploit synergy and reduce required dose of individual components	Complex PK/PD, manufacturing and regulatory classification

3. Bacteriophage therapy

3.1 Biology and therapeutic rationale

Bacteriophages are viruses that infect bacteria. Therapeutic development generally focuses on strictly lytic or virulent phages because they replicate in susceptible bacteria and lyse the host cell without requiring a stable lysogenic state. A therapeutic phage must be more than a virus that forms plaques in vitro: it should have a suitable host range, efficient bacterial killing, genomic characteristics compatible with clinical use, acceptable purity and a formulation that permits delivery to the infected site. Modern phage development therefore integrates microbiology, genomics, manufacturing, pharmacology and clinical infectious diseases. [3–5]

The conceptual advantage of phages is biological specificity. Unlike many antibiotics, a lytic phage may distinguish the target pathogen from unrelated organisms. This can be particularly attractive where preservation of the microbiome is clinically important or where broad-spectrum antibiotic exposure is itself contributing to selection pressure. Specificity, however, is a double-edged property: a phage that does not recognize the patient's isolate cannot be expected to work merely because the species is nominally susceptible.

The 2026 Nature Reviews Microbiology review emphasizes that clinical success cannot be reliably predicted from in-vitro testing alone and that therapeutic use requires an understanding of bacterial–phage–host interactions, in-vivo adaptation and the human immune response. [4] This is a crucial

consultant-level point: a plaque assay demonstrates a biological interaction; it does not establish clinical efficacy.

3.2 Phage selection and laboratory characterization

A practical phage-selection workflow should begin with a freshly obtained clinical isolate whenever feasible. The isolate should undergo species identification and susceptibility testing, while the phage laboratory assesses adsorption, plaque formation, killing kinetics and host range. Genomic sequencing is increasingly central to exclude undesirable genes and to characterize the phage with sufficient resolution for clinical-grade development. [3,5]

- Prefer strictly lytic phages for therapeutic use unless a specific engineered application has an alternative rationale.
- Perform complete genome sequencing and annotation; screen for lysogeny-associated elements, known toxin genes, antimicrobial-resistance determinants and other undesirable cargo.
- Characterize host range against the patient's isolate and, where relevant, a local panel of epidemiologically related isolates.
- Assess bacterial killing kinetics rather than relying only on plaque morphology.
- Characterize susceptibility stability because bacterial phage resistance can emerge rapidly.
- Use purified preparations with appropriate microbiological and biochemical quality control, including control of bacterial debris and endotoxin where relevant to the route of administration.

3.3 Phage cocktails, personalized phage therapy and engineered phages

Three broad clinical strategies are emerging. The first is a defined phage product with a relatively fixed composition. The second is a cocktail containing multiple phages to broaden coverage and reduce the probability of single-step phage escape. The third is individualized therapy in which phages are selected or adapted against a patient's isolate. Personalized therapy is particularly attractive for difficult-to-treat infections caused by unusual or highly resistant strains, but it creates challenges for manufacturing, release testing and regulation.

A multicentre, multinational observational analysis of 100 consecutive personalized phage-therapy cases reported clinical improvement in 77.2% of infections and eradication of the targeted bacterium in 61.3%. The most

common infection categories were lower respiratory tract, skin/soft-tissue and bone infections. Concomitant antibiotics were common, and eradication was less likely in cases without concomitant antibiotics. The study also documented phage resistance, in-vitro phage–antibiotic synergy and neutralizing immune responses. Importantly, the authors explicitly noted the uncontrolled nature of the dataset. [6] These findings support feasibility and hypothesis generation; they do not establish comparative efficacy.

Engineered phages can be designed to alter host range, reduce undesirable genes, improve lytic activity, deliver antimicrobial effectors or deliver CRISPR systems. The convergence of phage biology and CRISPR is particularly important because the phage provides a naturally evolved delivery mechanism while CRISPR supplies programmable sequence specificity. [7–9]

3.4 Phage–antibiotic combinations

Phage–antibiotic synergy is biologically plausible through several mechanisms. Antibiotics can change bacterial physiology or expose receptors that improve phage access; phages can reduce bacterial burden and alter phenotypic states; and phage-resistant mutants may incur fitness or virulence costs. The 100-case observational dataset found laboratory evidence of phage–antibiotic synergy in 90% of the small subset of evaluated patients, illustrating the potential of combination therapy but also the limited denominator and non-randomized nature of the evidence. [6]

Clinically, combination therapy should not be framed as an automatic rule. The consultant must consider antibiotic activity, infection site, phage susceptibility, pharmacokinetic compatibility, potential antagonism, bacterial density, source control and the likelihood of emergent resistance. The objective is not simply to add a phage to an antibiotic list but to construct a mechanistically coherent regimen.

3.5 Routes of administration and pharmacology

Phage administration can be intravenous, inhaled, oral, topical, local or delivered directly to an infected compartment. Route selection should reflect the anatomical site, bacterial burden, tissue penetration and local barriers. Intravenous administration introduces systemic pharmacokinetic questions and rapid clearance by the reticuloendothelial system, whereas inhaled delivery may achieve higher local exposure in airway infection but is influenced by mucus, airway inflammation and particle deposition. Local administration can produce high concentrations at a surgical site but depends on adequate drainage and tissue access.

Pharmacokinetic/pharmacodynamic thinking for phages differs from conventional antibiotics because phages can replicate in the presence of susceptible bacteria. This can produce a dynamic interaction between phage concentration, bacterial density, receptor availability, immune clearance and bacterial resistance. Nevertheless, 'self-amplification' should not be assumed to compensate for poor tissue penetration or low bacterial accessibility. Modern clinical development increasingly requires quantitative characterization of exposure, recovery of phage from biological samples, neutralizing antibodies and the relationship between dose, route and microbiological response. [4,5]

3.6 Phage resistance and evolutionary trade-offs

Bacteria can become phage resistant through receptor modification or loss, restriction–modification systems, CRISPR-associated immunity, abortive infection and other defence systems. From a clinical perspective, resistance is not necessarily equivalent to antibiotic resistance. A receptor that supports adhesion, capsule production, efflux or virulence may represent an evolutionary trade-off when altered to evade a phage. This creates the possibility of exploiting phage-driven evolutionary trajectories, although the magnitude and reproducibility of such trade-offs vary by pathogen and phage.

Practical countermeasures include cocktails, sequential phage use, isolation of additional phages, phage adaptation, receptor-aware phage selection and combination with antibiotics. The strategy should be reassessed when cultures remain positive or when susceptibility changes during treatment.

3.7 Safety, manufacturing and regulatory considerations

Potential adverse effects include infusion-related or inflammatory reactions, immunological neutralization, contamination with bacterial products and theoretical concerns related to horizontal gene transfer. Endotoxin control is particularly relevant for preparations produced in Gram-negative hosts and for systemic administration. Quality control must therefore extend beyond phage titre to purity, identity, genetic stability, sterility and route-specific safety attributes.

Regulation is evolving. EMA has published a draft guideline specifically addressing quality aspects of human phage-therapy medicinal products, including manufacture, characterization, specifications, analytical control and stability. The draft recognizes the distinctive characteristics of phages, including specificity, self-propagation, potential evolution and horizontal gene-transfer considerations. [10] In the United States, investigational phage therapy may be accessed through clinical trials or

expanded-access pathways when the regulatory criteria are met; FDA describes expanded access as use of investigational products outside clinical trials for serious or immediately life-threatening disease when no comparable satisfactory alternative exists. [11]

WHO/Europe has similarly emphasized that phages are currently used largely on compassionate grounds in many settings and that more clinical evidence is needed before routine widespread human use. [12] Therefore, consultants should treat phage therapy as a specialized investigational or regulated therapy whose availability depends on jurisdiction, product status and institutional oversight—not as a generally interchangeable antimicrobial prescription.

4. CRISPR-Cas-based precision antimicrobials

4.1 From bacterial immunity to therapeutic platform

CRISPR-Cas systems evolved as adaptive immune mechanisms in bacteria and archaea. Therapeutically, their most important feature is programmable sequence recognition. A guide RNA can direct a Cas nuclease toward a selected bacterial DNA or RNA sequence. Depending on the effector and design, the outcome can be lethal DNA damage, RNA degradation, transcriptional repression, removal of a resistance plasmid or selective alteration of a virulence determinant. [13–16]

The distinction between genome editing and antimicrobial killing is clinically important. A therapeutic CRISPR system can be designed to kill a bacterium by creating lethal DNA damage, to selectively eliminate a resistance plasmid so that the organism becomes antibiotic susceptible again, or to silence a gene without introducing a double-strand DNA break. CRISPR interference (CRISPRi) is particularly interesting because it uses targeting to repress gene expression rather than necessarily cleaving DNA.

4.2 Therapeutic targets

- Resistance genes: blaKPC, blaNDM, mcr-1 and other determinants can be targeted to remove or neutralize specific resistance phenotypes.
- Essential genes: targeting essential bacterial sequences can produce direct killing, although the possibility of target mutation and delivery failure remains.
- Virulence determinants: targeting toxin, adhesion, secretion or regulatory genes may reduce pathogenicity rather than immediately sterilize an infection.

- Plasmid backbones or replication functions: targeting the plasmid rather than the resistance gene alone may facilitate broader plasmid curing.
- RNA targets: Cas13 and related RNA-targeting systems provide an alternative to DNA cleavage and may be useful when transient activity is preferred.

A 2025 systematic review of CRISPR-Cas9 as a precision antimicrobial reported that most studies focused on Enterobacterales, particularly Escherichia coli, with frequent targeting of β-lactam resistance genes and mcr-1-mediated colistin resistance. Delivery included conjugative plasmids, electroporation, phage vectors and nanoparticles. Reported resensitization rates varied widely, reinforcing the point that laboratory efficacy depends heavily on the delivery system, bacterial strain and target architecture. [15]

4.3 Cas systems relevant to antimicrobial development

System	Primary substrate	Potential use	Important caveat
Cas9	DNA	Targeted cleavage; resistance-gene/plasmid targeting	Double-strand DNA break; delivery remains limiting
Cas3	DNA	Processive degradation and potentially strong bactericidal activity	Large/complex effector system; delivery and control challenges
Cas12	DNA	Alternative programmable DNA targeting	PAM and sequence constraints depend on system
Cas13	RNA	RNA degradation and transcript targeting	Transient RNA target; delivery and collateral effects require control
CRISPRi	DNA-guided transcriptional repression	Gene silencing without permanent editing	May inhibit rather than eradicate bacteria

4.4 The delivery problem

The most important translational bottleneck is delivery. A guide RNA and Cas protein cannot simply be administered systemically and expected to enter the relevant bacterial population. The payload must cross host barriers, reach the infection compartment, encounter the target bacterium, enter the bacterial envelope, express or release the CRISPR machinery, and retain activity long enough to alter the intended target.

Phages are attractive vectors because they naturally bind bacteria and deliver nucleic acid. Conjugative systems can transfer CRISPR cargo between bacteria, although their clinical use raises additional ecological and biosafety questions. Nanoparticles can protect nucleic acids and potentially provide targeting or controlled release. Outer-membrane vesicles and other biological delivery systems are also being explored. The choice of vector should be considered part of the therapeutic design rather than an afterthought. [13,14,16]

Delivery also determines selectivity. A CRISPR construct that targets a resistance plasmid may be useful only if it reaches the bacterial population carrying that plasmid. In polymicrobial infection, strain-level targeting may be an advantage, but it can also leave non-target pathogens untreated. The diagnostic workflow must therefore be tightly integrated with therapeutic design.

4.5 CRISPR-mediated reversal of antibiotic resistance

One of the most clinically appealing concepts is antibiotic resensitization. Instead of attempting to replace an antibiotic, CRISPR can remove the genetic determinant responsible for resistance, restoring susceptibility and allowing a conventional antimicrobial to become effective again. This creates a two-component therapeutic logic: precision molecular intervention followed by a familiar antibacterial.

However, successful resensitization in vitro does not guarantee clinical benefit. The resistance determinant may be carried on multiple plasmids, may coexist with chromosomal resistance mechanisms, or may be replaced by another resistance pathway. Furthermore, if the CRISPR system does not reach sufficient numbers of bacteria, the antibiotic component may continue to select for resistant subpopulations. Clinical development therefore requires serial genomic or molecular surveillance rather than relying solely on pre-treatment susceptibility testing.

4.6 Resistance to CRISPR itself and biosafety

Bacteria possess anti-phage and anti-CRISPR mechanisms that can reduce therapeutic activity. Target-site mutation is another obvious escape pathway. If the therapeutic target is a resistance gene, escape can theoretically occur through sequence alteration while retaining the resistant phenotype, depending on the mechanism. Multiplexed guides and conserved target selection may reduce this risk but also increase construct complexity.

Ecological safety requires particular attention when CRISPR systems are delivered through mobile genetic elements. A therapeutic system designed to alter bacterial populations should be evaluated for unintended transfer, persistence, recombination and effects on non-target microbiota. These are not merely theoretical laboratory issues: precision therapy changes the microbial ecosystem and therefore requires ecological as well as individual-patient risk assessment.

5. Nanomedicine for infectious diseases

5.1 What is meant by nanomedicine in AMR?

Nanomedicine is a broad term covering engineered materials and delivery systems at the nanoscale. In infectious diseases, the clinically relevant distinction is between nanomaterials that possess direct antimicrobial activity and nanocarriers whose principal function is to deliver an existing antimicrobial, peptide, nucleic acid or biologic to a desired site. The two may be combined.

Recent reviews describe liposomes, polymeric nanoparticles, lipid-based systems, nanoemulsions, dendrimers, metallic nanoparticles and other nanostructures as approaches capable of improving drug solubility, stability, tissue penetration and targeted delivery. They may also alter bacterial membrane interactions, generate reactive oxygen species, interfere with biofilms or increase local antimicrobial concentration. [17–20]

5.2 Nanocarriers versus intrinsically antimicrobial nanoparticles

Category	Examples	Principal mechanism	Consultant-level consideration
Lipid systems	Liposomes, lipid nanoparticles, nanoemulsions	Encapsulation and controlled delivery	Can improve distribution and reduce systemic exposure, but formulation stability and release kinetics matter
Polymeric systems	PLGA nanoparticles, polymeric micelles, dendrimers	Controlled release, targeting, intracellular delivery	Polymer degradation, particle size and reproducibility affect clinical translation
Metallic systems	Silver, gold, copper and related nanoparticles	Membrane damage, ROS, protein/DNA interactions	Direct antimicrobial action may coexist with host-cell toxicity
Metal-oxide systems	ZnO, TiO2, iron-oxide platforms	ROS, membrane and metabolic effects	Dose, redox biology and tissue accumulation require evaluation
Biological nanoparticles	Extracellular vesicles, outer-membrane vesicles, membrane-coated particles	Natural tropism, cargo delivery, immune interactions	Complex manufacturing, characterization and biological variability
Hybrid systems	Drug + nanoparticle + targeting ligand	Multimodal delivery	Highest potential complexity in CMC and regulatory classification

5.3 How nanomedicine addresses bacterial resistance

Nanocarriers can bypass several barriers described in the supplied reference chapter. Reduced membrane permeability can be addressed by increasing local drug concentration or using particles that interact directly with bacterial envelopes. Efflux-mediated resistance may be partly overcome by sustained intracellular delivery or by co-delivery of an efflux inhibitor. Biofilms can be targeted through matrix penetration, surface adhesion or local release. Intracellular pathogens may benefit from carrier systems capable of entering host cells and releasing antimicrobials near the intracellular bacterial niche.

These mechanisms should not be overinterpreted. A nanoparticle that kills bacteria in a planktonic culture does not automatically penetrate a mature biofilm in a human tissue, and improved cellular uptake does not guarantee therapeutic selectivity. The biological identity of a nanoparticle changes after exposure to plasma and tissue fluids because proteins and other biomolecules form a corona around the particle. This can alter targeting, biodistribution and immune recognition.

5.4 Nanoparticles as direct antimicrobials

Metal and metal-oxide nanoparticles can exert antimicrobial effects through membrane disruption, reactive oxygen species, interaction with thiol-containing proteins, interference with electron transport and damage to nucleic acids. Their multimodal activity may make simultaneous resistance more difficult than resistance to a single molecular target. However, the same physicochemical properties that injure bacteria can injure mammalian cells. Therefore, therapeutic windows must be demonstrated rather than inferred from antibacterial potency alone.

Consultant interpretation should therefore prioritize evidence from mammalian cytotoxicity, hemolysis, immunotoxicity, organ distribution, repeated-dose studies and pharmacokinetic analysis. 'Natural' or 'metal-based' should not be treated as synonyms for safe. Environmental persistence and disposal are additional considerations, particularly for widely used metal nanoparticles.

5.5 Nanomedicine and biofilms

Biofilms are clinically important because the extracellular polymeric matrix limits diffusion, creates metabolic gradients and supports persister populations. The supplied reference chapter identifies Pseudomonas

aeruginosa, Staphylococcus aureus, Acinetobacter baumannii and Candida species among clinically important biofilm-associated pathogens and highlights biofilms as facilitators of tolerance and horizontal gene transfer.

Nanocarriers can be engineered to increase residence within biofilms, respond to pH or enzymes, or release antimicrobial agents in a controlled fashion. Some systems combine antibiotic delivery with photothermal, photodynamic or ROS-generating effects. These strategies are promising but remain predominantly experimental. The key translational question is whether the particle can reach the relevant microenvironment at a concentration and duration that are safe for the host.

5.6 Nanoparticles as delivery vehicles for CRISPR

One of the most interesting convergence points is nanoparticle-mediated delivery of CRISPR cargo. Nanocarriers can protect nucleic acids from degradation and potentially enable controlled release. In principle, this creates a modular platform: the nanoparticle determines delivery and the CRISPR system determines sequence specificity. The combination could be particularly valuable where no suitable therapeutic phage exists or where phage immunogenicity is problematic.

At present, this remains an emerging field. Delivery into bacterial cells in vivo is substantially more difficult than delivery into mammalian cells, because bacterial envelopes, extracellular matrices and infection-site barriers must all be crossed. Translation will require quantitative demonstration of bacterial delivery, target engagement and therapeutic benefit—not simply evidence of nanoparticle uptake.

6. Comparative clinical framework for consultants

These technologies should be assessed using a common clinical framework rather than by novelty. The following sequence can be applied when conventional antibacterial therapy is failing or when a highly resistant pathogen has been identified.

1. Define the infection syndrome and establish source control. Drain collections, remove infected devices when appropriate, debride necrotic tissue and address obstruction. Novel therapeutics cannot compensate for uncontrolled anatomy.
2. Confirm the pathogen and resistance mechanism. Repeat cultures where appropriate, obtain susceptibility testing, and use molecular or genomic methods when they will change therapeutic selection.

3. Define the compartment that must be treated. Consider blood, lung, bone, joint, urinary tract, CNS, skin/soft tissue, prosthetic material and intracellular niches separately.
4. Review standard-of-care and salvage antibiotics with infectious-disease/microbiology input. Optimize dose, infusion strategy, combination therapy and PK/PD before declaring conventional therapy exhausted.
5. Identify whether a precision platform has a rational biological target. For phage therapy, this is demonstrated phage susceptibility; for CRISPR, a validated sequence target plus a feasible delivery route; for nanomedicine, a defined pharmacological or targeting problem that the formulation is designed to solve.
6. Assess access and regulatory status. Determine whether the product is approved, investigational, available through a clinical trial, or accessible only through an expanded-access/compassionate pathway in the relevant jurisdiction.
7. Establish microbiological endpoints before treatment. Define what constitutes success: clinical response, culture clearance, quantitative bacterial burden, resistance-gene loss, susceptibility restoration or another predefined endpoint.
8. Plan for evolutionary escape. Establish when repeat cultures, phage susceptibility testing or sequencing will be performed and what alternative intervention will be used if resistance emerges.
9. Monitor safety using platform-specific endpoints. For phages, consider inflammatory reactions, neutralization and preparation quality; for CRISPR, delivery, immune and off-target considerations; for nanoparticles, organ toxicity, hypersensitivity and biodistribution.
10. Document the intervention prospectively. Novel therapies should ideally be incorporated into institutional multidisciplinary review, pharmacovigilance and structured outcome reporting to strengthen the evidence base.

7. Patient scenarios where these approaches may become relevant

7.1 Difficult-to-treat Pseudomonas aeruginosa lung infection

In chronic airway infection, particularly cystic fibrosis or bronchiectatic disease, the clinical problem is often a high-density bacterial population within mucus and biofilm-like structures. Inhaled phage preparations are being studied because local delivery may increase exposure at the site of infection.

ClinicalTrials.gov lists a randomized, placebo-controlled phase 1b/2a study of inhaled AP-PA02 in cystic fibrosis patients with chronic P. aeruginosa infection. [21] This illustrates the transition from case reports to controlled clinical development, but the existence of a trial should not be equated with established efficacy.

7.2 MDR/XDR wound or bone infection

Chronic wounds, prosthetic infections and osteomyelitis are attractive contexts for phage research because they may involve biofilms, prolonged treatment courses and limited antibiotic options. Local or topical delivery can potentially achieve high concentrations. Nevertheless, debridement and removal of infected foreign material remain central. Phage therapy should be viewed as an adjunct to source control rather than a replacement for it.

7.3 Carbapenem-resistant Enterobacterales

CRE infections illustrate the potential logic of CRISPR-mediated resistance-gene targeting. A blaKPC- or blaNDM-directed system could, in principle, reduce resistance and restore susceptibility to a conventional antibiotic. The major unanswered clinical question is whether sufficient delivery to the infected bacterial population can be achieved safely. This is currently a translational research problem rather than routine bedside therapy.

7.4 Polymicrobial or diagnostically uncertain infection

High-specificity therapies are less attractive when the pathogen is unknown, when several pathogens contribute to disease, or when the target organism represents only part of the pathogenic process. Broad-spectrum antibiotics remain important in initial empiric therapy for severe sepsis when indicated. Precision approaches are more logically considered after microbiological clarification or in a highly characterized chronic infection.

8. Combining phages, CRISPR and nanomedicine: toward modular anti-infective platforms

The most important research direction may be convergence rather than competition. A phage can provide bacterial tropism; CRISPR can provide programmable molecular specificity; and a nanoparticle can provide protection, controlled release or alternative delivery. Such a system could theoretically be tailored to a resistance determinant in a defined pathogen while minimizing exposure of the surrounding microbiota.

Another model is sequential therapy. A phage may reduce bacterial burden or select for receptor changes, after which antibiotic susceptibility improves. CRISPR may remove a resistance plasmid, followed by an antibiotic whose activity has been restored. A nanocarrier may deliver an otherwise poorly penetrant antibiotic into an intracellular or biofilm-associated compartment. These are mechanistic hypotheses that require clinical validation.

Combination	Rationale	Potential benefit	Main uncertainty
Phage + antibiotic	Parallel or synergistic bacterial killing	Higher efficacy; possible resensitization	Timing, antagonism/synergy and resistance evolution
Phage + CRISPR	Phage-mediated delivery of programmable antimicrobial cargo	Sequence-specific killing or resistance-gene targeting	Delivery efficiency and genetic stability
Nanocarrier + antibiotic	Improved tissue/biofilm delivery	Higher local exposure, lower systemic dose	Clinical PK and safety
Nanocarrier + CRISPR	Protected nucleic-acid delivery	Precision targeting without reliance on phage tropism	Bacterial uptake and intracellular release
Phage + nanocarrier	Protect or localize phage preparations	Potentially improved stability or tissue residence	Effect on infectivity and manufacturing complexity
Phage + CRISPR + nanomedicine	Integrated targeting, payload and delivery	Highly modular precision therapy	Very high CMC, regulatory and biological complexity

9. Evidence hierarchy and current maturity

A recurring problem in novel anti-infective research is the mismatch between biological plausibility and clinical evidence. Consultant interpretation should explicitly separate evidence levels.

Evidence level	Typical evidence	What it can establish	What it cannot establish
Mechanistic / in vitro	Plaque assays, MIC changes, gene targeting, biofilm assays	Biological plausibility and mechanism	Clinical efficacy, human PK or safety
Animal model	Murine or other infection models	In-vivo activity, preliminary toxicity and delivery	Human efficacy and human PK/PD
Case report/series	Compassionate use or individualized treatment	Feasibility, signal detection, unusual safety observations	Comparative effectiveness or causal efficacy
Early-phase trial	Safety, tolerability, dose and pharmacology	Feasible clinical development pathway	Definitive clinical benefit
Randomized controlled trial	Comparator-controlled clinical outcomes	Efficacy and safety under defined conditions	Generalizability to all pathogens/settings
Real-world evidence	Registries, multicentre cohorts	Implementation, heterogeneity and rare outcomes	Causal inference without appropriate design

Phage therapy currently has a mixture of compelling mechanistic data, case-based experience, observational cohorts and early-phase trials. The 100-case personalized therapy cohort is an important real-world dataset, but it is retrospective and uncontrolled. [6] WHO/Europe has called for stronger evidence on efficacy, safety and feasibility, while EMA is developing quality expectations for human phage products. [10,12] CRISPR antimicrobials are earlier in the translational pathway; recent reviews emphasize that delivery remains a central barrier and that clinical application is still in its early stages. [13,14] Nanomedicine has a large preclinical literature, but human infectious-disease translation is constrained by toxicity, reproducibility, scale-up and regulatory classification. [17–20]

10. Manufacturing, quality and regulatory science

10.1 Chemistry, manufacturing and controls (CMC)

Novel anti-infective platforms cannot be evaluated clinically without robust CMC systems. For phages, the active biological entity is replicative and genetically variable by nature, so identity, purity, genomic stability, potency and contaminant control require specialized assays. For CRISPR products, the quality profile may include nucleic-acid sequence integrity, guide identity, Cas protein quality, vector characteristics and delivery-particle properties. For nanomedicines, particle size distribution, surface charge, morphology, encapsulation efficiency, release kinetics, aggregation, sterility and batch-to-batch reproducibility may all be clinically relevant.

Regulatory classification is also important. A product that combines a phage with a CRISPR cargo or a nanoparticle with a biological payload may cross conventional product categories. Early engagement with regulators can prevent late-stage redesign of manufacturing processes or clinical endpoints.

10.2 Pharmacovigilance

Post-treatment monitoring should include both clinical and microbiological safety. With phages, emergence of neutralizing antibodies may reduce repeat dosing efficacy; with CRISPR, persistence and unintended biological activity require attention; with nanomaterials, delayed tissue accumulation or organ toxicity may not be captured by short inpatient follow-up. Novel therapies therefore benefit from longitudinal registries with standardized adverse-event definitions.

11. One Health implications

The supplied reference chapter emphasizes that AMR is maintained across human, animal and environmental compartments and identifies conjugation, transformation and transduction as mechanisms that disseminate resistance genes. □filecite□turn0file0□L233-L257□ This is especially relevant to precision antimicrobials because phages, mobile genetic elements and nanoparticles can also interact with microbial ecosystems outside the treated patient.

Phage research already extends beyond human medicine into veterinary and environmental applications. WHO/Europe describes phages as potentially useful across human, animal and plant settings while emphasizing the need for evidence and governance. [12] The same One Health framework should be

applied to CRISPR and nanomedicine: therapeutic release into patients should be assessed not only for individual benefit but also for potential persistence, horizontal transfer, ecological exposure and downstream selection.

12. Consultant's practical checklist

Question	Phage therapy	CRISPR-Cas	Nanomedicine
Is the pathogen confirmed?	Essential; isolate-level susceptibility preferred	Essential; sequence target required	Important for targeted systems
Is there a defined biological target?	Phage receptor/host susceptibility	Specific DNA/RNA sequence	Tissue, cell or bacterial target
Is source control adequate?	Mandatory principle	Mandatory principle	Mandatory principle
Main delivery problem	Tissue access and immune clearance	Entry into target bacteria	Biodistribution and controlled release
Main resistance concern	Phage escape	Target mutation/anti-CRISPR	Biological adaptation plus formulation-dependent effects
Key safety issue	Purity, inflammatory response, neutralization	Off-target activity and delivery safety	Host toxicity and biodistribution
Current clinical maturity	Clinical trials + individualized/ compassionate use	Predominantly preclinical/early translational	Predominantly preclinical for AMR indications
Best current role	Specialist adjunct/investigational rescue	Research/clinical-trial setting	Targeted delivery research and selected approved nanomedicines, not routine AMR therapy

13. Future directions

Several developments are likely to determine whether these platforms become routine components of infectious-disease medicine.

- Rapid isolate-to-therapy pipelines: integration of whole-genome sequencing, phage susceptibility testing and computational matching could shorten the time required to select a therapeutic phage.
- AI-assisted phage and CRISPR design: computational prediction may improve host-range matching, guide selection and identification of conserved targets, but predictions require experimental validation.
- Adaptive and modular phage libraries: continuously updated libraries may make personalized therapy faster and reduce dependence on single fixed cocktails.
- Standardized clinical endpoints: future trials should distinguish microbiological eradication, clinical cure, recurrence, resistance emergence and microbiome effects.
- Quantitative PK/PD models: therapeutic development needs exposure-response relationships rather than simple dose escalation.
- Combination precision therapy: phage–antibiotic, CRISPR–antibiotic and nanoparticle–antibiotic combinations may be more practical than monotherapy.
- Regulatory harmonization: standardized approaches to manufacturing, release testing and individualized products are necessary for scalable clinical access.
- Global access: technologies that require expensive individualized manufacturing may widen inequity unless platform manufacturing, regional libraries and affordable diagnostics are developed in parallel.
- Microbiome-aware therapy: future precision antimicrobials should measure not only pathogen reduction but also collateral microbiome effects.
- One Health surveillance: therapeutic development should be integrated with genomic surveillance of resistance and ecological monitoring.

14. Conclusion

Novel anti-infective therapeutics are being developed because AMR is no longer adequately described as a shortage of new antibiotics. It is a problem of bacterial evolution, ecological selection, biofilm physiology, compartmental pharmacology, horizontal gene transfer and limitations in drug discovery. The supplied reference chapter provides this foundation by linking resistance

mechanisms to the need for alternative strategies and by identifying phages, CRISPR-Cas systems and nanotechnology among emerging approaches.

Phage therapy has the most direct clinical experience among the three platforms, particularly in individualized treatment of difficult-to-treat infections, but its specificity, resistance dynamics, immune neutralization and regulatory complexity prevent simple extrapolation from conventional antibiotics. CRISPR-Cas offers an unprecedented level of molecular precision and the possibility of directly targeting resistance genes or virulence determinants, yet delivery into clinically relevant bacterial populations remains the principal barrier. Nanomedicine provides a versatile engineering layer that can improve antimicrobial delivery, biofilm penetration and intracellular exposure, but the gap between compelling preclinical performance and clinical validation remains substantial.

For consultants, the key message is therefore pragmatic: these technologies should be selected for a defined biological problem, embedded within high-quality microbiology and source control, and evaluated according to the strength of human evidence available for the specific product and infection. The future of infectious-disease therapeutics is likely to be combinatorial and precision-based, with conventional antibiotics retained as essential tools while phages, CRISPR and nanomedicine expand the therapeutic repertoire against pathogens for which existing approaches are insufficient.

Key take-home points

1. AMR is a biological and ecological problem; novel therapeutics should be matched to the mechanism of resistance and the anatomical site of infection.
2. Phage therapy is highly specific and can be personalized, but susceptibility must be demonstrated and clinical efficacy cannot be inferred from plaque assays alone.
3. Phage resistance can emerge; cocktails, sequential therapy and antibiotic combinations are strategies under investigation.
4. CRISPR-Cas systems can target resistance genes, essential genes, virulence determinants or bacterial RNA, but delivery is the dominant translational challenge.
5. CRISPR-mediated antibiotic resensitization is conceptually different from conventional antibiotic killing and may be useful as a two-step precision strategy.

6. Nanomedicine can provide direct antimicrobial activity or improve the delivery of existing antibiotics and nucleic-acid therapeutics.
7. Nanoparticle toxicity, biodistribution, manufacturing reproducibility and regulatory classification must be considered as carefully as antimicrobial potency.
8. Current evidence is heterogeneous: phage therapy has entered clinical trials and real-world cohorts, whereas CRISPR antimicrobials and many nanomedicine applications remain primarily preclinical.
9. Source control, antimicrobial stewardship, rapid diagnostics and infection prevention remain foundational even when novel therapeutics are used.
10. One Health governance is essential because resistance genes, phages, mobile genetic elements and engineered biological systems can move across human, animal and environmental compartments.

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Emerging Zoonoses at the Human–Animal–Environment Interface

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Abstract

Emerging zoonotic diseases represent a major challenge to global public health because they arise from complex interactions among humans, animals, pathogens and the environment. Zoonoses are infectious diseases that can be naturally transmitted between animals and humans and may be caused by viruses, bacteria, fungi and parasites. Changes in land use, deforestation, agricultural intensification, urbanization, wildlife trade, climate change, biodiversity loss and increasing human–animal interactions can create opportunities for pathogens to cross species barriers. The concept of One Health recognizes that human health is closely connected to animal health and environmental health and therefore promotes integrated approaches to disease prevention, surveillance and control. This chapter discusses the emergence of zoonotic diseases at the human–animal–environment interface, mechanisms of pathogen spillover, major ecological and anthropogenic drivers, selected emerging zoonoses, climate change, antimicrobial resistance, surveillance, genomic technologies and the role of artificial intelligence. Case examples including avian influenza, Nipah virus, rabies and Ebola illustrate the importance of interdisciplinary approaches. The chapter concludes that effective prevention requires a shift from reactive outbreak response toward integrated, predictive and preventive One Health systems.

Keywords: Zoonoses, One Health, spillover, emerging infectious diseases, wildlife, climate change, surveillance, genomic epidemiology, artificial intelligence.

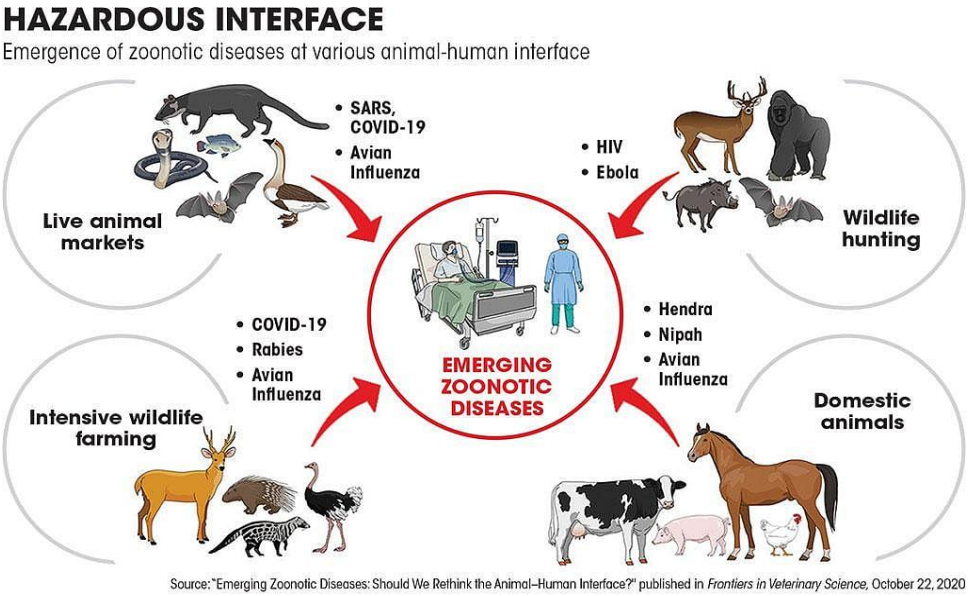
Introduction

Humans live within complex ecological systems shared with domestic animals, livestock, wildlife, insects, microorganisms and natural resources. This interconnectedness creates opportunities for pathogens to move between

species. Diseases that naturally transmit between animals and humans are known as zoonotic diseases or zoonoses**.**

Zoonoses may be transmitted by direct contact with infected animals, contaminated food or drink, vectors such as mosquitoes and ticks, respiratory droplets or aerosols, and contaminated ambient elements. The Centers for Disease Control and Prevention (CDC) report that around 3 out of 4 new or emerging infectious diseases in humans originate from animals, and that over 6 out of 10 recognized infectious diseases in people can spread from animals.

A zoonotic disease's appearance does not always indicate the appearance of a brand-new pathogen. When a pathogen spreads to a new area, infects a new host, creates a new route of transmission, or significantly rises in frequency, it might become an emergent concern. Consequently, the human environment is crucial. Therefore, understanding of new nfectious infectious infectious diseases.



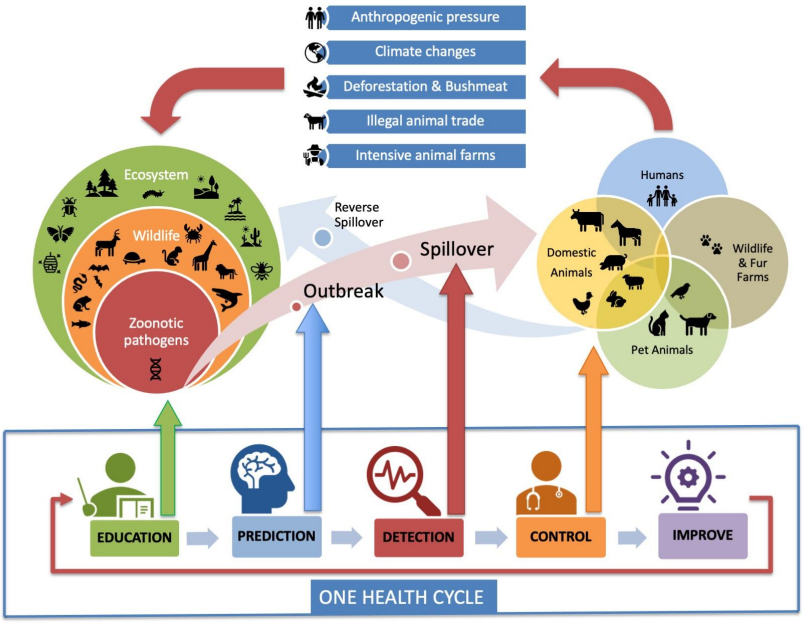


Figure1. Human–Animal–Environment Interface

Zoonotic Spillover

The movement of a pathogen from an animal reservoir or environmental source into humans is commonly referred to as **zoonotic spillover**.

A simplified spillover pathway can be represented as:

Animal reservoir → Human exposure → Spillover → Human infection → Transmission → Outbreak

However, spillover is not a single event. A pathogen must overcome several biological and ecological barriers before sustained human transmission can occur.

The factors are as follows:

- frequency of contact between humans and animals;
- pathogen abundance;
- route of exposure;
- ability of the pathogen to infect human cells;

- ability to replicate within humans;
- host susceptibility;
- environmental conditions; and
- availability of subsequent human-to-human transmission pathways.

Thus, the presence of a pathogen in wildlife does not automatically mean that an outbreak will occur**.**

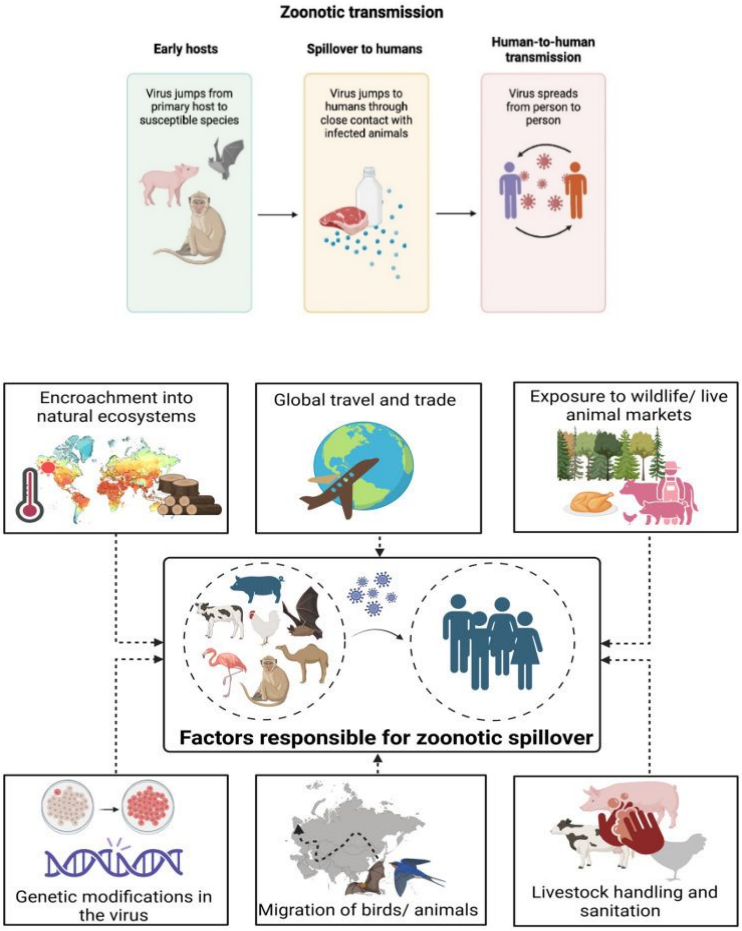


Figure 2. Zoonotic Spillover Cascade

Human–Animal–Environment Interface

The human–animal–environment interface represents locations and situations where humans, animals and ecosystems interact.

Important interfaces include:

- agricultural farms;
- livestock markets;
- wildlife habitats;
- forest margins;
- urban settlements;
- food-processing facilities;
- animal farms;
- water bodies;
- veterinary facilities; and
- households with companion animals.

Transmission may occur through multiple routes.

Transmission route	Examples
Direct contact	Bites, scratches, saliva, blood and body fluids
Foodborne	Contaminated meat, milk, eggs and other animal products
Waterborne	Contaminated drinking or recreational water
Vector-borne	Mosquitoes, ticks and fleas
Environmental	Contaminated soil, water and surfaces
Respiratory	Infectious droplets or aerosols
Fomites	Contaminated equipment, clothing and objects

The environment is therefore not merely a passive background. It can act as a **reservoir, transport medium and mediator**of disease transmission.

Drivers of Emerging Zoonoses

The emergence of zoonotic diseases is usually the result of several interacting factors rather than a single cause.

➢ Deforestation and Land-Use Change

Deforestation and conversion of natural ecosystems into agricultural land, roads and settlements can disturb wildlife habitats. Animals may relocate

in close proximity to livestock or human populations, which increases the risk of infection transmission.

The distribution and abundance of wildlife, vectors, and reservoir hosts can all be altered by forest fragmentation.

➢ Agricultural Intensification

Intensive livestock production can involve large animal populations living in close proximity.

Under inadequate biosecurity conditions, infectious agents can circulate efficiently within animal populations.

Preventive measures are as follows :

- animal vaccination;
- farm biosecurity;
- hygiene;
- veterinary surveillance;
- appropriate animal movement controls; and
- responsible antimicrobial use.

➢ Wildlife Trade and Human–Wildlife Interaction

Wildlife may harbor pathogens without showing obvious disease.

Activities involving capture, transportation, processing and close human contact can increase opportunities for pathogen transmission.

Wildlife conservation and disease prevention should therefore be considered together rather than treated as completely separate issues.

➢ Urbanization

Rapid urban growth changes the ecological relationships between humans, domestic animals and wildlife.

Urban environments may support:

- rodents;
- mosquitoes;
- stray animals;
- contaminated water;

- foodborne pathogens.

Poor sanitation and inadequate waste management can further increase exposure.

➢ Climate Change

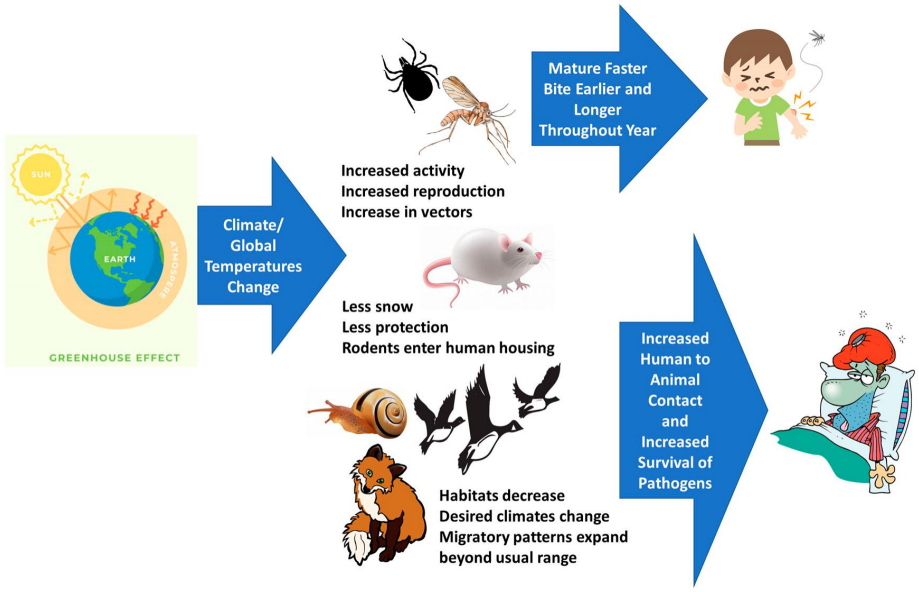
Climate change can modify the distribution and seasonal activity of vectors, wildlife and pathogens.

Changes in:

- temperature;
- rainfall;
- humidity;
- flooding;
- drought; and
- vegetation

can influence disease transmission.

WHO identifies climate change, biodiversity loss, land-use change and pollution as important environmental factors affecting One Health.



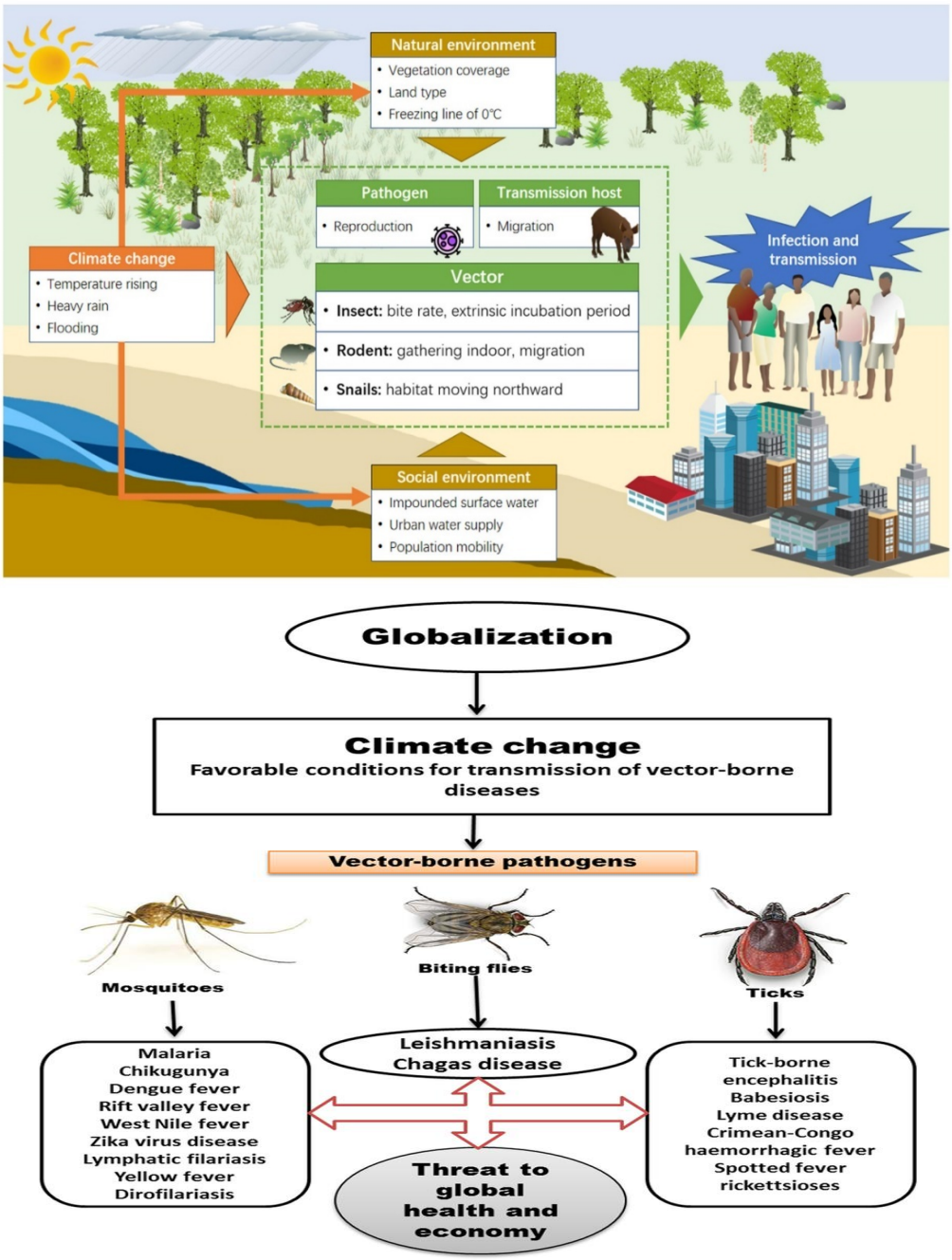


Figure 3. Climate Change and Zoonotic Disease Risk

Selected Emerging and Re-emerging Zoonoses

➢ Avian Influenza

Avian influenza viruses circulate among wild and domestic birds. Some strains can infect mammals, including humans.

The ecology of avian influenza demonstrates the interaction between:

Wild birds → Poultry → Farm environments → Humans

The disease highlights the importance of animal surveillance, farm biosecurity and monitoring of viral evolution.

➢ Nipah Virus

Nipah virus is an important example of a zoonotic disease involving wildlife reservoirs and human–animal interactions.

Fruit bats are recognized as natural hosts. Transmission pathways may involve infected animals, contaminated food or direct contact.

Nipah outbreaks have demonstrated the importance of:

- wildlife surveillance;
- infection prevention;
- rapid diagnosis;
- contact tracing;
- community awareness.

➢ Rabies

Rabies is a classic example of a zoonotic disease that can be prevented through effective animal and human health interventions.

Dogs are responsible for the vast majority of human rabies transmission globally.

Important interventions include:

- mass dog vaccination;
- responsible dog population management;
- immediate wound care;
- post-exposure prophylaxis;

- public education.

Rabies demonstrates why animal vaccination can be an essential component of human disease prevention.

➢ Ebola Virus Disease

Ebola illustrates the complex interaction between wildlife, humans, health systems and environmental conditions.

The disease has highlighted the importance of:

- early detection;
- infection prevention and control;
- community engagement;
- laboratory diagnosis;
- surveillance;
- safe and culturally appropriate outbreak response.

➢ Rift Valley Fever

Rift Valley fever demonstrates the importance of vector ecology, livestock health, rainfall and environmental conditions.

Mosquito populations can increase under suitable environmental conditions, creating opportunities for transmission among animals and humans.

One Health Approach

The **One Health approach** recognizes that the health of humans, animals and ecosystems is interconnected.

The modern One Health framework involves collaboration among:

- medical professionals;
- veterinarians;
- microbiologists;
- epidemiologists;
- environmental scientists;
- ecologists;
- agricultural scientists;

- public-health professionals;
- data scientists;
- policymakers; and
- local communities.

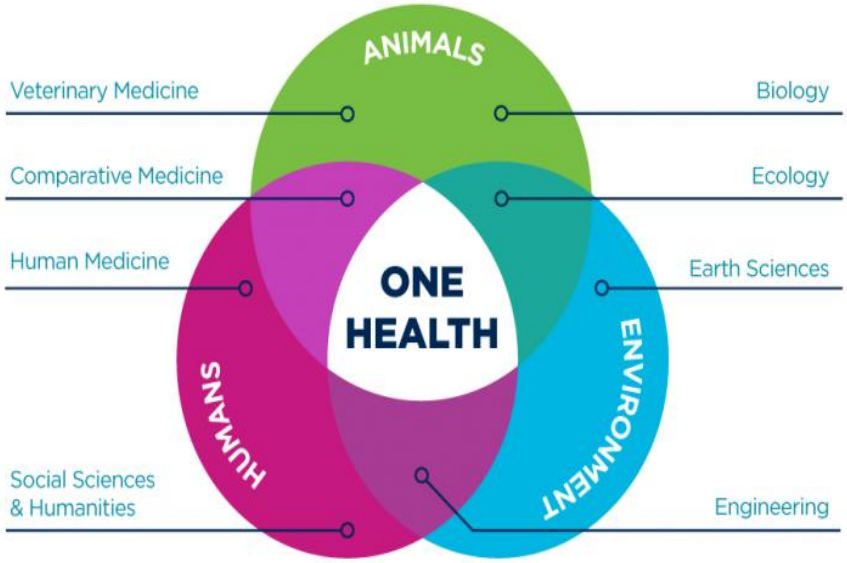


Figure 4. One Health integrates human, animal and environmental health.

A successful One Health strategy should address four major stages:

Prevent → Detect → Respond → Recover

Prevention

- vaccination;
- sanitation;
- biosecurity;
- safe food handling;
- vector control;
- environmental protection.

Detection

- clinical surveillance;
- veterinary surveillance;
- wildlife monitoring;
- laboratory diagnosis;
- genomic surveillance.

Response

- outbreak investigation;
- rapid communication;
- infection control;
- animal-health measures;
- community engagement.

Recovery

- ecosystem restoration;
- economic recovery;
- evaluation of interventions;
- strengthening of preparedness systems.

Genomic Surveillance

Modern sequencing technologies have transformed infectious disease surveillance.

Pathogen genomic data can provide information regarding:

- genetic variation;
- evolutionary relationships;
- emergence of variants;
- geographical distribution;
- transmission patterns.

A simplified genomic surveillance workflow is:

Sample collection → Genome sequencing → Bioinformatics → Phylogenetic analysis → Epidemiological interpretation → Public-health response

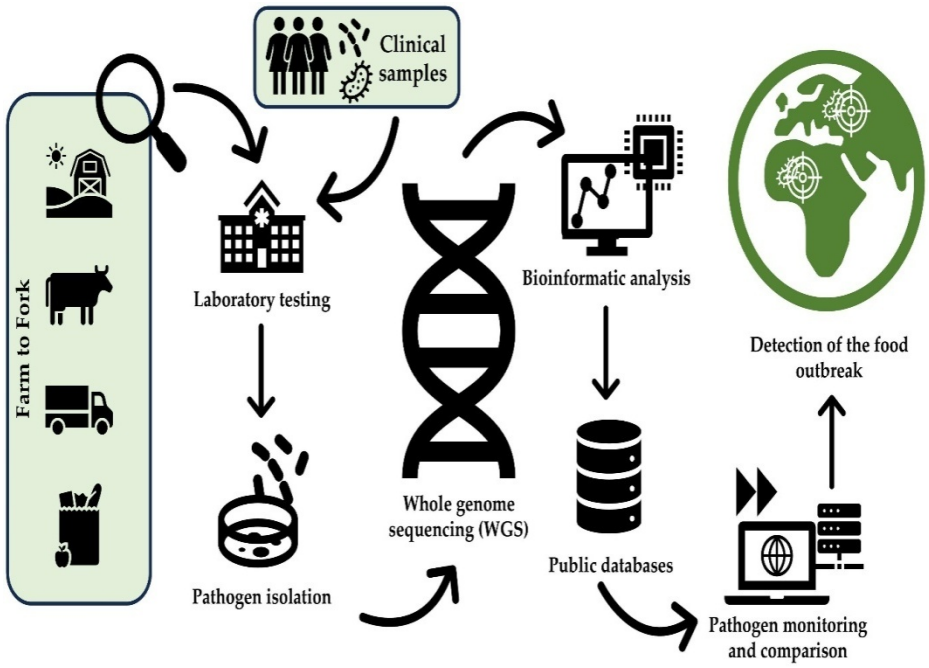


Figure 5. Genomic Surveillance Workflow

Genomic information becomes especially powerful when combined with epidemiological, environmental and animal-health data.

Climate Change and the Future of Zoonoses

Climate change is likely to influence zoonotic disease ecology in several ways.

Environmental change

↓

Temperature/rainfall changes

↓

Changes in vectors and reservoirs

↓

Changes in pathogen survival

↓

Changes in human exposure

↓

Altered disease risk

For example, changes in temperature and rainfall can influence mosquito and tick populations and therefore affect vector-borne disease transmission.

However, disease emergence cannot be attributed to climate change alone. Land use, human behavior, socioeconomic conditions, healthcare access and pathogen characteristics also influence disease patterns.

Community Participation

Communities are an essential component of One Health surveillance.

Farmers, animal owners, forest workers, veterinarians and local healthcare workers may notice unusual events before they appear in formal surveillance systems.

Examples include:

- unexplained animal deaths;
- unusual wildlife behavior;
- increased mosquito abundance;
- unusual human illness;
- contamination of water sources.

Effective communication should be:

- scientifically accurate;
- culturally appropriate;
- transparent;
- locally understandable;
- sensitive to uncertainty.

Emerging zoonoses develop at the nexus of environmental, animal, and human health. Ecological upheaval, land-use change, agricultural practices, wildlife interactions, urbanization, climate change, and global connection all have an impact on their emergence.

The central lesson is that zoonotic diseases cannot be effectively controlled through medical interventions alone. Disease prevention must extend from hospitals to farms, forests, wildlife habitats, food systems, water systems and communities.

A framework for combining these areas is offered by the One Health approach. Artificial intelligence, remote sensing, genetic surveillance, and environmental monitoring are examples of contemporary methods that can improve risk assessment and early identification.

In order to respond to current zoonoses and lessen the likelihood that future spillover incidents would turn into serious public health emergencies, it is ultimately crucial to comprehend the human–animal–environment interface.

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Emerging Trends in Medical Mycology: Diagnosis, Management, and Antifungal Resistance

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Abstract

Fungal infections have transitioned from opportunistic secondary complications to major global health threats. Driven by expanding immunocompromised populations, environmental changes, and intense agricultural fungicide use, emerging multidrug-resistant fungal pathogens—most notably *Candida auris* and triazole-resistant *Aspergillus fumigatus*—pose substantial challenges to healthcare systems. This chapter explores recent diagnostic paradigm shifts towards molecular testing, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and point-of-care assays; reviews therapeutic advances, including novel antifungal classes such as rezafungin, ibrexafungerp, fosmanogepix, and olorofim; and delves into the molecular mechanisms driving antifungal resistance across azoles, echinocandins, and polyenes. Finally, an integrated One Health framework is presented to curb environmental drivers of resistance and safeguard clinical antifungal efficacy.

Keywords: Medical mycology, *Candida auris*, triazole resistance, antifungal stewardship, novel antifungals, One Health.

Introduction

The field of medical mycology has undergone a significant paradigm shift over the past two decades. Historically considered rare or opportunistic pathogens affecting limited clinical cohorts, fungal diseases now account for substantial morbidity and mortality globally. Invasive candidiasis, invasive aspergillosis, mucormycosis, and cryptococcosis contribute substantially to global morbidity and mortality.

The evolution of clinical fungal epidemiology is accelerated by three main factors:

* Expansion of susceptible host populations: Increased use of targeted immunosuppressive therapies, solid-organ and hematopoietic stem-cell transplantation, intensive care advancements, and conditions like post-viral hyper-inflammation (e.g., COVID-19-associated pulmonary aspergillosis [CAPA] and mucormycosis).
* Emergence of Multidrug-Resistant (MDR) Pathogens: Species such as *Candida auris* exhibit intrinsic or rapidly acquired resistance to multiple antifungal classes, persisting on hospital surfaces and driving nosocomial outbreaks.
* Environmental Resistance Drivers: Broad-spectrum demethylation inhibitor (DMI) fungicides used in crop protection have driven cross-resistance to clinical triazoles in species like *Aspergillus fumigatus*.

Diagnostic Innovations in Medical Mycology

Prompt and accurate diagnosis remains crucial for reducing mortality in invasive fungal diseases. Conventional diagnostic methods—microscopy and culture—often suffer from low sensitivity and extended turnaround times. Recent technological advancements have revolutionized early detection:

1. Mass Spectrometry & Phenotypic Identification

* MALDI-TOF MS: MALDI-TOF MS has increasingly complemented and, in appropriately equipped laboratories, replaced conventional biochemical identification methods for rapid fungal identification. Updated reference spectral libraries allow accurate identification of cryptic species (e.g., differentiating *Candida auris* from *Candida haemulonii*, or *Aspergillus lentulus* from *Aspergillus fumigatus*).

2. Molecular & Genomic Diagnostics

* Real-Time Polymerase Chain Reaction (qPCR): Standardized assays enable sensitive detection of fungal DNA directly from blood, bronchoalveolar lavage (BAL) fluid, and tissue specimens.
* Multiplex PCR & Syndromic Panels: Commercial assays detect common fungal pathogens simultaneously alongside specific resistance markers (e.g., CYP51A mutations in Aspergillus) directly from clinical samples.

* Next-Generation Sequencing (NGS): Metagenomic NGS (mNGS) aids in identifying rare, unculturable, or novel fungal pathogens from complex clinical matrices without requiring preliminary target selection.

3. Biomarker Assays & Point-of-Care (POC) Testing

* beta-D-Glucan (BDG): beta-D-glucan is a serum biomarker that can support the diagnosis of selected invasive fungal infections; however, it is not specific for fungal infection and should be interpreted together with clinical findings and other laboratory investigations.
* Galactomannan (GM) Antigen: Widely utilized in BAL and serum for invasive aspergillosis screening.
* Lateral Flow Assays (LFA): Rapid POC tests for Cryptococcal Antigen (CrAg) and galactomannan (GM) antigen provide rapid results with minimal infrastructure requirements.

Molecular Mechanisms of Antifungal Resistance

Antifungal resistance may be intrinsic or acquired (arising via point mutations, gene duplication, or transcriptional upregulation).

1. Azole Resistance

Azoles act by inhibiting 14\alpha-demethylase (encoded by ERG11 in yeasts and CYP51A/B in moulds), disrupting ergosterol biosynthesis.

* Target Site Alterations: Point mutations in ERG11 (e.g., Y132F, K143R in *Candida*) reduce azole binding affinity. In *Aspergillus fumigatus*, tandem repeats in the promoter region combined with point mutations—notably TR_{34}/L98H and TR_{46}/Y121F/T289A——can result in increased CYP51A expression and resistance to multiple medical triazoles via both patient therapy and agricultural fungicide exposure.
* Efflux Pump Overexpression: Upregulation of ATP-binding cassette (ABC) transporters (e.g., CDR1, CDR2) or Major Facilitator Superfamily (MFS) transporters (e.g., MDR1) significantly lowers intracellular azole concentrations.

2. Echinocandin Resistance

Echinocandins inhibit beta-(1,3)-D-glucan synthase, thereby disrupting fungal cell-wall synthesis.

* FKS Mutations: Resistance is primarily driven by point mutations in specific "hotspot" regions of the FKS1 (and FKS2 in *Candida glabrata*)

genes, such as substitutions at position S639 in *Candida auris*. These mutations alter the catalytic subunit of glucan synthase, drastically increasing minimum inhibitory concentrations (MICs).

3. Polyene Resistance

Polyenes (e.g., amphotericin B) bind directly to membrane ergosterol, forming lethal ion-permeable pores.

* Ergosterol Pathway Alterations: Alterations in the ergosterol biosynthetic pathway can modify membrane ergosterol composition and reduce susceptibility to amphotericin B; the specific mechanisms vary among Candida species.

Management Challenges & Novel Therapeutics

Treating multidrug-resistant fungal infections is complicated by a limited armoury of antifungal drug classes. However, several novel agents targeting distinct fungal pathways offer improved therapeutic options, spanning both regulatory-approved treatments and investigational-stage drugs:

Rezafungin–

1. Next-Gen Echinocandin .
2. Inhibits beta-(1,3)-D-glucan synthase .
3. Approved (FDA / EMA approved for invasive candidiasis).
4. Long half-life allowing once-weekly IV dosing; enhanced tissue penetration. |

Ibrexafungerp |-

1. Triterpenoid
2. Non-competitive inhibitor of beta-(1,3)-D-glucan synthase |
3. Approved (FDA approved for vulvovaginal candidiasis; Phase III for invasive candidiasis)
4. Oral bioavailability; active against many echinocandin-resistant FKS-mutant isolates.

Oteseconazole-

1. Tetrazole
2. Selective fungal CYP51 inhibitor
3. Approved (FDA approved for recurrent vulvovaginal candidiasis)
4. Higher specificity for fungal CYP51 over human CYP enzymes; reduced drug interactions. |

Fosmanogepix-

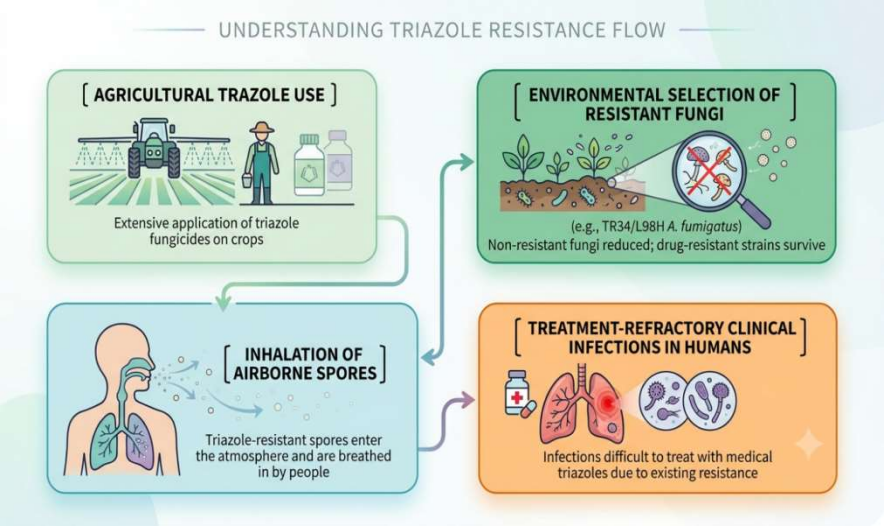
1. Gwt1 Enzyme Inhibitor
2. Inhibits fungal Gwt1 enzyme, disrupting GPI-anchored protein maturation
3. Investigational (Phase III clinical trials)
4. Broad-spectrum activity against *Candida, Aspergillus, Fusarium*, and Mucorales; oral & IV formulations.

Olorofim–

1. Orotomide
2. Inhibits dihydroorotate dehydrogenase (DHODH), blocking pyrimidine synthesis |
3. Investigational (Phase III clinical trials; FDA Breakthrough Therapy designation)
4. Excellent activity against resistant moulds (*Aspergillus, Scedosporium, Lomentospora*); inactive against yeasts.

The One Health Perspective in Medical Mycology

Antifungal resistance is not confined to healthcare settings. The widespread use of triazole fungicides (e.g., tebuconazole, difenoconazole) in agriculture has created environmental reservoirs for resistant fungi. Spores of *Aspergillus fumigatus* carrying environmental resistance alleles (such as TR_{34}/L98H) are inhaled by azole-naïve patients, leading to primary recalcitrant invasive aspergillosis.



Addressing this challenge requires:

* Cross-sectoral surveillance integrating agricultural, environmental, and clinical fungal isolate genomics.
* Antifungal stewardship programs across healthcare settings to prevent off-target selection pressure.
* Development of non-cross-reactive fungicide classes for crop protection.

Conclusion

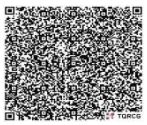
Emerging fungal pathogens and escalating antifungal resistance represent a major challenge in modern clinical practice. Strengthening diagnostic networks through rapid molecular and mass spectrometry tools, adopting new and emerging antifungal agents, where clinically appropriate, enforcing strict hospital infection control, and implementing integrated One Health surveillance are crucial steps to safeguard patient outcomes and preserve antifungal efficacy for the future.

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Microbial Threats, Disease Prevention and Population Health

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Abstract

Microbiology provides the scientific foundation for understanding infectious diseases and developing measures to protect population health. Microorganisms influence human well-being through their roles in infection, immunity, food production, environmental processes and the maintenance of normal microbial communities. Public health microbiology connects laboratory investigations with epidemiological surveillance, disease prevention and health policy. This chapter examines major microbial groups of public health importance, mechanisms of disease transmission, host–pathogen interactions and the contribution of microbiological methods to diagnosis and outbreak investigation. It discusses water and food safety, vaccination, infection prevention, antimicrobial resistance and laboratory biosafety. Particular attention is given to the One Health approach, which recognises connections among human, animal and environmental health. Emerging applications, including genomic surveillance, wastewater monitoring and rapid molecular diagnostics, are considered alongside their limitations. The chapter emphasises that effective disease control requires reliable laboratory services, timely interpretation of evidence, equitable access to preventive measures and coordinated action across sectors. Integrating microbiology into public health systems is essential for reducing infectious disease burdens and strengthening preparedness for future health emergencies.

Keywords: Public health microbiology; infectious diseases; epidemiology; antimicrobial resistance; vaccination; microbial surveillance; One Health; sanitation.

Introduction

Microbiology is the study of microorganisms and their activities. It includes bacteriology, virology, mycology, protozoology and related fields concerned with microbial structure, physiology, genetics and interactions. Public health applies scientific knowledge and organised community action to prevent disease, prolong life and improve health.The relationship between these disciplines is fundamental. Microbiology identifies infectious agents and explains their biological behaviour, while public health investigates how diseases occur in populations and determines how transmission can be interrupted. A microbiological result becomes especially valuable when it guides an intervention, such as improving a contaminated water supply, controlling a hospital outbreak or selecting an appropriate antimicrobial treatment.Public health microbiology therefore extends beyond the examination of individual clinical specimens. It includes the investigation of food, water, wastewater, animals and environmental reservoirs. It also supports surveillance systems that detect changes in pathogen distribution, antimicrobial susceptibility and outbreak patterns.

Modern public health requires laboratory findings to be connected with clinical and epidemiological information. The World Health Organization's genomic surveillance strategy similarly emphasises the integration of sampling, diagnostics, analysis and data sharing within broader surveillance systems [1].

Microorganisms of Public Health Importance

Microorganisms differ in their organisation, environmental survival, routes of transmission and susceptibility to control measures. These differences determine how they are investigated and managed.

Group	General characteristics	Examples of public health importance	Associated diseases or concerns
Bacteria	Prokaryotic organisms; some produce toxins, spores or biofilms	*Mycobacterium tuberculosis*, *Vibrio cholerae*, *Salmonella enterica*	Tuberculosis, cholera and salmonellosis
Viruses	Acellular infectious agents that require host cells for replication	Influenza viruses, measles virus, hepatitis B virus, SARS-CoV-2	Respiratory infections, measles and hepatitis

Fungi	Eukaryotic organisms occurring as yeasts, moulds or other forms	*Candida* species, *Aspergillus* species, dermatophytes	Invasive fungal infections and superficial mycoses
Protozoa	Unicellular eukaryotic organisms; several have complex life cycles	*Plasmodium* species, *Giardia duodenalis*, *Entamoeba histolytica*	Malaria, giardiasis and amoebiasis
Helminths	Multicellular parasitic worms; their eggs and larvae are commonly examined in diagnostic laboratories	*Ascaris lumbricoides*, hookworms and *Schistosoma* species	Intestinal and tissue parasitic diseases

Helminths are not microorganisms in the strict sense, but medical parasitology is commonly included within public health laboratory services.

Microbial presence does not always indicate danger. Many microorganisms contribute to food fermentation, wastewater treatment, nutrient cycling and normal host functions. Public health microbiology must distinguish beneficial organisms, harmless colonisers, indicators of contamination and true pathogens.

Host–Microbe Relationships and Disease Development

Colonisation occurs when microorganisms establish themselves on or within a host without necessarily causing tissue injury or symptoms.Infection involves the establishment and multiplication of an infectious agent in a host. Infection may remain asymptomatic.Infectious disease develops when infection produces clinically significant dysfunction, tissue damage or symptoms.These distinctions are important when interpreting laboratory findings. Detection of an organism on the skin or in the intestine may represent normal carriage, whereas detection in a normally sterile site may have a different significance.Pathogenicity refers to an organism’s capacity to cause disease. Virulence describes the degree of disease-causing ability under particular conditions.

Microbial characteristics that contribute to disease include:

a. Adhesion to host surfaces.

b. Invasion of tissues.

c. Production of toxins or tissue-damaging enzymes.

d. Avoidance of immune responses.

e. Survival within host cells.

f. Formation of protective biofilms.

Disease severity also depends on the infectious dose, route of exposure, host immunity, nutritional status, age and underlying health conditions.The human microbiota consists of microbial communities associated with sites such as the intestine, skin and oral cavity. These communities can contribute to nutrient metabolism, immune development and resistance to pathogen colonisation.Changes in microbial communities, often described as **dysbiosis**, have been associated with several diseases. However, an association between a microbial pattern and illness does not establish causation. Microbiome findings require careful evaluation before they can support population-level interventions.

Transmission of Infectious Diseases

The chain of infection consists of an infectious agent, a reservoir, a portal of exit, a mode of transmission, a portal of entry and a susceptible host. Prevention aims to interrupt one or more of these links.Direct contact includes physical transfer between individuals. Indirect contact occurs through contaminated objects, equipment or surfaces. The importance of contaminated surfaces varies according to the pathogen, its survival outside the host and opportunities for transfer.Respiratory pathogens can spread through infectious particles released during breathing, speaking, coughing or sneezing. Exposure depends on particle characteristics, distance, ventilation, duration of contact and the surrounding environment.Preventive measures may include vaccination, improved ventilation, respiratory hygiene and appropriate protective equipment.Enteric pathogens spread when material contaminated with faeces reaches another person's mouth, often through water, food or hands. Inadequate sanitation and unsafe drinking water increase opportunities for transmission.WHO identifies contaminated drinking water as an important route for diseases including diarrhoeal infections, cholera, dysentery, typhoid and polio [2]. Vectors transfer infectious agents between hosts. Mosquitoes transmit malaria parasites and several viruses, while ticks and other arthropods transmit additional pathogens.Vector control requires understanding vector ecology, breeding conditions, host interactions and local patterns of disease.Some pathogens spread through infected blood, sexual contact or

transmission from mother to child during pregnancy, delivery or breastfeeding. Prevention depends on the specific organism and may include screening, vaccination, safe injection practices, barrier methods and appropriate treatment.

Microbiological Diagnosis and Public Health Laboratories

Public health laboratories identify pathogens, detect resistance, investigate outbreaks and monitor environmental hazards. Their findings must be accurate, timely and interpreted in context.Diagnostic quality begins before testing. Specimens must represent the suspected infection site and be collected, labelled, transported and stored appropriately.Poor sampling, contamination, delayed transport or unsuitable storage can produce misleading results. Prior antimicrobial exposure may also reduce recovery of viable organisms.Microscopy provides information about microbial morphology, staining characteristics and the presence of parasites or inflammatory cells. It can provide rapid preliminary evidence, although its sensitivity and specificity vary among applications.Culture allows viable organisms to multiply under suitable laboratory conditions. It supports identification, antimicrobial susceptibility testing and further investigation of isolates.Its limitations include slow growth, demanding nutritional requirements and failure to recover some organisms after treatment or unsuitable transport.Antigen tests detect microbial components, while antibody tests detect host immune responses. Their interpretation depends on the timing of infection, test performance and clinical context.An antibody result may indicate previous exposure or vaccination rather than current infection. Some antigen tests offer rapid results but may be less sensitive than molecular methods.Polymerase chain reaction and related methods detect specific nucleic acid sequences. Reverse transcription is used when RNA is the target.Molecular methods can improve detection and shorten turnaround times. However, detection of microbial nucleic acid does not always demonstrate viable organisms, active disease or infectiousness.Laboratory quality requires validated methods, internal controls, trained personnel, equipment maintenance and external quality assessment. Results must also reach clinicians and public health authorities quickly enough to influence decisions.

Surveillance and Outbreak Investigation

Surveillance is the systematic collection, analysis, interpretation and communication of health-related information for action. Microbiological surveillance adds evidence about the organisms responsible for disease.An outbreak investigation generally involves:

a. Confirming that an unusual increase in disease has occurred.
b. Developing a case definition.
c. Describing cases by person, place and time.
d. Collecting appropriate clinical and environmental samples.
e. Investigating possible sources and transmission routes.
f. Implementing control measures.
g. Evaluating whether transmission has declined.

Laboratory evidence can connect cases to a common pathogen or source. Nevertheless, matching organisms alone may not establish the direction of transmission or prove that a particular exposure caused the outbreak.Whole-genome sequencing provides detailed information about pathogen relatedness and genetic variation. It can support the investigation of clusters, monitor pathogen evolution and identify resistance-associated genetic changes.Genomic findings are strongest when combined with exposure histories, sampling dates and other epidemiological information. WHO's strategy treats genomic surveillance as part of an integrated laboratory and surveillance system [1].

Water, Sanitation and Environmental Health

Water safety is a central application of public health microbiology. Contamination may arise from sewage, animal waste, agricultural runoff, damaged distribution systems or unsafe household storage.Testing every possible pathogen in every water sample is impractical. Indicator organisms are therefore used to assess contamination and treatment performance.*Escherichia coli* is widely used as an indicator of faecal contamination. However, indicator testing does not guarantee the absence of every pathogen. Some viruses and protozoan stages differ from indicator bacteria in their persistence and response to treatment.Effective water protection involves source management, appropriate treatment, distribution-system maintenance and safe storage. A preventive approach considers hazards throughout the supply chain rather than relying solely on occasional testing of finished water.WHO drinking-water guidance provides a framework for health-based targets, water safety management and surveillance [3]. Untreated or inadequately treated wastewater can introduce pathogens into rivers, ponds and other receiving environments. Environmental microorganisms also perform beneficial functions by degrading organic matter during biological

treatment.Environmental investigations should distinguish the detection of contamination from demonstrated human exposure and disease risk.

Food Microbiology and Public Health

Food can transmit pathogens through contaminated ingredients, unsafe processing, inadequate cooking, poor storage or cross-contamination.

Foodborne illness may result from:

- Infection after ingestion of viable pathogens.
- Ingestion of toxins formed in food.
- Ingestion of organisms that subsequently produce toxins in the intestine.

Microbiology supports food safety through testing, process validation and investigation of contamination sources. However, testing a small number of samples cannot establish the safety of an entire batch when contamination is unevenly distributed.WHO's Five Keys to Safer Food emphasise cleanliness, separation of raw and cooked foods, thorough cooking, safe storage temperatures and the use of safe water and ingredients [4]. Preventive systems should identify hazards and control them at relevant stages of production, processing, transport and preparation. For fish and other aquatic foods, this includes attention to water quality, harvesting conditions, hygienic handling and storage.

Vaccination and Population Protection

Vaccination prepares the immune system to respond to particular infectious agents or their harmful products. Depending on the vaccine, it may reduce infection, clinical disease, severe outcomes or transmission.At population level, high vaccination coverage can reduce circulation of some pathogens and indirectly protect susceptible individuals. The extent of this protection depends on vaccine effectiveness against transmission, pathogen characteristics and the distribution of immunity.Microbiology contributes to vaccination by identifying protective antigens, characterising circulating strains and monitoring pathogen changes.Successful immunisation programmes require reliable supply, appropriate storage, trained staff, safety monitoring and community trust. WHO's Immunisation Agenda 2030 places equitable access and the inclusion of underserved populations at the centre of vaccination strategy [5].Antimicrobial resistance occurs when microorganisms are no longer adequately inhibited or killed by medicines to which they were previously susceptible. Antibiotic resistance is the bacterial component of this

broader problem.Resistance can arise through mutation or the acquisition of genetic material. Mobile genetic elements can facilitate its spread between bacteria.Antimicrobial exposure creates selection pressure favouring resistant organisms. Misuse and overuse contribute to this process, while inadequate sanitation and infection control facilitate transmission.Resistant infections can restrict treatment options, prolong illness and increase demands on health services. WHO identifies AMR as a major health and development threat requiring coordinated prevention and control [6]. Control requires appropriate prescribing, reliable diagnostics, susceptibility testing, infection prevention and access to quality-assured medicines.An antibiogram summarises susceptibility patterns among selected local isolates and can support treatment-policy decisions. Its usefulness depends on representative sampling, appropriate analysis and regular updating.Stewardship aims to improve antimicrobial use while ensuring that patients who need effective treatment can obtain it.

Healthcare-Associated Infections

Healthcare-associated infections arise in connection with healthcare delivery and may involve wounds, the bloodstream, urinary tract or respiratory system.Risk can increase with invasive devices, surgery, prolonged hospitalisation and impaired host defences. Resistant organisms create additional challenges.Infection prevention includes hand hygiene, appropriate precautions, safe injection practices, equipment processing, environmental cleaning and suitable management of invasive devices.Microbiology supports these measures by identifying organisms, detecting unusual clusters and monitoring resistance. WHO's core-component guidelines emphasise organised programmes, evidence-based guidance, training, surveillance and implementation support [7]. Laboratory data should guide investigation and action. Routine environmental sampling without a clear question may produce results that are difficult to interpret.The One Health approach recognises that human, animal and environmental health are interconnected. Microorganisms move through shared environments, food systems and contact networks.Zoonotic infections may arise through animal contact, contaminated animal products, vectors or environmental exposure. Their prevention requires cooperation among human health services, veterinary services and environmental authorities.Joint activities can include coordinated outbreak investigation, animal vaccination, food-chain surveillance, monitoring of antimicrobial use and environmental risk assessment.The joint One Health plan developed by FAO, UNEP, WHO and WOAH provides a framework for collaboration across these sectors [8]. A district-level application could connect

observations of animal illness, unusual human infections and contamination of shared water sources. Such integration helps identify hazards that might be missed when each sector works independently.

Emerging Approaches and Research Priorities

Wastewater monitoring detects microbial targets shed by people within a contributing population. It can complement clinical surveillance, including when testing access or healthcare-seeking behaviour changes. Interpretation requires attention to shedding patterns, rainfall, wastewater flow, sampling methods and population coverage. A wastewater signal generally cannot identify infected individuals or directly determine the number of cases. CDC emphasises its use alongside other surveillance information [9]. Metagenomics examines genetic material from microbial communities without requiring a separate culture for every organism. It offers opportunities to investigate complex samples and infections that remain unexplained after targeted testing.Challenges include contamination, incomplete databases, analytical costs and uncertainty about whether a detected organism caused disease.Portable molecular and antigen tests can shorten the interval between sampling and action. Their value depends on accuracy, affordability, quality assurance and connection to treatment or prevention services.Combining laboratory results with clinical, geographical and environmental information can improve situational awareness. Analytical tools require validation, representative data and protection of personal information.Future research should prioritise practical questions: which tests improve decisions, how surveillance can reach underserved populations and which interventions reduce transmission under local conditions.

Laboratory biosafety protects workers, communities and the environment from unintended exposure. Biosecurity addresses the prevention of loss, theft or misuse of biological materials and associated information.Appropriate safeguards depend on the agent, procedure, exposure route and working environment. WHO’s fourth-edition laboratory biosafety manual emphasises evidence-based risk assessment rather than a uniform approach to all activities [10]. Ethical practice also requires confidentiality, responsible data sharing and avoidance of stigma. Public communication should explain what findings show, what remains uncertain and what actions people can take.Effective public health microbiology requires sustained investment in people, systems and infrastructure. Priorities include dependable specimen referral, trained staff, quality-assured laboratories and timely reporting.Advanced technologies should be introduced where they address a

clear need. Sequencing capacity has limited impact if sampling is unrepresentative or results arrive after decisions have been made.Equity must guide service design. Rural communities and populations with limited access to healthcare should be included in surveillance and diagnostic networks. Locally useful programmes may integrate drinking-water testing, food safety, hospital surveillance and animal-health reporting.Performance should be judged by practical outcomes, including earlier detection, shorter reporting times, improved outbreak control and reduced disease burden.

Conclusion

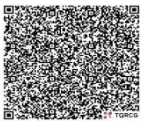
Microbiology provides the scientific foundation for protecting public health by explaining how microorganisms cause disease, spread through populations and interact with hosts and environments. Its applications extend beyond clinical diagnosis to water quality assessment, food safety, environmental monitoring and the prevention of healthcare-associated infections. Reliable microbiological investigations enable early identification of pathogens, support outbreak control and guide appropriate treatment. Effective disease prevention requires the integration of laboratory evidence with epidemiological surveillance, vaccination, sanitation, hygiene and community education. Antimicrobial resistance highlights the need for responsible prescribing, accurate susceptibility testing and coordinated efforts to reduce unnecessary antimicrobial use. Equally important are accessible diagnostic services, trained personnel and consistent laboratory quality assurance. Advances in molecular diagnostics, genomic sequencing and wastewater surveillance offer opportunities to detect emerging threats and monitor microbial transmission. However, these technologies must be interpreted alongside clinical and epidemiological information, recognising that microbial detection alone does not always establish disease causation or infectiousness. Their public health value depends on whether findings produce timely and effective action.The One Health approach strengthens prevention by recognising connections among human, animal and environmental health. Collaboration across these sectors is essential for addressing zoonotic infections, environmental contamination and resistant microorganisms.Ultimately, strengthening public health microbiology requires sustained investment, ethical data management, clear communication and equitable access to preventive and therapeutic services. Combining scientific innovation with practical community interventions can reduce infectious disease burdens, improve preparedness and build resilient health systems.

Microbiology therefore remains indispensable to safeguarding population health and advancing sustainable well-being across communities and future generations.

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Emerging and re-emerging bacterial pathogens: Current research and clinical challenges

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Introduction

Infectious diseases remain a major cause of morbidity and mortality worldwide despite remarkable advances in antimicrobial therapy, vaccination, sanitation, diagnostic microbiology and public health. Bacteria that were previously uncommon may become important causes of human disease, while pathogens that had been controlled for decades may return as significant public health threats.

An emerging bacterial pathogen is a bacterial organism or bacterial disease that has newly appeared in a population, has been recognized for the first time as a human pathogen, or is rapidly increasing in incidence, geographic distribution, host range or clinical importance.

A re-emerging bacterial pathogen is a previously recognized pathogen whose incidence or geographic distribution has increased after a period of decline or effective control.

Emergence may result from the appearance of a genuinely new pathogen, acquisition of new virulence determinants, expansion into a new host or geographic region, or improved recognition through modern diagnostic methods. Re-emergence commonly occurs because of deterioration in vaccination coverage, antimicrobial resistance, inadequate public health infrastructure, environmental change, population movement or breakdown of infection-control measures.

Antimicrobial resistance (AMR) has become one of the most important drivers of the clinical significance of bacterial pathogens. The WHO Bacterial Priority Pathogens List 2024 places carbapenem-resistant Acinetobacter baumannii, carbapenem resistant Enterobacterales and rifampicin-resistant Mycobacterium tuberculosis in the critical-priority category. Several resistant

strains of Salmonella, Shigella, Enterococcus faecium, Pseudomonas aeruginosa, Neisseria gonorrhoeae and Staphylococcus aureus are categorized as high priority.

Thus, emergence today should not be understood only as the appearance of a new bacterial species. The emergence of antimicrobial resistance, hypervirulent strains, new transmission patterns and new ecological niches is equally important.

Important definitions

Emerging bacterial pathogen

An emerging pathogen may represent:

- A Newly recognized bacterial species causing human disease.
- A Known organism appearing in a new geographic region.
- A Pathogen causing disease in a new population or host.
- A Bacterium showing a sudden increase in incidence.
- A Pathogen acquiring increased virulence.
- A Previously uncommon organism becoming clinically important.
- A Bacterial organism developing clinically significant multidrug resistance.

Re-emerging bacterial pathogen

Re-emergence refers to the return or resurgence of a previously controlled or declining bacterial infection. Examples include: Tuberculosis, Cholera, Diphtheria, Pertussis, Plague, Typhoid fever, Gonorrhoea, Syphilis, Leptospirosis, Melioidosis and Anthrax.

Emerging resistance

A pathogen may be well established epidemiologically but become "emerging" from a clinical perspective when resistance significantly compromises treatment. Examples include:

- Carbapenem-resistant Klebsiella pneumoniae
- Carbapenem-resistant Acinetobacter baumannii
- Carbapenem-resistant Pseudomonas aeruginosa

- Extended-spectrum β-lactamase-producing Escherichia coli
- Vancomycin-resistant Enterococcus faecium
- Methicillin-resistant Staphylococcus aureus
- Extensively drug-resistant Mycobacterium tuberculosis
- Drug-resistant Neisseria gonorrhoeae

Factors responsible for emergence and re-emergence

Microbial evolution

Bacteria undergo mutation, genetic recombination, horizontal gene transfer and selection which can produce organisms with increased virulence, Antimicrobial resistance, Enhanced transmissibility

Antimicrobial resistance

Antibiotic pressure selects resistant organisms. Major mechanisms include:

- Enzymatic drug destruction- ESBLs, AmpC β-lactamases, Carbapenemases
- Modification of antibiotic targets - Altered penicillin-binding proteins,Ribosomal modification, DNA gyrase mutations
- Reduced permeability- alteration or loss of porins can decrease antibiotic entry.
- Efflux pumps can expel multiple antimicrobial classes and contribute to multidrug resistance.

Biofilms and persistent infections

Biofilm formation can reduce antimicrobial penetration, promote bacterial persistence and contributes to the emergence and persistence of bacterial infections such as catheter-associated UTI, prosthetic joint infections, central-line-associated bloodstream infections, prosthetic valve infections,chronic wounds and cystic fibrosis. Important organisms includes pseudomonas aeruginosa, staphylococcus aureus, acinetobacter baumannii, klebsiella pneumoniae and enterococcus species.

Environmental and climatic Factors

Increased temperature, flooding, changes in rainfall and coastal water conditions may influence bacterial survival and exposure. For example flooding can increase the risk of cholera, leptospirosis, enteric infections and melioidosis.

Urbanization and population movement

Rapid urbanization may produce overcrowding, inadequate sanitation, poor waste disposal and increased healthcare-associated transmission. International travel and migration facilitate rapid geographic dissemination of resistant organisms.

Zoonotic transmission

Many emerging bacteria originate in animal reservoirs. Important zoonotic bacterial pathogens include:

Pathogen	Major reservoir/exposure	Important disease
Brucella spp.	Cattle, goats, sheep	Brucellosis
Leptospira spp.	Rodents, animals	Leptospirosis
Yersinia pestis	Rodents/fleas	Plague
Burkholderia pseudomallei	Soil/water	Melioidosis
Campylobacter spp	Poultry/animals	Gastroenteritis
Salmonella spp.	Animals/food	Enteric disease

Important emerging and re-emerging bacterial pathogens

Mycobacterium tuberculosis

Tuberculosis remains one of the most important re-emerging bacterial diseases.

The major concern is the increasing prevalence of Multidrug-resistant tuberculosis (MDR-TB), Rifampicin-resistant TB, Pre-XDR-TB and XDR-TB. Resistance may result from inadequate treatment, poor adherence, interrupted therapy. Incorrect drug combinations and transmission of resistant strains.

Acinetobacter baumannii

Carbapenem-resistant A. baumannii is the most important healthcare-associated emerging bacterial threats. It commonly causes ventilator-associated pneumonia, bloodstream infection, wound infection, urinary tract infection,

postoperative infection and meningitis. Important resistance mechanisms includes OXA-type carbapenemases, metallo-β-lactamases, efflux mechanisms and porin alterations

Carbapenem-resistant Enterobacterales

Important organisms includes Klebsiella pneumoniae, Escherichia coli, Enterobacter cloacae complex, Citrobacter freundii complex and Serratia marcescens. Important carbapenemases are KPC, NDM, VIM, IMP, OXA-48-like enzymes.These organisms may cause sepsis, pneumonia, UTI, intra-abdominal infection, surgical-site infection and device-associated infections

Pseudomonas aeruginosa

P. aeruginosa is an important opportunistic pathogen. It is associated with hospital-acquired pneumonia, burn wound infection, UTI, bacteremia, otitis externa and keratitis. Resistance may involve efflux pumps, AmpC hyperproduction, porin loss, carbapenemases and biofilm formation.

Staphylococcus aureus

Staphylococcus aureus remains a major community and healthcare-associated pathogen. Important emerging phenotypes include MRSA, Community-associated MRSA

Vancomycin-intermediate S. aureus and Vancomycin-resistant S. aureus. Clinical manifestations include skin and soft-tissue infections, pneumonia, osteomyelitis, septic arthritis, endocarditis, bacteremia and device-associated infection.

Enterococcus faecium

Vancomycin-resistant E. faecium (VRE) is an important healthcare-associated pathogen. Risk factors include Prolonged hospitalization, ICU admission, broad-spectrum antibiotic exposure, immunosuppression and indwelling devices.Clinical infections include UTI, bacteremia, endocarditis and intra-abdominal infection.

Neisseria gonorrhoeae

Gonorrhoea represents an important re-emerging sexually transmitted bacterial infection. Resistance has developed against penicillins, tetracyclines, macrolides, fluoroquinolones and sulfonamides. Reduced susceptibility or resistance to extended-spectrum cephalosporins is of particular concern because therapeutic options are limited. Complications include pelvic

inflammatory disease, infertility, ectopic pregnancy, epididymitis, disseminated gonococcal infection and neonatal conjunctivitis.

Salmonella enterica

Important emerging/re-emerging groups include: Salmonella typhi, Salmonella paratyphi, Non-typhoidal salmonella. Fluoroquinolone resistance in S. Typhi has become a major clinical concern. Extensively drug-resistant typhoid strains demonstrate how antimicrobial resistance can transform an established pathogen into a major emerging threat.

Vibrio cholerae

Cholera is a classic re-emerging bacterial disease. Major pathogenic serogroups are: O1 and O139. The El Tor biotype of O1 has been responsible for the seventh pandemic.

Vibrio vulnificus

Vibrio vulnificus is an emerging marine-associated pathogen. It causes severe wound infections, necrotizing soft-tissue infection and septicemia. Risk is increased in individuals with chronic liver disease, iron overload and immunocompromised states.

Campylobacter jejuni and Campylobacter coli

Campylobacteriosis is one of the major bacterial causes of gastroenteritis. Important sources include poultry, raw milk, contaminated water, animal contact. Fluoroquinolone resistance is an important clinical problem.

Elizabethkingia species

Elizabethkingiaanophelis has emerged as an important opportunistic pathogen. It is associated with bacteremia, neonatal infection, meningitis and pneumonia. The organism can be intrinsically resistant to many antimicrobial agents, making laboratory identification and susceptibility testing particularly important.

Leptospira species

Leptospirosis is a re-emerging zoonotic infection. Outbreaks may follow flooding, heavy rainfall, cyclones, occupational exposure and contaminated water. Clinical disease ranges from mild febrile illness to severe weil disease with jaundice, renal failure, hemorrhage and pulmonary involvement.

Brucella species

Brucellosis is a zoonotic infection associated with unpasteurized dairy products, livestock and laboratory exposure. Important species includes Brucella melitensis, Brucella abortus, Brucella suis, Brucella canis. Clinical disease may include undulant fever, arthritis, hepatitis, endocarditis and neurobrucellosis.

Yersinia pestis

Plague is a classic example of a bacterial disease that has re-emerged locally despite historical control. Transmission occurs primarily through infected fleas associated with rodent reservoirs. Major forms include: bubonic, septicemic and pneumonic plague.

Bacillus anthracis

Anthrax is a zoonotic disease caused by Bacillus Anthracis. Clinical forms include cutaneous, gastrointestinal and inhalational anthrax. Environmental spores can persist for prolonged periods. Anthrax remains important because of natural outbreaks, occupational exposure. Animal disease and public health preparedness concerns.

Corynebacterium diphtheriae

Diphtheria is a classic re-emerging infection when vaccination coverage decreases.

Clinical manifestations include pharyngitis, pseudomembrane formation, cervical lymphadenopathy, myocarditis and neuropathy. Re-emergence is strongly associated with inadequate immunization and disruption of healthcare systems.

Bordetella pertussis

Pertussis has re-emerged in several populations despite widespread vaccination. Factors include waning immunity, incomplete vaccination and changes in circulating strains. Disease is particularly severe in young infants.

Antimicrobial resistance as a driver of emergence

Antimicrobial resistance has transformed the clinical importance of many established bacterial pathogens. The WHO identifies 24 antibiotic-resistant bacterial pathogens across 15 families and classifies them into critical, high and medium priority groups

Critical-priority pathogens	High-priority pathogens	Medium-priority pathogens
• Carbapenem-resistant Acinetobacter baumannii • Carbapenem-resistant Enterobacterales • Third-generation-cephalosporin-resistant Enterobacterales. • Rifampicin-resistant Mycobacterium tuberculosis	• Fluoroquinolone-resistant Salmonella Typhi • Fluoroquinolone-resistant Shigella • Vancomycin-resistant Enterococcus faecium • Carbapenem-resistant Pseudomonas aeruginosa • Fluoroquinolone-resistant non-typhoidal Salmonella • Resistant Neisseria gonorrhoeae • Methicillin-resistant Staphylococcus aureus	• Macrolide-resistant Group A Streptococcus • Macrolide-resistant Streptococcus pneumoniae • Ampicillin-resistant Haemophilus influenza • Penicillin-resistant Group B Streptococcus

Healthcare-associated emerging pathogens

Hospitals provide an ideal environment for the selection and transmission of resistant organisms. Major healthcare-associated emerging pathogens include:

- MRSA
- VRE
- Carbapenem-resistant Enterobacterales
- Carbapenem-resistant A. baumannii
- Carbapenem-resistant P. aeruginosa
- ESBL-producing Enterobacterales
- Multidrug-resistant Stenotrophomonas maltophilia
- Elizabethkingia spp.

Community-associated emerging pathogens

Emerging resistant pathogens are no longer restricted to hospitals.Community-associated problems include:

- CA-MRSA
- Drug-resistant gonorrhoea
- Extensively drug resistant E. coli
- Fluoroquinolone resistant Salmonella
- Fluoroquinolone resistant Shigella
- Drug-resistant tuberculosis

This represents an important epidemiological shift because community transmission can make containment considerably more difficult.

Role of molecular diagnostics and genomic surveillance

Molecular diagnostics have transformed the detection of emerging pathogens. PCR is useful for Rapid identification and detection of organisms. Multiplex PCR allows simultaneous detection of multiple pathogens. Whole-genome sequencing provides information about species identification, strain typing, transmission pathways, resistance and virulence genes. Metagenomic sequencing allows detection of microbial DNA or RNA directly from clinical samples without requiring conventional culture.Genomic surveillance is increasingly important in identifying emerging bacterial clones. It can detect new resistance genes, clonal expansion, transmission clusters, geographic spread virulence determinants and plasmid dissemination. A major concern is the movement of resistance genes across species through mobile genetic elements. For example, carbapenemase genes can disseminate through plasmids among different members of the Enterobacterales. However, challenges include cost, bioinformatics requirements, interpretation of colonization versus infection, need for validated workflows and data management.

Antimicrobial stewardship

Antimicrobial stewardship is a central component of controlling emerging resistant bacteria. Major principles include correct indication, appropriate antimicrobial selection, correct dose, correct route, correct duration, de-escalation according to culture results, avoidance of unnecessary broad-spectrum therapy. Microbiology laboratories play a major role through

timely culture reporting, antimicrobial susceptibility testing, resistance surveillance, alert reporting, infection-control communication and cumulative antibiograms.

Infection prevention and control

Control of emerging pathogens requires rapid interruption of transmission. Important interventions includes

Standard precautions	Transmission-based precautions	Environmental control
• Hand hygiene • Appropriate PPE • Safe injection practices • Respiratory hygiene • Sharps disposal • Biomedical waste management	• Contact precautions E.g- MRSA • Droplet precautions E.g - Haemophillus influenzae • Airborne precautions E.g Mycobacterium tuberculosis	• Cleaning • Disinfection • Sterilization

One health approach

The One Health approach recognizes the interconnection between human health, animal health and environmental health. This is particularly relevant to bacterial emergence. Antibiotic-resistant organisms may circulate between Humans, animals, food and environment. Important examples include: Salmonella, Campylobacter, E. coli, Enterococcus Staphylococcus aureus, Brucella and Leptospira. One Health surveillance therefore requires collaboration between clinicians, microbiologists, veterinarians, epidemiologists, environmental scientists and public health authorities.

Vaccines and emerging bacterial diseases

Vaccination remains one of the most effective strategies against bacterial disease. Important vaccines include those against:

- Corynebacterium diphtheriae toxin
- Bordetella pertussis
- Streptococcus pneumoniae
- Haemophilus influenzae type b

- Salmonella Typhi

Future research is focused on vaccines against pathogens for which effective vaccines are currently unavailable or insufficient. Potential targets include:

- Staphylococcus aureus
- Klebsiella pneumoniae
- Group A Streptococcus
- Clostridioides difficile
- Gonococcus
- Tuberculosis

Current research directions

Newer antibiotics

Research and development of newer antibacterial agents is focusing on novel mechanisms of action and improved activity against multidrug-resistant organisms. Some of them are listed below

Current research areas	Example	Mechanism
Novel molecular targets	Gepotidacin	A triazaacenaphthylene antibiotic that inhibits bacterial DNA replication by targeting DNA gyrase and topoisomerase IV. It received FDA approval in 2025 for uncomplicated urinary tract infections.
New antibiotic classes	Lefamulin	A pleuromutilin antibiotic that inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit. It is used for community-acquired bacterial pneumonia.
β-lactam/β-lactamase inhibitor combinations	Ceftazidime–avibactam	Combines a third-generation cephalosporin with avibactam and is particularly useful against selected β-lactamase-producing Gram-negative organisms.

Siderophore antibiotics	Cefiderocol	A siderophore cephalosporin that utilizes bacterial iron-transport systems to facilitate entry into Gram-negative bacteria
New tetracycline derivatives	Eravacycline	A fluorocycline with activity against a broad range of Gram-positive, Gram-negative and anaerobic bacteria.

Bacteriophage therapy

Bacteriophages are viruses that infect bacteria. Potential applications include difficult-to-treat infections caused by Pseudomonas, Acinetobacter, Klebsiella and Staphylococcus. Challenges include narrow host range, phage resistance, regulatory issues, pharmacokinetics and immune responses.

Antimicrobial peptides

Antimicrobial peptides may act through membrane disruption, intracellular target interference, immunomodulation. They represent an active research area for multidrug-resistant bacteria.

Anti-virulence therapy

Instead of killing bacteria directly, anti-virulence strategies attempt to prevent disease by targeting toxins, adhesion, quorum sensing, biofilm formation and iron acquisition. This may impose less selective pressure for resistance than conventional bactericidal therapy.

CRISPR-based approaches

CRISPR-associated technologies are being investigated for:

- Targeted elimination of resistance genes
- Bacterial genome editing
- Rapid molecular diagnostics
- Selective targeting of resistant bacteria

Major challenges in low- and middle-income countries

Emerging bacterial infections pose particular challenges in resource-limited settings. Major problems include:

- Limited laboratory infrastructure
- Delayed diagnosis
- Inadequate surveillance
- Antibiotic misuse
- Limited access to newer antibiotics
- Poor sanitation
- Inadequate vaccination
- Overcrowding
- Inconsistent infection-control practices
- Limited genomic sequencing capacity

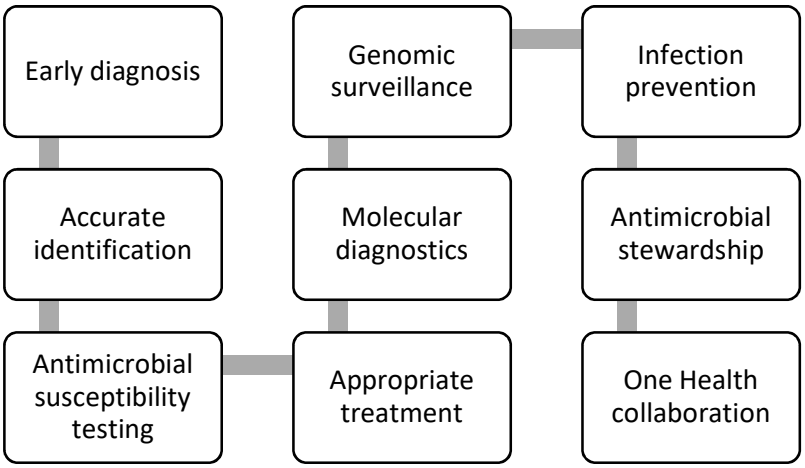
Conclusion

Emerging and re-emerging bacterial pathogens represent a constantly changing challenge for modern medicine. Emergence may result from microbial evolution, antimicrobial resistance, environmental changes, zoonotic transmission, globalization, population movement, healthcare practices and deficiencies in public health infrastructure.

The modern concept of bacterial emergence extends beyond the appearance of entirely new organisms. Multidrug-resistant, extensively drug-resistant, hypervirulent and geographically expanding strains of established pathogens are equally important emerging threats.

The WHO 2024 bacterial priority pathogens list emphasizes the extraordinary importance of resistant Gram-negative bacteria, resistant M. tuberculosis, MRSA, VRE, resistant Salmonella, Shigella, N. gonorrhoeae and other resistant bacterial pathogens.

Effective control requires an integrated approach involving:



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