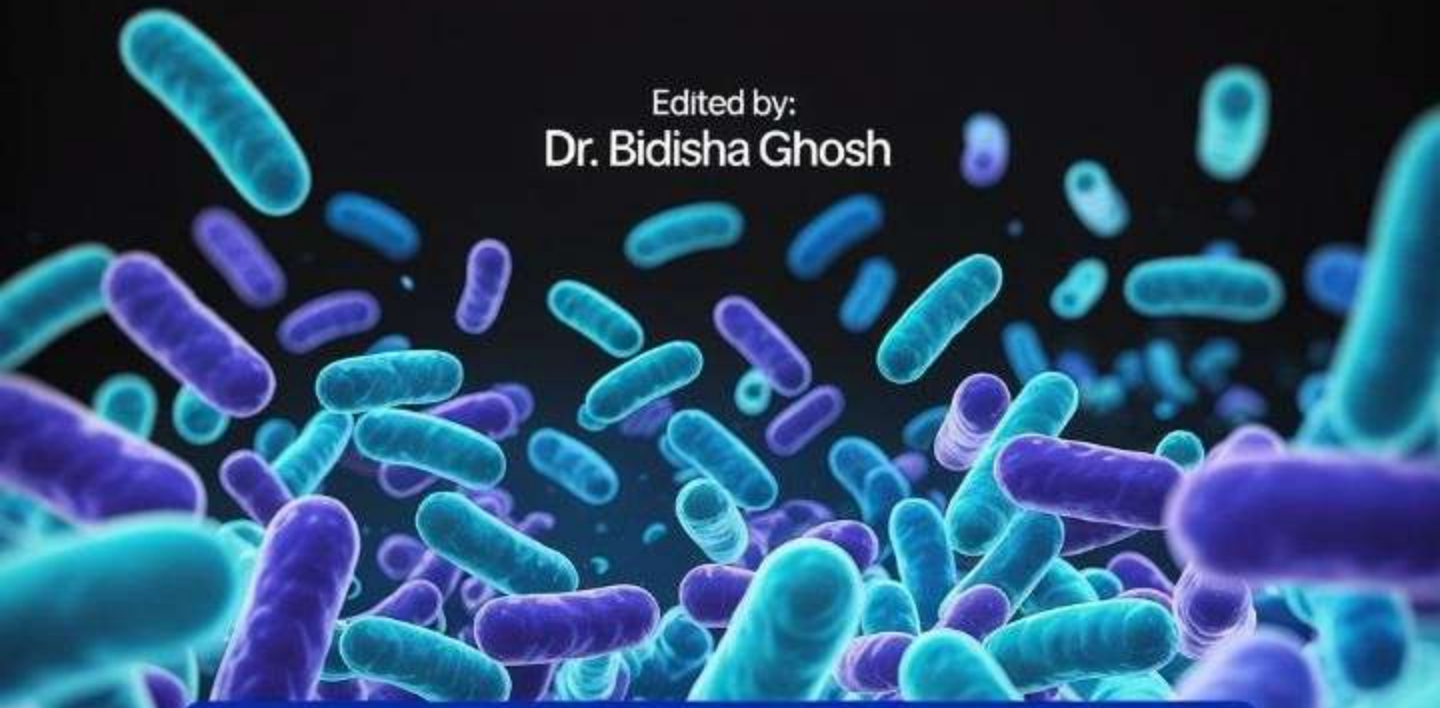
Essentials of Microbiology

Unearthing the Unseen



Edited by:
Dr. Bidisha Ghosh

Department of Microbiology
Swami Vivekananda University
Telimpaa, Barsat-Barackpore Rd
Bara Kanthalia, West Bengal–700121

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PREFACE

Microorganisms constitute the invisible foundation of life on Earth. From shaping global biogeochemical cycles to influencing human health and disease, these microscopic entities play roles that are both profound and far-reaching. *Essentials of Microbiology: Unearthing the Unseen* has been written with the aim of guiding readers through the fascinating world of microorganisms while connecting classical microbiological principles with emerging interdisciplinary areas of modern biological science.

This book seeks to present microbiology not merely as the study of microbes but as a dynamic field that intersects with diverse scientific disciplines. Alongside fundamental concepts such as microbial structure, physiology, genetics, and ecology, the text explores contemporary developments that demonstrate how microbiology integrates with material science, environmental studies, and biomedical research. In recent decades, advances in biomaterials and nanotechnology have opened new possibilities for the biomedical applications of materials, including antimicrobial surfaces, drug delivery systems, and tissue engineering approaches that rely on an understanding of microbial interactions.

Environmental challenges also demand a deeper understanding of microbial processes. Heavy metal contamination, for instance, represents a growing global concern due to its toxic effects on ecosystems and human tissues. This book therefore introduces readers to the biological implications of heavy metal exposure, the microbial mechanisms involved in metal transformation and detoxification, and the broader environmental consequences of metal pollution.

Equally important is the exploration of therapeutic strategies that mitigate inflammation and disease. The text highlights the role of anti-inflammatory drugs and naturally occurring phytochemicals, emphasizing their mechanisms of action, biological significance, and potential integration with microbiological and biomedical research. Such discussions aim to bridge traditional pharmacological knowledge with insights derived from microbial and molecular biology.

Designed primarily for undergraduate and postgraduate students of life sciences, this book also serves as a useful reference for researchers and educators seeking a concise yet comprehensive overview of microbiology in the context of modern scientific advancements. By bringing together foundational knowledge and interdisciplinary perspectives, *Essentials of Microbiology: Unearthing the Unseen* aspires to inspire curiosity about the microbial world and encourage readers to explore the unseen forces that shape life, health, and the environment.

ACKNOWLEDGEMENT

The successful completion of *Essentials of Microbiology: Unearthing the Unseen* has been possible through the collective effort, dedication, and expertise of many individuals. I would like to extend my sincere gratitude to all the contributing authors who generously shared their knowledge, research insights, and valuable time in preparing the chapters of this book. Their scholarly contributions have greatly enriched the content and helped present microbiology in a comprehensive and interdisciplinary perspective.

I am equally thankful to the reviewers whose critical evaluations, thoughtful suggestions, and constructive feedback significantly improved the clarity, accuracy, and scientific quality of the manuscript. Their guidance has played a vital role in shaping the final form of this book.

Special appreciation is extended to the publishing team for their continuous support, professional guidance, and meticulous efforts throughout the editing, design, and production process. Their commitment and efficiency have ensured the smooth and timely publication of this work.

I am grateful to Dr Suranjana Sarkar, Assistant Professor, Dept of Microbiology, Swami Vivekananda University for the beautiful illustration of the frontispiece.

Dr Bidisha Ghosh

Assistant Professor

Department of Biotechnology

Swami Vivekananda University

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Mycobacterium tuberculosis as a Chronic Architect of the Tumor-Permissive Microenvironment

Aishwarya Guha*

[1]*Department of Biotechnology Sciences, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata -700121*

**Corresponding author: aishbrabourne@gmail.com*

Abstract

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains a major global health challenge and is increasingly recognized for its long-term consequences beyond acute infection. Accumulating evidence suggests that TB may contribute to cancer development through persistent biological alterations that endure after microbiological cure. This study synthesizes current epidemiological and mechanistic evidence linking TB with increased cancer risk, with particular emphasis on lung cancer, while also considering extrapulmonary malignancies. Epidemiological studies consistently demonstrate a higher incidence of lung cancer among individuals with prior TB, an association that persists after adjustment for major confounders such as smoking and socioeconomic status. Emerging data further suggest systemic effects of TB that may elevate the risk of cancers at distant sites. Mechanistically, TB induces chronic inflammation, sustained cytokine signaling and excessive production of reactive oxygen and nitrogen species, promoting DNA damage, genomic instability and epigenetic reprogramming. Concurrent immune dysregulation including macrophage polarization, impaired antigen presentation and T-cell exhaustion may compromise long-term immune surveillance, facilitating malignant transformation. In lungs, TB-associated fibrosis, scarring and tissue remodelling creates a pro-tumorigenic microenvironment characterized by hypoxia and dysregulated growth factor signaling, supporting the concept of post-tuberculous carcinoma. Interactions between TB and cancer therapies further complicate clinical management, particularly in TB-endemic regions. Overall, the evidence positions TB as a chronic inflammatory condition capable of modifying long-term cancer risk. Recognizing TB survivors as a population at increased oncologic risk has important implications for cancer prevention, surveillance and integrated infectious and non-communicable disease care strategies worldwide.

Keywords: Tuberculosis, Cancer risk, Chronic inflammation, Lung cancer, Immune dysregulation, Fibrosis and tissue remodelling

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is a chronic infectious disease that continues to exert a profound global health burden and remains a leading cause of infection related mortality worldwide (WHO, 2023). Beyond its acute clinical manifestations, TB induces long-lasting biological alterations characterized by persistent immune activation, chronic inflammation, metabolic reprogramming and structural tissue remodelling that may persist long after apparent microbiological cure (Allwood et.al.2021). These features place TB within a growing group of chronic inflammatory conditions increasingly recognized as potential drivers of carcinogenesis.

Cancer development is a multistep process shaped by cumulative genetic and epigenetic alterations occurring within a permissive tissue microenvironment. Chronic inflammation is now regarded as a central enabling hallmark of cancer, promoting genomic instability, aberrant cell signalling, angiogenesis and immune evasion (Hanahan, Weinberg, 2011). Infectious agents collectively account for approximately 13–15% of cancers worldwide, underscoring the importance of infection driven oncogenic pathways (de Martel C et.al 2020). While viral oncogenesis has been extensively characterized at the molecular level, the contribution of chronic bacterial infections such as TB to tumor initiation and progression remains comparatively under investigated.

Epidemiological studies increasingly support a link between prior TB and elevated cancer risk, most consistently for lung cancer (Liang et al. 2009). Importantly, this association persists after adjustment for major confounders including smoking, age and socioeconomic status, suggesting that TB-associated biological processes may independently contribute to malignant transformation (Wu CY et al. 2011). Associations with extrapulmonary malignancies, including cancers of the head and neck, gastrointestinal tract and haematological system, have also been reported, raising the possibility of systemic effects of TB-related immune and inflammatory dysregulation (Simonsen et al. 2014).

At the molecular level, *M. tuberculosis* infection establishes a persistent inflammatory milieu characterized by sustained production of cytokines, chemokines and reactive oxygen and nitrogen species, all of which can induce DNA damage, epigenetic reprogramming and altered cell fate decisions (Elinav E et al. 2013). TB-associated immune remodelling,

including T-cell exhaustion, macrophage polarization and impaired antigen presentation, may further compromise immune surveillance and facilitate the survival and expansion of premalignant clones. In pulmonary TB, repeated cycles of tissue injury and repair lead to fibrosis and scarring, creating a structurally and biologically altered niche that may favour malignant transformation through dysregulated growth factor signalling and extracellular matrix remodelling.

In parallel, TB and cancer share overlapping social, environmental and behavioural determinants, including smoking, malnutrition, poverty and limited access to healthcare, which may synergize with TB-induced molecular alterations to amplify cancer risk (Lonnroth K et al. 2009). Disentangling these shared risk factors from TB-specific biological effects remains a major challenge but is essential for establishing causality. Given the substantial global overlap between TB endemic regions and rising cancer incidence, elucidating the molecular and cellular mechanisms linking TB to carcinogenesis has important implications for cancer prevention, risk stratification and long-term survivorship care. This study examines current epidemiological evidence and emerging molecular insights into TB-associated cancer risk, with a focus on inflammatory signalling, immune dysregulation and tissue micro-environmental changes that may connect chronic infection to malignant transformation.

1. Global Burden of Tuberculosis and Cancer

Tuberculosis and cancer together represent two of the most significant contributors to global morbidity and mortality, with an increasingly important overlap in many regions of the world. Tuberculosis continues to affect millions of individuals annually, with the highest burden concentrated in low- and middle-income countries, where delayed diagnosis, treatment interruptions and recurrent disease are common (WHO 2023). Although effective antimicrobial therapy has reduced TB-related mortality, a growing population of TB survivors now lives with long-term sequela, including chronic lung disease, systemic inflammation and immune dysfunction (Allwood BW et al. 2021). This expanding survivor population has brought renewed attention to the long-term, non-infectious consequences of TB.

In parallel, cancer incidence is rising globally, driven by population aging, urbanization, lifestyle changes and improved survival from infectious and chronic diseases. Importantly, the cancer burden is increasing most rapidly in regions where TB remains endemic, creating a convergence of infectious and non-communicable disease risks. In these settings, individuals may experience prolonged exposure to inflammatory stimuli, environmental carcinogens and socioeconomic stressors that collectively shape cancer susceptibility. Infection-associated cancers account for a substantial proportion of malignancies worldwide and while viral pathogens dominate this category, chronic bacterial infections such as TB may contribute indirectly through sustained tissue damage and immune perturbation (de Martel C et al. 2020). The intersection of TB and cancer is particularly evident in pulmonary disease. Pulmonary TB frequently results in permanent structural lung abnormalities, including fibrosis, bronchiectasis and cavitation, which can persist long after treatment completion. These pathological changes alter the local tissue microenvironment, potentially facilitating carcinogen retention, aberrant epithelial repair and dysregulated growth signalling. Given that lung cancer remains the leading cause of cancer-related death globally, understanding how post-TB lung pathology contributes to cancer risk is of major clinical and public health relevance. The growing global population of TB survivors therefore represents an emerging at-risk group for cancer development that has not been adequately addressed in current cancer prevention strategies.

2. Epidemiological Evidence Linking Tuberculosis and Cancer

A substantial body of epidemiological research has examined the association between tuberculosis and subsequent cancer risk, with the strongest and most consistent evidence observed for lung cancer. Multiple cohort and case control studies across diverse populations have demonstrated that individuals with a prior history of pulmonary TB have a significantly higher incidence of lung cancer compared with the general population (Liang HY *et* al 2009, Wu *et* al 2011). Notably, this increased risk persists after adjustment for major confounders such as smoking, age, sex and socioeconomic status, suggesting that TB-related biological effects may independently contribute to carcinogenesis rather than merely reflecting shared risk factors.

Temporal analysis indicate that cancer risk is highest within the first several years following TB diagnosis but may remain elevated for decades, supporting both short-term and long-term

pathogenic mechanisms. Early excess risk may reflect reverse causation or diagnostic overlap, as lung cancer can initially mimic TB clinically and radiologically. However, sustained long-term risk strongly implicates post-infectious processes such as chronic inflammation, fibrosis and immune remodelling as contributors to malignant transformation. The concept of TB-related 'scar carcinoma' is supported by radiological and pathological studies demonstrating preferential tumor development in areas of prior tuberculous scarring. Beyond lung cancer, epidemiological studies have reported associations between TB and extrapulmonary malignancies, including cancers of the head and neck, gastrointestinal tract, lymphatic system and genitourinary organs (Simonsen DF et al. 2014). Although these associations are less consistent across studies and populations, they suggest that TB may exert systemic effects on cancer risk, potentially mediated by chronic immune activation, altered cytokine profiles, or long-term impairment of immune surveillance. Large nationwide registry studies have further demonstrated increased overall cancer incidence among TB patients compared with matched controls, reinforcing the hypothesis that TB represents a marker of increased cancer susceptibility rather than a site-specific phenomenon alone.

3. Biological Mechanisms

The biological link between tuberculosis and cancer is increasingly understood as a consequence of sustained host-pathogen interactions that remodel cellular, molecular and tissue-level processes in ways that favour malignant transformation (Elinav E et al. 2013). Unlike acute infections, *Mycobacterium tuberculosis* establishes long-term persistence within the host, driving chronic inflammation, immune dysregulation and structural tissue damage that collectively create a pro-tumorigenic microenvironment. Chronic inflammation represents a central mechanistic axis connecting TB to carcinogenesis. Persistent activation of innate immune cells during TB infection results in prolonged secretion of pro-inflammatory cytokines such as tumor necrosis factor-α, interleukin-6 and interleukin-1β, alongside increased production of reactive oxygen and nitrogen species (Hussain SP et al. 2007). These mediators can induce oxidative DNA damage, impair DNA repair mechanisms and promote genomic instability in surrounding epithelial cells. Over time, repeated cycles of tissue injury and repair driven by unresolved inflammation may increase the likelihood of accumulating oncogenic mutations and epigenetic alterations that initiate tumorigenesis.

Immune dysregulation further contributes to cancer susceptibility in individuals with current or prior TB. *M. tuberculosis* employs multiple immune evasion strategies that alter both innate and adaptive immunity, including modulation of macrophage polarization, impairment of antigen presentation and induction of T-cell exhaustion (O Garra et al. 2013). These changes may persist beyond active infection, leading to long-term defects in immune surveillance. Reduced capacity to recognize and eliminate premalignant or malignant cells allows transformed clones to survive and expand, thereby facilitating cancer development. Structural tissue remodeling following TB infection provides an additional mechanistic link, particularly in the lung. Pulmonary TB frequently leads to fibrosis, bronchiectasis, and scarring, which alter normal tissue architecture and disrupt epithelial-mesenchymal interactions. Fibrotic tissue is characterized by aberrant extracellular matrix deposition, altered growth factor signalling and hypoxic conditions, all of which are known to promote malignant transformation and tumor progression (Wynn TA et al. 2008). The phenomenon of post-tuberculous 'scar carcinoma' highlights the importance of localized tissue damage as a driver of lung cancer risk (Yu YH et al.2011).

Finally, TB-induced systemic effects, including chronic immune activation and metabolic alterations, may contribute to extrapulmonary cancer risk. Persistent inflammatory signalling and cytokine imbalance can affect distant organs, potentially influencing stem cell niches, promoting epigenetic reprogramming and altering systemic immune homeostasis (Grivennikov SI et al.2010). Together, these mechanisms support a biologically plausible model in which TB acts as a long-term modifier of cancer risk through interconnected inflammatory, immune, and tissue-remodelling pathways.

4. Tuberculosis, Cancer and Treatment Interactions

The relationship between tuberculosis and cancer is further complicated by interactions between disease treatments and host immunity. Effective anti-tuberculosis therapy reduces bacterial burden and resolves active inflammation. However, treatment does not fully reverse immune dysregulation or structural tissue damage, particularly in advanced or recurrent disease. As a result, TB survivors may remain at increased cancer risk despite microbiological cure. Conversely, cancer therapies can profoundly influence TB risk and outcomes. Cytotoxic chemotherapy, corticosteroids and targeted therapies suppress immune function and can lead to reactivation of latent TB infection. More recently, immune

checkpoint inhibitors, which enhance antitumor immunity, have been associated with paradoxical immune reconstitution and TB reactivation, highlighting the complex interplay between cancer immunotherapy and host defense against M. tuberculosis. These interactions underscore the need for careful TB screening and monitoring in cancer patients, particularly in TB endemic settings. In addition, overlapping toxicities and diagnostic challenges complicate clinical management. Radiological findings such as lung nodules, cavitation or lymphadenopathy may be difficult to distinguish between TB and malignancy, leading to delays in diagnosis or inappropriate treatment. Integrated clinical approaches are therefore essential to optimize outcomes for patients affected by both conditions.

5. Signalling Mechanisms Linking Tuberculosis to Carcinogenesis

At the molecular level, tuberculosis drives carcinogenesis through sustained activation of multiple pro-inflammatory and pro-survival signalling pathways that are well established in cancer biology. Central among these is the NF-κB signalling pathway, which is persistently activated during *Mycobacterium tuberculosis* infection. Engagement of pattern recognition receptors such as Toll-like receptors (TLR2, TLR4 and TLR9) on macrophages and epithelial cells leads to NF-κB nuclear translocation and chronic transcription of genes encoding tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2) and anti-apoptotic proteins including BCL-2 and survivin (Hayden MS et al.2011). Prolonged NF-κB activation promotes resistance to apoptosis, sustained cell proliferation and genomic instability, thereby creating a permissive environment for malignant transformation.

IL-6/STAT3 signalling represents another critical axis linking TB-associated inflammation to cancer risk. Elevated IL-6 levels have been consistently documented in active and post-treatment TB patients. Chronic IL-6 exposure activates STAT3 in epithelial and stromal cells, driving transcriptional programs associated with cell cycle progression, angiogenesis (via VEGF), epithelial-mesenchymal transition (EMT) and immune evasion (Yu H et al.2009). Persistent STAT3 activation is a hallmark of many solid tumors, including lung cancer and may provide a mechanistic explanation for the long-term oncogenic effects observed after TB resolution.

TB infection also induces transforming growth factor-β (TGF-β) signalling, particularly during the fibrotic repair phase of pulmonary disease. TGF-β promotes fibroblast activation, extracellular matrix deposition and tissue scarring, while simultaneously exerting

immunosuppressive effects by inhibiting cytotoxic T-cell function. In epithelial cells, aberrant TGF-β signalling can facilitate EMT, enhance invasiveness and promote tumor progression, particularly within fibrotic lung tissue (Massagué Jet al.2009).

Oxidative stress related signalling further contributes to carcinogenesis. Chronic production of reactive oxygen and nitrogen species (ROS/RNS) during TB activates DNA damage response pathways, including ATM/ATR and p53. However, persistent oxidative stress may overwhelm repair mechanisms, leading to mutations, microsatellite instability and epigenetic alterations such as DNA methylation changes. ROS-mediated activation of MAPK pathways (ERK, JNK and p38) additionally promotes proliferation and survival signalling in damaged epithelial cells (Reuter Sal et al. 2010). Emerging evidence also implicates hypoxia-inducible factor-1α (HIF-1α) signalling in TB-associated cancer risk. Granulomatous lesions and fibrotic lung tissue are characterized by hypoxic microenvironments that stabilize HIF-1α, driving metabolic reprogramming toward glycolysis, angiogenesis and resistance to apoptosis. These adaptations mirror metabolic hallmarks of cancer and may facilitate the survival and expansion of premalignant cells within post-tuberculous tissue niches.

Collectively, sustained activation of NF-κB, IL-6/STAT3, TGF-β, MAPK and HIF-1α signalling pathways provides a mechanistic framework linking chronic TB-induced inflammation, immune dysregulation and tissue remodelling to carcinogenesis. These convergent signalling networks highlight how a persistent bacterial infection can reprogram host cellular pathways in ways that resemble and ultimately promote malignant transformation.

6. Conclusion

The evidence synthesized in this study supports a meaningful association between tuberculosis and increased cancer risk, particularly lung cancer, while also suggesting broader systemic effects that may predispose to extrapulmonary malignancies. Importantly, this relationship appears to extend beyond shared behavioural and socioeconomic determinants, implicating TB-specific biological processes as contributors to carcinogenesis. The persistence of elevated cancer risk years after successful TB treatment underscores the importance of long-term host alterations rather than active infection alone. From a mechanistic standpoint, tuberculosis represents a paradigm of inflammation-driven cancer risk. Chronic immune activation, sustained cytokine signalling and excessive production of

reactive oxygen and nitrogen species create conditions conducive to DNA damage, genomic instability and epigenetic reprogramming. In pulmonary disease, TB-induced fibrosis and scarring generate a remodelled tissue microenvironment characterized by hypoxia, aberrant extracellular matrix deposition and dysregulated growth factor signalling, all of which are recognized promoters of malignant transformation. These processes provide a biologically plausible explanation for the consistent epidemiological association between prior TB and lung cancer, including the phenomenon of post-tuberculous scar carcinoma.

Immune dysregulation further links TB to cancer susceptibility. Persistent alterations in macrophage function, antigen presentation and T-cell exhaustion may compromise long-term immune surveillance, allowing premalignant clones to escape immune control. Systemic immune and metabolic effects of TB may also influence cancer risk at distant sites, supporting the observation of associations with extrapulmonary malignancies. Clinically, these findings highlight the need to move beyond a narrow focus on microbiological cure and to recognize TB survivors as a population at potential long-term cancer risk. Targeted surveillance, particularly for lung cancer in individuals with residual pulmonary damage, may improve early detection. From a public health perspective, integrating TB control with non-communicable disease prevention strategies is increasingly important in TB endemic regions. Future longitudinal and molecular studies will be essential to clarify causality, identify high risk subgroups and inform evidence-based prevention and survivorship care strategies.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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Hydrogel incorporated with phytochemicals: A smart green approach towards wound healing

Sovan Patra [1], Raj Manna[1], Subhasis Sarkar[2], Bidisha Ghosh[1]*

[1]*Department of Biotechnology, School of life Sciences, Swami Vivekananda University, Barrackpore, Kolkata 700121*

[2]*Department of Microbiology, School of life Sciences, Swami Vivekananda University, Barrackpore, Kolkata 700121*

**Corresponding author*: bidishag@svu.ac.in

Abstract

The intricate, multi-phase process of wound healing is frequently hampered by oxidative stress, infection, and inadequate angiogenesis. Three-dimensional hydrophilic polymer networks, or hydrogels, offer a moist microenvironment and a regulated delivery system, while phytochemicals, which are tiny compounds originating from plants, have regenerative, antioxidant, and antibacterial qualities. This review discusses design techniques, mechanisms of action, and medical results to highlight the beneficial use of hydrogels combined with phytochemicals in wound care. Ending with future thoughts on intelligent and multifunctional hydrogel systems for clinical translation, we highlight issues like stability, controlled release, and regulatory considerations. The objective was Optimizing antibacterial action and identifying the phytochemical component.

Keywords: Wound healing, Hydrogels, Phytochemicals, Antibacterial activity, Controlled release, Clinical translation.

Introduction

The biological process of wound healing is intricate and dynamic, restoring the continuity and functionality of damaged tissues. It goes through phases that overlap: remodelling, proliferation, inflammation, and haemostasis. The human body can naturally heal minor wounds, but a number of internal and environmental variables, including oxidative stress, chronic disorders like diabetes, and infections, can hinder or delay the healing process, particularly in chronic wounds (Deng et al. 2021). Advanced biomaterials that facilitate quicker and more effective healing have replaced conventional wound dressings like gauze and cotton in modern wound care. Among these, hydrogels have drawn interest because of their special qualities, which include a high-water content, biocompatibility, and a porous structure that allow for the exchange of nutrients and oxygen while maintaining a moist wound environment. Their gel-like consistency supports tissue regeneration and cellular migration by imitating the extracellular matrix (Yu et al. 2022).

In parallel, traditional medicine has made great use of phytochemicals—natural bioactive compounds derived from medicinal plants—for the treatment of wounds. Aloe vera extract, quercetin, tannic acid, and curcumin (from Curcuma longa) are among the compounds that have several medicinal uses, such as collagen-promoting, anti-inflammatory, antioxidant, and antibacterial effects (Sivamani et al. 2012). The usefulness of many phytochemicals when administered directly to wounds is limited by their low water solubility, poor absorption, and quick degradation, despite their promising pharmacological properties. Researchers have created hydrogel-based delivery methods that encapsulate phytochemicals to prevent degradation and allow for regulated, continuous release at the wound site in order to overcome these difficulties (Mirrezaei et al. 2020). These composite solutions provide a natural, secure, and multipurpose method of wound treatment by combining the healing capacity of phytochemicals with the structural and protective qualities of hydrogels. In order to determine how well phytochemical-loaded hydrogels promote wound healing, this thesis will investigate their synthesis, characterization, and therapeutic performance using both animal and laboratory model studies (Qadir et al. 2021).

1.1 Hydrogel as a Treatment for Wounds

Soft, jelly-like substances called hydrogels are created from networks of hydrophilic

polymers. These polymers' unique quality is their capacity to keep and hold a lot of water, sometimes up to multiple times their dry weight. This water-rich composition forms a cool, calming layer when applied to a wound, which not only soothes the patient but also aids in the healing process. Hydrogels keep the area wet, in contrast to dry dressings that may adhere to the wound surface and hurt to remove. This moist state is crucial because it promotes cell migration, keeps tissues from drying out, and speeds up the skin's natural healing process (Nuutila et al. 2021).

The capacity of hydrogels to encourage autolytic debridement is another crucial characteristic. To put it simply, the hydrogel facilitates the growth of new, healthy tissue by allowing the body's natural enzymes and fluids to soften and break down dead or damaged tissue in the wound. This lessens the need for unpleasant manual wound washing and lowers the risk of subsequent trauma while changing dressings (Waycaster et al. 2013).

It is possible to create hydrogels using both synthetic and natural polymers. Because they are biocompatible and frequently have extra biological advantages like antibacterial or antioxidant properties, natural polymers including chitosan, alginate, gelatine, and hyaluronic acid are especially appealing. Conversely, synthetic polymers such as polyvinyl alcohol (PVA) and polyethylene glycol (PEG) provide superior control over their physical properties, stability, and great mechanical strength. Scientists can create hybrid hydrogels that combine the benefits of both natural and synthetic materials, such as durability and biological activity (Zhao et al. 2023).

The ability to precisely control the porosity and crosslinking structure of hydrogels is one of their intriguing features. Researchers can manipulate the rate at which molecules flow through the gel by changing the way the polymers are joined. Hydrogels are therefore a great platform for regulated medication release. Bioactive substances like growth hormones, antibiotics, or herbal extracts, for instance, might be included into the hydrogel and thereafter released gradually at the wound site. In addition to lowering the danger of infection, this local and sustained administration promotes tissue regeneration and eliminates the need for repeated therapy (Shan & Wu 2024).

Scientists have developed "smart" hydrogels in recent years, going beyond ordinary hydrogels. Certain triggers in the wound environment can cause these sophisticated mechanisms to react. For example, certain hydrogels are made to detect pH variations, which are frequently higher in infected wounds. Others may react to naturally occurring

enzymes during tissue breakdown or to reactive oxygen species (ROS), which are produced in excess during inflammation. At the precise moment when they are most needed, these smart hydrogels can alter their structure, expand or shrink, and release their therapeutic chemicals. As a result, they become active participants in the healing process rather than merely passive dressings (Wu et al. 2022).

All things considered, hydrogels are a fantastic advancement in wound care. They are considerably superior to traditional dressings because of their capacity to maintain the wound moist, promote natural cleaning, provide therapeutic chemicals in a controlled manner, and even react intelligently to the wound's changing condition. Hydrogels are anticipated to evolve further with continued study, providing patients with improved protection against problems, reduced discomfort, and quicker recovery (Du et al. 2025).

1.2 ***Phytochemicals in the Healing of Wounds***

Plants naturally produce bioactive substances called phytochemicals to defend against environmental stressors, infections, and other threats. Many of these plant-derived compounds have also been found to have substantial therapeutic utility for humans throughout time, particularly in the area of wound healing. Phytochemicals have a broad and synergistic influence on the intricate process of tissue regeneration because, in contrast to synthetic medications, they frequently work through many pathways simultaneously. Several key mechanisms can be used to categorize their actions:

1.2.1 Underlying antimicrobial action of phytochemicals in the treatment of wounds:

Since bacteria and fungi can readily colonize the surface of an open wound, infection is one of the main challenges in wound care. Some phytochemicals exhibit exceptional antibacterial qualities, including eugenol, thymol, and berberine. By rupturing microbial cell membranes, these substances make it harder for microorganisms to thrive. Thymol and eugenol change the permeability of microbial membranes, allowing necessary substances to escape. Berberine, for example, disrupts bacterial DNA replication and protein synthesis. In an era of increasing antibiotic resistance, this natural antibacterial effect helps prevent wound infections without relying solely on synthetic antibiotics (Herman & Herman 2023).

1.2.2 The Activity of Antioxidants

Oxidative stress—brought on by an excess of reactive oxygen species (ROS)—can harm healthy cells surrounding the wound site and postpone the healing process. Strong antioxidants such as flavonoids, quercetin, and resveratrol are found in phytochemicals that can counteract these dangerous substances. They prevent oxidative damage to proteins, DNA, and biological membranes by scavenging free radicals. In addition to providing basic defense, antioxidants aid in reestablishing the balance of cellular signalling pathways, which is essential for healthy wound healing. Antioxidant phytochemicals, therefore, help the wound return to a healing-friendly environment rather than allowing it to stay in a protracted state of stress (Zulkefli et al. 2023).

1.2.3 Reduction of Inflammation

Although severe or prolonged inflammation can damage new tissue formation and result in chronic wounds, it is a normal aspect of the wound healing process. Certain phytochemicals, such as curcumin and baicalin, have substantial anti-inflammatory properties. These compounds inhibit nuclear factor-kappa B (NF-κB), a key signalling pathway that controls the release of inflammatory cytokines and chemokines. In doing so, they reduce unnecessary inflammation, decrease edema and redness, and improve conditions for new tissue formation. Because of this, they are particularly beneficial for chronic wounds where inflammation is often unmanageable (Shah & Amini-Nik 2017).

1.2.4 Benefits of Regeneration

Healing necessitates rebuilding in addition to eliminating stress and combating germs. During this stage, phytochemicals from *Centella asiatica*, also referred to as gotu kola, such as Asiaticoside and madecassoside, are essential. By encouraging fibroblast proliferation, these substances increase the number of cells that can produce new tissue. Additionally, they stimulate production of collagen, which is necessary to give the healed skin strength and structure, and also promote angiogenesis (growth of new blood vessels), which enhances nutrient and oxygen supply to the wound bed. Together, these regenerative processes hasten wound closure and improve the quality of repaired tissue (Arribas-López et al. 2022).

2. Systems of Hydrogel and Phytochemistry

One of the most intriguing developments in wound care is the concept of mixing hydrogels and phytochemicals. Although phytochemicals alone have a great deal of therapeutic potential, their application is sometimes restricted since many of them are poorly soluble in water, unstable in the presence of light or oxygen, and rapidly broken down by the body. Hydrogels, with their water-rich and protective environment, present a good answer to these difficulties. Hydrogels become more stable, their release can be precisely regulated, and their healing potential can be fully utilized at the wound site when phytochemicals are added.

2.1 Techniques for Encapsulating Hydrophobic Phytochemicals

Curcumin and resveratrol are two examples of potent phytochemicals that are inherently hydrophobic, meaning they do not dissolve readily in water. When placed directly on wounds, this hinders their ability to be absorbed efficiently. In order to get around this, researchers have devised methods that include first encasing these hydrophobic substances in micelles or nanoparticles. By acting as protective shells, these carriers improve the phytochemicals' water-friendliness and prevent them from breaking down too soon. The phytochemicals can then be inserted into the hydrogel matrix after being encapsulated. Instead of delivering a brief, transient therapeutic effect at the wound site, our dual method guarantees that the compounds are released gradually in a stable and active state (Mirrezaei et al. 2020).

2.2 Direct Hydrogel Backbone Conjugation

The direct attachment of phytochemicals to the hydrogel's polymer chains is another cutting-edge method. This method involves chemically attaching the phytochemical molecules to the hydrogel's backbone, a process known as conjugation. This guarantees that the bioactive ingredients stay anchored in the dressing and are not readily removed by washing. These phytochemicals are released gradually and under control when the hydrogel interacts with the wound environment over time. By preventing needless loss of activity and enhancing the stability of sensitive substances, this technique enables phytochemicals to function precisely where and when they are required (Zheng et al. 2024).

3. **Benefits of the Combination Working Together: Synergism of Phytochemical incorporated Hydrogel:**

The synergistic link between hydrogel and phytochemical systems is their real strength. Significant wound-healing advantages are already provided by hydrogels alone: they keep the area wet, avoid dehydration, lessen discomfort, and create a gentle cushioning layer that shields the tissue. Conversely, phytochemicals provide specific biological functions such as tissue regeneration, antioxidant defence, antibacterial activity, and anti-inflammatory support. The combined effect of these two systems is significantly more potent than either would be on its own. The phytochemicals actively promote healing through their various biological actions, while the hydrogel acts as a stable and supportive delivery system. When combined, they produce a clever, multipurpose dressing that may simultaneously treat several issues, including tissue restoration, oxidative stress reduction, infection prevention, and moisture management (Lynch et al. 1987).

4. **Moving Towards Smart Intelligent Wound Care**

Hydrogel–phytochemical systems are developing into smart wound dressings that can adapt to the changing conditions of the wound thanks to advancements in material science. In order to detect infection, for example, scientists are creating hydrogels that release phytochemicals more quickly when the wound environment becomes more acidic. Others are being developed to react to particular enzymes generated during inflammation or the presence of reactive oxygen species. In this sense, the hydrogel functions almost like a sensor, modifying the release of phytochemicals in response to the demands of the wound. Because of its dynamic function, the system becomes an active collaborator in healing rather than only a passive bandage (Wang et al. 2025).

5. **Obstacles and Prospects for the Future**

Despite their impressive potential in lab and animal experiments, hydrogel–phytochemical systems have a difficult path from research to practical medicinal use.

Before these cutting-edge dressings are regularly utilized in clinics and hospitals, a number of obstacles must be removed. Their successful integration into contemporary wound care will depend on our ability to comprehend these issues and seek out creative solutions.

5.1 Plant Extract Variability

The unpredictability of molecules derived from plants is one of the main obstacles. A plant's phytochemical content can vary greatly based on its growing environment, harvest season, soil type, and even the extraction method. For example, the potency and purity of curcumin derived from turmeric cultivated in one area may not be the same as that derived from turmeric grown in another. It is challenging to guarantee consistent quality and predictable treatment results due to batch-to-batch variability, which is a crucial prerequisite for clinical approval. Because variations in composition can result in inconsistent wound healing efficacy, clinicians may be reluctant to depend on these systems without standardization (Dhami & Mishra 2015).

5.2 Regulatory Uncertainties

The regulatory environment is another significant barrier. Since hydrogels are medical devices and phytochemicals fall somewhere in the middle of the spectrum between pharmaceutical medications and herbal cures, it is challenging to classify the combined product. This raises questions about long-term monitoring, safety evaluations, and approval processes. Strict proof of safety, effectiveness, and reproducibility is required by regulatory bodies; yet, because phytochemicals are inherently complex, demonstrating this can be difficult. Clinical use of hydrogel–phytochemical systems will continue to be delayed and incur additional expenditures until clear guidelines are established (Parveen et al. 2015).

5.3 Limitations in Manufacturing and Scale-Up

Although hydrogel–phytochemical dressings have been successfully prepared on a modest scale in laboratories; it is far more difficult to scale up production for commercial usage. The accuracy required to maintain uniform phytochemical loading, distribution,

and release qualities in large batches is sometimes lacking in current manufacturing procedures. Furthermore, maintaining mechanical strength, biocompatibility, and sterility during large-scale production presents additional challenges. These technologies might not make it to pharmacy shelves, remaining restricted to experimental research in the absence of reliable large-scale production techniques (Siafaka et al. 2025).

5.4 Issues with Stability

It is well known that when exposed to environmental stress, phytochemicals become unstable. When many substances come into contact with oxygen, light, heat, or enzymes in the wound bed, they break down rapidly. Long-term stability during storage and transit is still a major challenge, even when shielded by a hydrogel. The therapeutic effectiveness of a hydrogel–phytochemical dressing is reduced if it loses its bioactivity before it even reaches the patient. To ensure dependable performance, it will be crucial to develop novel stabilization strategies such as nanoencapsulation, protective polymer coatings, or antioxidant co-formulations (Azizah et al. 2023).

6. Delving deep in to the Future: Towards Cutting edge engineering strategies for smart wound dressings

Looking ahead, technological integration and clever design are key to the success of hydrogel–phytochemical systems. It will be essential to standardize phytochemical formulations in order to ensure clinical trust and reproducibility. Furthermore, hydrogels are being developed to become multi-responsive systems, meaning they may release their cargo in response to multiple wound signals simultaneously, such as pH, temperature, or reactive oxygen levels. Cutting-edge fabrication technologies, especially 3D bioprinting, make it possible to create wound dressings with unique structures, forms, and release patterns for each patient.

The application of artificial intelligence (AI) to formulation design is another fascinating area. AI can assist in predicting the optimal combinations and optimizing release profiles more effectively than conventional trial-and-error techniques by evaluating sizable datasets on polymer architectures, phytochemical stability, and wound healing outcomes.

Lastly, researchers must concentrate on developing hydrogel–phytochemical dressings that are both affordable and biodegradable in order to guarantee widespread use. In addition to

being reasonably priced for broad adoption, such systems would cut down on medical waste and satisfy the rising need for ecologically friendly healthcare options.

7. Conclusion

In the area of wound care, hydrogel–phytochemical composites are a noteworthy advancement. These cutting-edge solutions combine the many biological activities of plant-derived chemicals with the structural and functional advantages of hydrogels, in contrast to traditional dressings that mostly act as passive barriers. When combined, they produce multipurpose platforms that have the ability to actively fight infection, neutralize oxidative stress, lessen damaging inflammation, and hasten the formation of new, healthy tissue. This makes them ideal for dealing with the intricate problems of acute and chronic wounds, which frequently call for coordinated action on several fronts.

These systems' capacity to connect contemporary biomaterials research with traditional herbal medicine is their real strength. Phytochemicals have been utilized for centuries in traditional wound-healing therapies, but because to problems such low solubility, instability, and uncontrolled administration, their therapeutic influence has been limited. By providing a stable, water-rich, and protective environment that allows phytochemicals to be encapsulated, stored, and released in a targeted and sustained manner, hydrogels help to overcome many of these obstacles. In this way, the precision and dependability of engineered materials are seamlessly combined with the wisdom of natural healing to create therapies that are both biologically inspired and scientifically sound.

But in the end, clever design and thorough validation will be necessary for hydrogel–phytochemical systems to succeed. The selection of polymers, the process of incorporating phytochemicals, and the system's adaptability to the wound's changing conditions all require careful thought. Thorough preclinical and clinical testing is equally crucial in order to guarantee that these novel compounds are safe, repeatable, and effective. Such composites could advance beyond experimental research and become dependable, generally recognized choices in clinical wound care if they fulfil these requirements.

These hybrid technologies have the potential to revolutionize the treatment of wounds in the future. They represent a new generation of multifunctional wound remedies rather than just minor advancements over current dressings. Through the simultaneous treatment of infection, oxidative damage, and hindered tissue regeneration, they offer a more comprehensive and patient-centred method of healing. With continued developments in drug delivery, biomedical engineering, and material science, hydrogel–phytochemical composites could soon establish a new standard for wound dressings, which would eventually benefit patients all over the world by improving their quality of life, accelerating their recovery, and improving their results.

Conflict of Interest:

The authors declare that they have no competing interest.

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Alphafold: Decoding Nature's Blueprint and Redefining the Future of Structural Biology

Vinitha S.[1], Debjit De[2]

[1]*Department of Microbiology, Swami Vivekananda University, Barrackpore, Kolkata -700121*

[2]*Department of Biotechnology, Swami Vivekananda University, Barrackpore, Kolkata-700121*

[*]*Corresponding author*: debjitd@svu.ac.in

Abstract

The elucidation of protein three-dimensional structures from amino acid sequences has remained one of the most formidable challenges in molecular biology for over half a century. AlphaFold, an artificial intelligence system developed by DeepMind, has revolutionized this field by achieving near-experimental accuracy in protein structure prediction through sophisticated deep learning architectures. This breakthrough has catalyzed unprecedented advances in drug discovery, disease mechanism elucidation, protein engineering, and fundamental biological research. This review examines the technological underpinnings of AlphaFold, its transformative impact across multiple scientific disciplines, current applications in addressing global challenges, and the emerging landscape of computational structural biology. We discuss the integration of predictive models with experimental methodologies, address current limitations including intrinsically disordered proteins and conformational dynamics, and explore future directions that promise to revolutionize our understanding of protein function, molecular interactions, and cellular organization. The democratization of structural information through AlphaFold represents a paradigm shift that is accelerating scientific discovery and opening unprecedented opportunities for therapeutic development, sustainable biotechnology, and the fundamental understanding of life's molecular machinery.

Keywords: AlphaFold, protein structure prediction, deep learning, artificial intelligence, structural biology, computational biology, drug discovery

1. Introduction

Proteins constitute the fundamental machinery of life, orchestrating virtually every biological process from enzymatic catalysis to cellular signalling and structural organization. The three-dimensional architecture of a protein is inextricably linked to its function, a principle that has driven structural biology since the pioneering determination of myoglobin's crystal structure in 1958 (Kendrew et al., 1958). However, experimental structure determination through X-ray crystallography, nuclear magnetic resonance spectroscopy, and cryo-electron microscopy remains resource-intensive, technically demanding, and time-consuming, leaving millions of protein sequences without corresponding structural information.

The protein folding problem—predicting a protein's native three-dimensional structure solely from its amino acid sequence—has challenged scientists since Cyrus Levinthal articulated the paradox in 1969 (Levinthal, 1969). Despite the astronomical number of theoretically possible conformations, proteins consistently fold into specific structures within milliseconds. Christian Anfinsen's groundbreaking experiments established that the native structure is thermodynamically determined by the amino acid sequence alone, providing the theoretical foundation for computational structure prediction (Anfinsen, 1973).

The advent of AlphaFold represents a watershed moment in addressing this grand challenge. Introduced at the Critical Assessment of protein Structure Prediction (CASP) competition, AlphaFold achieved unprecedented accuracy at CASP14 in 2020, with performance approaching experimental methods (Jumper et al., 2021). The subsequent release of AlphaFold2 and the AlphaFold Protein Structure Database, containing predictions for over 200 million proteins, has democratized access to structural information and accelerated research across diverse fields (Varadi et al., 2022).

This review examines the architecture and methodology underlying AlphaFold, its profound impact on structural biology and related disciplines, current applications addressing global challenges, inherent limitations, and the future trajectory of computational structure prediction in the post-AlphaFold era.

2. The Alphafold Revolution: Technical Architecture and Innovation

2.1 Deep Learning Foundations

AlphaFold employs a sophisticated neural network architecture that integrates evolutionary information, physical constraints, and geometric principles to predict protein structures with remarkable accuracy. Unlike traditional template-based approaches that rely heavily on homologous structures, AlphaFold learns the fundamental principles governing protein folding from the Protein Data Bank's experimentally determined structures (Senior et al., 2020). The AlphaFold2 architecture comprises three interconnected modules: an input processing system generating multiple sequence alignments (MSAs), an Evoformer network processing spatial and evolutionary relationships through attention mechanisms, and a structure module iteratively refining three-dimensional atomic coordinates (Jumper et al., 2021).

2.2 Evolutionary Intelligence: Multiple Sequence Alignments

Central to AlphaFold's success is its exploitation of evolutionary information encoded in MSAs. By analyzing co-evolutionary patterns across homologous sequences from diverse organisms, the algorithm identifies residue pairs that maintain spatial proximity in folded structures (Marks et al., 2011). This co-evolutionary signal provides powerful geometric constraints. AlphaFold constructs MSAs by searching extensive genetic databases including UniRef90, MGnify, and the Big Fantastic Database, capturing evolutionary relationships spanning billions of years of molecular evolution.

2.3 The Evoformer: Attention Mechanisms Meet Structural Biology

The Evoformer module represents a pivotal innovation, employing attention mechanisms—originally developed for natural language processing—to model relationships between residues in both MSA and structural representations simultaneously (Jumper et al., 2021). This dual representation enables seamless integration of evolutionary and geometric information. The Evoformer iteratively updates two representations: the MSA representation capturing evolutionary patterns and the pair representation encoding pairwise residue relationships including distances and orientations. This architecture allows the network to leverage evolutionary information while respecting the physical constraints of protein folding.

2.4 Structure Module and Confidence Estimation

The structure module converts processed representations into three-dimensional atomic coordinates through an equivariant transformer architecture that respects rotational and translational symmetries of three-dimensional space. Critically, AlphaFold provides quantitative confidence metrics including per-residue confidence scores (pLDDT) and predicted aligned error (PAE) matrices, enabling researchers to assess prediction reliability (Jumper et al., 2021). These confidence measures are essential for downstream applications, particularly in drug discovery where structural accuracy is paramount.

Table 1: Comparative Analysis of Protein Structure Prediction Methodologies

Method	**Approach**	**Average Accuracy (TM-score)**	**Computational Time**	**Primary Limitations**
Homology Modeling	Template-based comparison	0.5-0.7	Hours	Requires close homologs
Ab Initio Methods	Physics-based simulation	0.4-0.6	Days-Weeks	Computationally prohibitive
Rosetta	Energy-based optimization	0.5-0.65	Days	Limited accuracy for large proteins
AlphaFold2	Deep learning (MSA + attention)	0.85-0.95	Minutes-Hours	Requires evolutionary information
AlphaFold3	Enhanced deep learning	0.90-0.96	Minutes	Limited for novel folds

Note. TM-score ranges from 0 to 1, with values >0.5 indicating correct topology and >0.8 indicating high-quality predictions comparable to experimental structures. Adapted from Jumper et al. (2021) and Abramson et al. (2024).

3. Transformative Impact Across Scientific Disciplines

3.1 Revolutionizing Drug Discovery and Therapeutic Development

AlphaFold has fundamentally transformed early-stage drug discovery by providing structural information for therapeutic targets previously inaccessible to experimental methods. Structure-based drug design, which relies on detailed three-dimensional protein architecture to design selective small molecule inhibitors, has been dramatically accelerated (Thornton et al., 2021). Pharmaceutical companies have rapidly integrated AlphaFold predictions into their discovery pipelines, reducing timelines and costs associated with lead identification and optimization.

Notable applications include structure predictions for challenging targets in neglected tropical diseases, oncology, and infectious diseases. During the COVID-19 pandemic, AlphaFold predictions of SARS-CoV-2 protein structures accelerated therapeutic development efforts (Callaway, 2020). The ability to predict structures of membrane proteins, G-protein coupled receptors, and other traditionally difficult targets has opened new avenues for therapeutic intervention.

3.2 Protein Engineering and Synthetic Biology

The availability of accurate structural models has revolutionized rational protein engineering (Dauparas et al., 2022). Researchers can now design enzyme variants with enhanced catalytic activity, improved stability, or altered substrate specificity by leveraging structural insights. This capability is crucial for developing industrial biocatalysts, biosensors, and novel biomaterials. In synthetic biology, structural predictions enable the design of protein circuits, molecular switches, and artificial metabolic pathways with unprecedented precision.

3.3 Illuminating Disease Mechanisms and Precision Medicine

AlphaFold enables structural interpretation of disease-associated genetic variants, facilitating mechanistic understanding of pathogenic mutations. By modeling how amino acid substitutions affect protein structure and stability, researchers can predict variant pathogenicity with improved accuracy (Buel & Walters, 2022). This structural perspective enhances genomic medicine by enabling more sophisticated interpretation of patient sequencing data, supporting personalized therapeutic strategies and improved diagnostic accuracy.

3.4 Complementing and Accelerating Experimental Structural Biology

Rather than replacing experimental techniques, AlphaFold serves as a powerful complementary tool that enhances experimental structural biology. Cryo-electron microscopy reconstructions often require

initial molecular models, and AlphaFold predictions provide highly accurate starting points (Kryshtafovych et al., 2021). Crystallographers employ AlphaFold models for molecular replacement in challenging structure determination problems. The synergistic integration of computational predictions with experimental data has created hybrid approaches that leverage the strengths of both methodologies, accelerating structural characterization across the field.

Table 2: Applications of AlphaFold Across Scientific Domains

Scientific Domain	**Specific Applications**	**Measurable Impact**	**Representative Examples**
Drug Discovery	Target identification, structure-based design, virtual screening	40-60% reduction in lead optimization time	SARS-CoV-2 therapeutics, cancer targets
Protein Engineering	Enzyme optimization, de novo design, stability enhancement	5-10× acceleration in variant screening	Industrial biocatalysts, biosensors
Disease Biology	Variant interpretation, mechanism elucidation, biomarker discovery	Enhanced diagnostic accuracy	Rare genetic diseases, cancer genomics
Evolutionary Biology	Ancient protein reconstruction, functional divergence analysis	Novel insights into protein evolution	Ancestral enzyme reconstruction
Synthetic Biology	Pathway design, regulatory circuit engineering	Accelerated design-build-test cycles	Metabolic engineering, genetic circuits
Agricultural Biotechnology	Crop improvement, stress resistance engineering	Enhanced nutritional content, climate resilience	Nitrogen fixation, drought tolerance

Note. Impact metrics represent approximate improvements compared to pre-AlphaFold methodologies based on published case studies. Adapted from Thornton et al. (2021) and Varadi et al. (2022).

4. Expanding Frontiers: Beyond Single-Chain Predictions

4.1 Protein Complexes and Molecular Interactions

While AlphaFold2 initially focused on single-chain predictions, subsequent developments extended its capabilities to multi-chain complexes. AlphaFold-Multimer enables prediction of quaternary structures and protein-protein interaction interfaces, opening avenues for understanding cellular machinery including signal transduction complexes, transcriptional regulatory assemblies, and metabolic enzyme complexes (Evans et al., 2021). This capability is transformative for systems biology, enabling structural modeling of entire interaction networks and facilitating understanding of how disease mutations disrupt protein interactions.

4.2 AlphaFold3: Modeling Biomolecular Complexity

AlphaFold3, released in 2024, represents a significant expansion by incorporating predictions of protein interactions with small molecules, nucleic acids, ions, and post-translational modifications (Abramson et al., 2024). This advancement enables modeling of proteins bound to drug molecules, metabolites, and cofactors, as well as protein-DNA and protein-RNA complexes. Such capabilities are transformative for understanding gene regulation, RNA processing, and CRISPR mechanisms. The ability to predict ligand binding sites with accuracy approaches that of experimental methods, dramatically accelerating structure-based drug design.

4.3 Addressing Intrinsically Disordered Proteins

A significant frontier involves modeling intrinsically disordered proteins (IDPs) and flexible regions lacking stable structure. IDPs play crucial roles in cellular signaling and regulation, yet their conformational heterogeneity poses challenges for both experimental and computational methods (Ruff & Pappu, 2021). While AlphaFold's confidence metrics can identify disordered regions, predicting their conformational ensembles remains an active research area. Future developments must integrate molecular dynamics simulations, enhanced sampling methods, and experimental data to capture the conformational landscapes of flexible proteins.

5. Current Limitations And Ongoing Challenges

5.1 Confidence Calibration and Validation Requirements

While AlphaFold provides confidence scores, interpretation requires careful consideration. Regions with high predicted confidence generally exhibit accurate geometry, but the algorithm can occasionally produce overconfident predictions for incorrect structures (Akdel et al., 2022). Experimental validation

remains essential, particularly for therapeutic applications. The scientific community continues developing improved confidence calibration methods and validation protocols to enhance prediction reliability.

5.2 Orphan Proteins and Limited Evolutionary Information

AlphaFold's performance depends heavily on homologous sequence availability. For proteins with sparse evolutionary information—including de novo designed proteins, some viral proteins, and proteins from uncultured organisms—prediction accuracy decreases substantially. These "orphan" proteins represent a blind spot necessitating development of algorithms that can predict structures from first principles with limited evolutionary data (Baek et al., 2021).

5.3 Conformational Dynamics and Functional States

Current structure prediction methods provide static snapshots rather than dynamic conformational ensembles. Many proteins adopt multiple functional conformations, and understanding these dynamics is crucial for comprehensive functional characterization. Integrating AlphaFold predictions with molecular dynamics simulations and experimental techniques like NMR and single-molecule spectroscopy represents an important frontier (Del Alamo et al., 2022).

5.4 Computational Resources and Global Accessibility

Despite democratization efforts through public databases and servers, running AlphaFold locally requires substantial computational resources including high-end GPUs and extensive sequence databases. This creates barriers for researchers in resource-limited settings, though ongoing optimization efforts aim to reduce computational requirements and enhance accessibility worldwide.

Table 3 Emerging Frontiers in Post-AlphaFold Computational Structural Biology

Frontier Area	Current Development Stage	Technical Challenges	Potential Applications
Conformational Ensemble Prediction	Early development	Sampling efficiency, scoring accuracy	Allosteric drug design, functional mechanism elucidation
De Novo Protein Design	Active research	Sequence-structure relationship, functionality	Therapeutic proteins, novel enzymes, biomaterials
Whole-Cell Structural Models	Proof-of-concept	Scale, integration complexity	Systems biology, cellular engineering
Metabolite-Protein Interactions	Emerging tools	Chemical diversity, binding mode prediction	Metabolic engineering, drug metabolism
Membrane Protein Complexes	Improved accuracy	Lipid interactions, oligomeric states	Drug targeting, signal transduction
Allosteric Mechanism Prediction	Limited capabilities	Dynamic coupling, long-range effects	Allosteric drug discovery, regulation

Note. Development stages reflect current status as of 2024. Challenges and applications represent consensus views from recent literature. Adapted from Dauparas et al. (2022) and Ruff & Pappu (2021).

6. The Future of Computational Structural Biology

6.1 Integration with Artificial Intelligence Ecosystems

AlphaFold's success has catalyzed development of complementary AI tools for protein design, function prediction, and molecular interaction modeling. Systems like RoseTTAFold (Baek et al., 2021), ESMFold (Lin et al., 2023), and ProteinMPNN (Dauparas et al., 2022) represent alternative approaches with distinct advantages. The future lies in ensemble methods combining multiple algorithms to improve accuracy and enable end-to-end protein design pipelines that seamlessly integrate structure prediction, function annotation, and experimental validation.

6.2 Toward Whole-Cell Structural Biology

A grand challenge involves constructing spatially resolved structural models of entire cells, integrating structure predictions with imaging data and biochemical information (Goodsell et al., 2020). Such whole-cell models would enable systems-level understanding of cellular organization, metabolic flux, and signaling networks. AlphaFold predictions provide the structural foundation for these efforts, supplying coordinates for the majority of cellular proteins and enabling construction of comprehensive cellular atlases.

6.3 Precision Medicine and Therapeutic Development

The ability to predict how genetic variants affect protein structure enables sophisticated interpretation of genomic data in clinical contexts. Structural pharmacogenomics—combining structure prediction with pharmacological data—promises to enhance prediction of drug response variability across patient populations (Buel & Walters, 2022). This structural dimension of precision medicine will enable truly personalized therapeutic strategies optimized for individual genetic backgrounds.

6.4 Sustainable Biotechnology and Global Challenges

Computational structure prediction accelerates development of enzymes for sustainable chemistry, including biocatalysts for plastic degradation, carbon capture, and green chemical synthesis. Agricultural applications include engineering crop proteins for enhanced nutrition and developing climate-resilient varieties (Thornton et al., 2021). The ability to rapidly screen and optimize proteins supports efforts addressing food security, environmental sustainability, and climate change adaptation.

6.5 Ethical Considerations and Responsible Innovation

The power of AI-driven structural biology raises important ethical considerations including biosecurity risks, equitable access, and responsible development. The potential for misuse in designing harmful biological agents necessitates thoughtful governance frameworks balancing open science with biosecurity (Jumper et al., 2021). Ensuring global access, particularly for researchers in low- and middle-income countries, requires continued investment in computational infrastructure, education, and open-access resources. The scientific community must proactively address these challenges to maximize benefits while minimizing potential risks.

7. Conclusion

AlphaFold represents a transformative breakthrough that has fundamentally reshaped structural biology and catalyzed progress across numerous scientific disciplines. By solving the protein structure prediction problem with unprecedented accuracy, it has democratized access to structural information and enabled research previously considered impractical or impossible. The technology has already demonstrated profound impact in drug discovery, disease understanding, protein engineering, and fundamental biological research, accelerating scientific discovery and therapeutic development.

However, AlphaFold marks the beginning rather than the culmination of a new era in computational structural biology. Significant challenges persist, including accurate modeling of conformational dynamics, prediction of complex cellular assemblies, and integration of diverse data types. The future will likely witness continued evolution of artificial intelligence methods, development of hybrid computational-experimental approaches, and expansion toward modeling entire biological systems at atomic resolution.

The post-AlphaFold era offers unprecedented opportunities to translate structural insights into practical applications addressing global challenges in health, sustainability, and food security. As computational methods continue advancing, the integration of structure prediction with functional characterization, molecular design, and systems biology promises to unlock deeper understanding of life's molecular machinery. The ultimate vision extends beyond predicting protein shapes to comprehensively understanding the fundamental principles governing biological organization, enabling rational design of novel functions, and harnessing this knowledge to benefit humanity while advancing our understanding of the molecular basis of life itself.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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Bioaccumulation of Arsenic (As) in Red Lentil (*Lens Culinaris*): Uptake Pathways, Tissue-Specific Deposition, Phytotoxicity and Public Health Implications in India

Ankana Ghosh[1], Pritha Pal[2]*

[1] *Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal- 700121*

[2] *Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal- 700121*

* *Corresponding author*: prithap@svu.ac.in

Abstract

Arsenic (As), a toxic and carcinogenic metalloid, enters agricultural systems mainly through irrigation with contaminated groundwater, posing serious risks to crop quality and human health. *Lens culinaris* (red lentil), a nutritionally important staple legume in South and Southeast Asia, exhibits a pronounced propensity for Arsenic bioaccumulation, readily accumulates arsenic, making it a significant dietary exposure route in Arsenic-endemic regions such as West Bengal and Bangladesh. This review is based on the current knowledge on uptake mechanisms of As (V) via phosphate transporters and As (III) via aquaporin channels and its reduction, sequestration, and tissue-specific accumulation (roots > shoots > grains) and also the mechanisms of arsenic toxicity in *Len culinaris*. Bioaccumulation of Arsenic disrupts energy metabolism, induces oxidative stress, impairs photosynthesis, reduces yield, and degrades grain quality. Chronic consumption of contaminated lentils is linked to increased cancer risk and other systemic non-malignant diseases such as diabetes and cardiovascular diseases. This review highlights integrated agronomic, genetic, and biotechnological interventions designed to reduce the risks to public health in As-endemic agroecological zones by reducing the accumulation of Arsenic in *Lens culinaris*.

***Keywords*:** Arsenic, *Lens culinaris*, Bioaccumulation, Uptake mechanism, Phytotoxicity, Public health.

Introduction

Arsenic (As) is a carcinogenic metalloid, released into the environment by both natural and anthropogenic (i.e., caused by human activities) sources. One of the primary ways by which humans are exposed to As is through the transfer of As in soil-plant systems (Li et al.,2014). The arsenic mobilizes into agricultural systems is mainly through the irrigation with contaminated groundwater that can result in genotoxic effects on human (Banerjee et al.,2013). In India, arsenic-poisoned (arsenicosis) patient was first spotted in 1983 in West Bengal. Groundwater containing arsenic is frequently used for crop irrigation in South and Southeast Asia, especially in nations like Bangladesh, Nepal, and India causing arsenic to build up in soils and then in food crops. However, in addition of being utilized for irrigation, the arsenic-contaminated water is also used for drinking, washing, and food preparation. Arsenic and other toxic metals can accumulate in plants and contaminate food crops when agricultural soils are irrigated with water contaminated with arsenic over an extended period of time. In West Bengal and Bangladesh, contaminated tube wells may result in dietary exposure to arsenic and other harmful substances that could have an adverse effect on health (Rahaman et al.,2013). Nine districts in West Bengal, eleven districts in Bihar, and seventeen districts in Uttar Pradesh have so far affected with arsenic toxicity, with arsenic levels exceeding the 50 ppb nationally recognized limit. It is estimated that over 100 million people worldwide are chronically exposed to arsenic through drinking water. The arsenic contaminated groundwater leads to the contamination of food chain and it is suggested that arsenic in rice, lentil and other food sources would contribute to approximately 30% of the total arsenic intake (Alam et al. 2019). Arsenate (As is easily absorbed by plants via high-affinity phosphate transporters since it is an analogue of phosphate. Large quantities of arsenic build up in the edible parts of major crops like cereals and legumes cultivated in As-contaminated fields, which can be highly hazardous to human health.

Among the impacted food crops, Red Lentil (*Lens culinaris*), a staple legume, has demonstrated a tendency to bioaccumulate arsenic. One of the most ancient legume crops to be cultivated is red lentils majorly growing in the countries like Bangladesh, Canada, China, India, Iran, Nepal, Syria, Turkey, and USA (Alihan et al.,2013). Red lentil is the vital source of proteins and fibres as well as it contains multiple vitamins and minerals, including iron, zinc, folate, and magnesium. Additionally, red lentil possesses the phytochemicals like saponins and tannins that have anti-inflammatory and anti-oxidant properties respectively,

suggesting red lentil may have anti-cancer effects (Mudryj et al.,2014). The mechanisms of arsenic uptake, transport, and accumulation in red lentils, the detrimental effects on plant physiology, and the degree of arsenic contamination in lentil-growing regions are the main objectives of this review. It also emphasizes the need for agronomic and genetic measures to reduce arsenic buildup in this vital crop and the possible health hazards linked to eating lentils contaminated with arsenic.

1. Arsenic (As) Accumulation in the Tissues of *Lens culinaris*

In red lentil (*Lens culinaris*), arsenic accumulation is tissue specific. The plant exhibits distinctly different concentrations in root, shoot and grain tissues. According to experimental evidence, the pattern of distribution of arsenic in red lentil plants occurs in the following order: roots> shoots> grains (Garg and Shingla.,2011).

Table-1: Bioaccumulation and Physiological Effects of Arsenic in *Lens culinaris* Tissues (Garg and Shingla, 2011)

Red lentil plant tissue	Uptake Mechanism	Major physiological effects
1. Roots	• Arsenate (AsV) enters roots via phosphate transporters • Arsenite (AsIII) enters roots via aquaporin channels	Inhibits root extension and proliferation.
2. Shoots	Via Xylem vessels.	Constraints physiological growth.
3. Seeds (Grains)	Via Phloem translocation.	Chronic exposure risk.

2. Pattern Of Movement of Arsenic from Soil To The Root of *Lens culinaris*

Arsenic from contaminated groundwater or irrigation release into the soil solution, where it can be absorbed by the roots of the lentil plants. In the root cells, phosphate transporters allow roots to absorb arsenate As(V), whereas aquaporin channels allow arsenite

As (III) to enter. As (V) is converted to As (III) inside the root cells, where it is either retained in vacuoles or transported to the shoots via the xylem. Arsenic accumulates in leaves and stems, which may inhibit photosynthesis and results in oxidative stress. A minor percentage travels to the developing seeds via the phloem. Even while grain has lower quantities of arsenic than roots or shoots, prolonged exposure can still affect food safety.

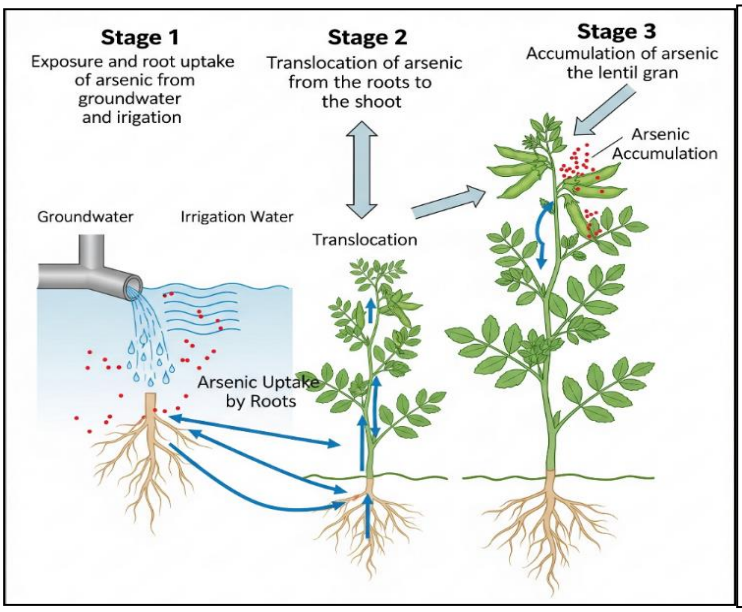

Figure-1: Three stages of Arsenic Bioaccumulation in *Lens culinaris*

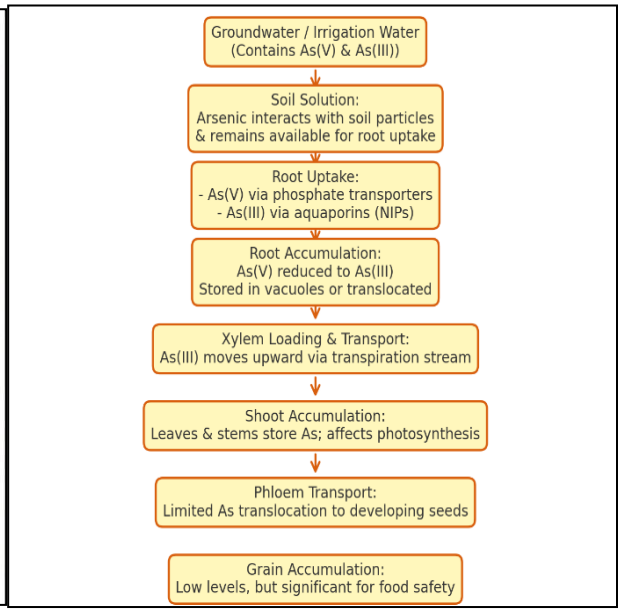

Figure-2 : Pathway of Arsenic Transport in *Lens culinaris*

4.Molecular Mechanism of Phytotoxic Effects of Arsenic on *Lens culinaris*

Elevated concentrations of Arsenic disrupt vital metabolism processes, which may result in the death of the lentil plants (Song et al.,2014). In lentil (*Lens culinaris*), arsenite As (III) enters through aquaporin channels such nodulin-26-like intrinsic proteins (NIPs) of the root cells, whereas arsenate As (V) is taken up by roots via phosphate transporters because of its structural resemblance to phosphate. Arsenate reductases rapidly convert As (V) to the more toxic As III) in the cytosol of the root cells. The arsenate competes with phosphate for ATP synthesis and produces ADP-As instead of ATP which leads to inhibition of phosphorylation-dependent metabolic processes like protein phosphorylation and also results in severe energy depletion. Due to accumulation of arsenic in biomass of *Lens culinaris*, oxidative stress increases in the plant resulting excessive production of reactive oxygen species (ROS)

including superoxide radicals (O_2^{-}•), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH) which cause lipid peroxidation, electrolyte leakage, H_2O_2 accumulation, and root oxidizability (Talukdar,2013). Additionally, the activity of the antioxidant enzymes (Superoxide dismutase, Catalase, Ascorbate peroxidase, Peroxidase, Glutathione reductase) drastically changes in response to stress which restricts the ROS detoxification system and increases oxidative damage (Singh et al.,2007). Arsenic changes osmotic balance via interfering with ion transporters and ion channels, leading to decreased turgor pressure, water use efficiency and stomatal conductance. Arsenic reduces the efficiency of light harvesting and electron transport by degrading chlorophyll a and b, inhibiting chloroplast enzymes, and damaging photosystem II (PSII) causing reduced growth and pigment content which finally leads to impairment of photosynthesis ((Srivastava et al.,2013). Reduced meristematic activity and cell cycle arrest brought on by arsenic result in shorter roots and shoots. With decreased pollen viability, abnormal ovule development, and incomplete seed filling, reproductive tissues are especially sensitive.

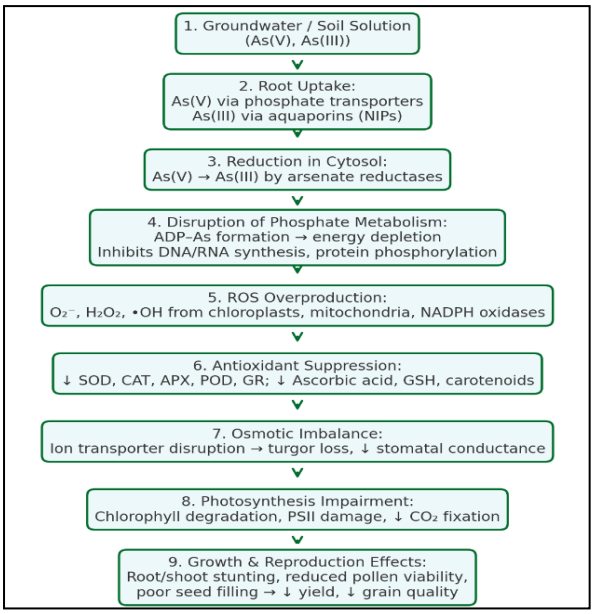

Figure-3: Molecular mechanism of Phytotoxic effects of Arsenic in *Lens culinaris*

Particularly, due to the imposition of arsenic in food crops, the amount of total chlorophyll, catalase, and ascorbic acid is significantly decreased. These effects lead to a significant yield loss and decreased grain quality (Talukdar & Dibyendu,2013).

5.Public Health Impact and Disease Prevalence in India from Dietary Arsenic Exposure Through *Lens culinaris* Consumption

Cancer is one of the serious diseases that can result from consuming arsenic. However, because of geological processes and human activities, this metalloid is ubiquitous in the environment. Lentil plants absorb arsenic from the soil, which accumulated in edible parts (like lentil grains) and is then consumed by humans and other higher-ranking organisms in the food chain (Alam et al.,2011). Millions of people are currently at risk of being exposed to food contaminated with As, particularly in South and Southeast Asia (Verbruggen et al.,2009). Overconsumption of As from lentil grains causes tissues to accumulate As and reduces the activity of cellular enzymes. The primary routes by which humans are exposed to As are through ingestion, inhalation, and skin contact. Chronic consumption of As has been linked to skin cancer and there is substantial evidence that it raises the risk of bladder, lung, kidney, liver, colon, and prostate cancers. According to recent research, As is also linked to a variety of non-neoplastic conditions, such as diabetes mellitus, heart disease, cerebrovascular disease, pulmonary disease, and diseases of the arteries, arterioles, and capillaries. People who are malnourished, have a protein deficit, or have a chronic Hepatitis B infection may be more vulnerable to the effects of arsenic (Engel and Smith,2004).

Among all the regions where lentils are grown, many of them have high levels of As contamination. In particular, Bangladesh and West Bengal together make up the second-largest As-contaminated area globally. Approximately 8% area of the United States is impacted.

Table-2: District having arsenic in groundwater in different States of India (Ghritlahre and Singh, 2022)

State	**Parts of Districts having As >0.05 mg/lit**
West Bengal	Hooghly, Malda, Murshidabad, North 24 Parganas, South 24 Parganas, Nadia
Bihar	Godda, Khagaria, Munger, Begusarai
Punjab	Amritsar, Ropar, Taran
Haryana	Ambala, Jhajjhar
Uttar Pradesh	Bahraich, Deoria, Lakhimpur, Azamgarh, Maunath Bhanjan

6. Conclusion

Arsenic contamination in agricultural soils poses a serious threat to food safety, especially in lentil-growing regions like Bangladesh and West Bengal. *Lens culinaris* (red lentil), a staple legume and a valuable protein source, are susceptible to arsenic uptake through phosphate and aquaporin transporters, leading to tissue-specific accumulation with the highest concentration in roots, followed by shoots and grains. Although grains contain lower arsenic concentrations than roots and shoots, chronic exposure through consumption remains a significant concern. Studies reveal that arsenic disrupts key physiological processes such as ATP synthesis, photosynthesis, and antioxidant defence resulting in oxidative stress, reduced biomass, and impaired grain quality. Experiments suggests that selective breeding could be a viable strategy to reduce arsenic accumulation in the lentils. Significantly, there is significant potential for lowering arsenic uptake via the application of arbuscular mycorrhizal fungi (AMF). AMF inoculation resulted in quantifiable decreases in arsenic concentrations in roots, shoots, and grains as well as it increases plant biomass and shoot length under arsenic stress. As an illustration of the fungi's role in regulating arsenic transport and improving stress tolerance, AMF-treated lentil plants that were cultivated in soils contaminated with 45 mg/kg arsenic showed reduced arsenic accumulation when compared to non-AMF controls (Alam et al.,2019). According to a randomized controlled study, arsenic toxicity in human is considerably decreased by a dietary intervention using selenium-enriched lentils. Selenium-rich lentil consumption was associated with improved methylation efficiency with higher percentages of DMA (Dimethylarsinic acid) and lower percentages of iAs (inorganic As), increased blood and urine selenium levels, and increased urinary excretion of arsenic and its metabolites (DMA and MMA-monomethyl arsonic acid), all of which confirmed effective absorption. Crucially, the intervention resulted in a modest but significant increase in BMI, indicating improved nutritional status despite ongoing arsenic exposure, and a decreased incidence of asthma and allergy, which were attributed to selenium's antioxidant and immunomodulatory effects (Smit et al.,2019). Future directions include molecular studies on arsenic transport, development of dual-trait lentil cultivars with low arsenic and high selenium content, microbial interventions like AMF to reduce arsenic uptake, and policy-driven outreach to promote arsenic-safe agriculture. These integrated strategies offer a promising path toward cultivating arsenic-resilient lentils that protect food security and public health in affected regions.

Conflict of Interest

The authors declare that they have no known competing financial interests in the work reported in this paper.

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The Interplay of Hypoxia And Gut Microbiota: Mechanisms, Physiological Adaptations, And Disease Implications

Debaprasad Koner*

**Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Brrrackpore-700121.*

**Corresponding author: debzoology@gmail.com*

Abstract

The gastrointestinal tract is a unique environment characterized by a steep oxygen gradient, creating a state of physiological hypoxia that is essential for maintaining homeostasis. This review synthesizes current understanding of the intricate, bidirectional relationship between hypoxia and the vast microbial communities inhabiting the gut. We delve into the molecular mechanisms, centred on the master regulator Hypoxia-Inducible Factor-1 (HIF-1), that govern the cellular response to low oxygen tension. We explore how the gut microbiota, through the production of metabolites like short-chain fatty acids (SCFAs), actively shapes the hypoxic microenvironment, thereby influencing host cell metabolism and reinforcing mucosal barrier integrity. Conversely, we studied how hypoxic conditions whether physiological, occurring at high altitudes, or pathological, in diseases like cancer and inflammatory bowel disease (IBD) act as a powerful selective force that modulates the composition and diversity of the gut microbiota. We discuss the crosstalk between the hypoxia-microbiota axis and the host, focusing on its role in regulating intestinal epithelial cell (IEC) function and orchestrating mucosal immune responses via cells such as type-3 innate lymphoid cells (ILC3s). Finally, we highlight the clinical implications of this interplay in various human diseases, including IBD, necrotizing enterocolitis (NEC), and COVID-19, and identify key outstanding questions to guide future research in this burgeoning field. Understanding this complex relationship is paramount for developing novel therapeutic strategies that target the hypoxia-microbiota axis to restore intestinal homeostasis and mitigate disease.

Keywords: Hypoxia; Gut microbiota; HIF-1 signaling; Short-chain fatty acids; Intestinal epithelial cells; ILC3s; Inflammatory bowel disease

1. Introduction

The human gut harbors a complex and dynamic ecosystem of over 100 trillion microorganisms, collectively known as the gut microbiota, which plays a pivotal role in host physiology, metabolism, and immunity (Faith et al., 2013). The symbiotic relationship between the host and its resident microbes is maintained within a tightly regulated microenvironment. A defining, yet often underappreciated, feature of the intestinal lumen is its profound lack of oxygen, a state termed physiological hypoxia (Pral et al., 2021). This low-oxygen environment is not a passive condition but is actively maintained by the high metabolic activity of both host intestinal epithelial cells (IECs) and the luminal microbiota itself.

Hypoxia is a powerful physiological and pathological stimulus that triggers adaptive responses in virtually all cells. The primary molecular mechanism governing this response is the Hypoxia-Inducible Factor (HIF) signaling pathway (Han et al., 2021). HIF-1, the principal isoform, is a transcription factor that regulates a vast array of genes involved in angiogenesis, metabolism, cell survival, and inflammation, allowing cells to adapt to and function in low-oxygen conditions (Pral et al., 2021).

Recent evidence has unveiled a complex and bidirectional crosstalk between intestinal hypoxia and the gut microbiota. The microbiota and its metabolic byproducts, such as short-chain fatty acids (SCFAs), can modulate host cell oxygen consumption, thereby influencing the stability of HIF-1 and reinforcing the hypoxic state (Kelly et al., 2015). In turn, hypoxia acts as a critical environmental pressure that shapes the composition, diversity, and function of the microbial community (Han et al., 2021). This dynamic interplay has profound implications for the maintenance of mucosal barrier function, the education and regulation of the host immune system, and overall gut homeostasis. Disruptions in this delicate balance have been implicated in a range of human pathologies, from inflammatory and infectious diseases to cancer (Pral et al., 2021; Han et al., 2021). This review aims to synthesize the current literature, drawing from mechanistic reviews and recent experimental findings, to provide a comprehensive overview of the relationship between hypoxia and the gut microbiota. We will discuss the molecular sensing of hypoxia via HIF-1, the modulatory role of the microbiota, the temporal and site-specific microbial adaptations to hypoxic exposure, and the functional consequences of this interplay for host physiology in health and disease.

1. **The Physiological Hypoxia of the Gut**

The gastrointestinal tract is characterized by a steep physiological oxygen gradient. While the vasculature of the submucosal layer is relatively oxygen-rich (pO2 ~40 mmHg), oxygen levels drop dramatically across the epithelium, culminating in a functionally anoxic (pO2 < 1 mmHg) environment within the lumen of the large intestine (Pral et al., 2021). This gradient is the result of two main processes: i) oxygen delivery via the bloodstream to the intestinal tissue, and ii) rapid oxygen consumption by both host IECs and the dense population of facultative and obligate anaerobic microorganisms residing in the gut (Albenberg et al., 2014). Butyrate and other SCFAs produced by microbial fermentation of dietary fibers are a primary energy source for colonocytes. The metabolism of these SCFAs via beta-oxidation is a highly oxygen-consumptive process, which further depletes oxygen at the epithelial surface and contributes significantly to the maintenance of luminal hypoxia (Kelly et al., 2015).

1. HIF-1: The Master Molecular Sensor of Hypoxia

The cellular adaptation to low oxygen is primarily orchestrated by the Hypoxia-Inducible Factor (HIF) family of transcription factors. The main isoform, HIF-1, is a heterodimer composed of a constitutively expressed HIF-1β subunit and a highly regulated HIF-1α subunit (Pral et al., 2021). In the presence of sufficient oxygen (normoxia), HIF-1α is hydroxylated by prolyl-hydroxylase domain (PHD) enzymes, leading to its recognition by the von Hippel-Lindau (VHL) E3 ubiquitin ligase complex, subsequent ubiquitination, and rapid proteasomal degradation. Under hypoxic conditions, the lack of oxygen inhibits PHD activity, allowing HIF-1α to stabilize, accumulate, and translocate to the nucleus. There, it dimerizes with HIF-1β and binds to hypoxia-response elements (HREs) in the promoter regions of hundreds of target genes (Han et al., 2021; Pral et al., 2021).

These target genes orchestrate a multi-faceted adaptive response, including a metabolic shift from oxidative phosphorylation to anaerobic glycolysis, promotion of angiogenesis to improve oxygen supply, and regulation of cell survival and apoptosis. Importantly, the microbiota-driven, SCFA-fueled consumption of oxygen by IECs directly contributes to the inhibition of PHDs and the subsequent stabilization of HIF-1α. This establishes a crucial feedback loop where the microbiota helps maintain the very hypoxic signal that the host epithelium senses and adapts to (Kelly et al., 2015).

2. Impact of Hypoxia on Gut Microbiota Composition

Given that the gut microbiota is predominantly composed of obligate anaerobes, changes in oxygen tension serve as a powerful selective pressure that can restructure the entire microbial community. This is observed in various contexts, from short-term exposure to high altitudes to chronic disease states.

2.1. Short-Term Hypoxia and Acute Adaptation

Exposure to acute or short-term hypoxia, such as that experienced during the initial days at high altitude, induces significant and rapid shifts in the gut microbiota. Experimental studies in mice exposed to simulated high-altitude conditions (5,500 m) reveal a biphasic pattern of microbial alteration (Liao et al., 2025). During the first 1–3 days of exposure, there is a marked reduction in overall microbial richness and diversity (α- and β-diversity) in both the stomach and small intestine. This initial "suppression phase" is characterized by a pronounced expansion of facultative anaerobes. In particular, the relative abundance of the phylum *Firmicutes*, especially the genus *Lactobacillus*, increases dramatically. Concurrently, there is a significant decline in phyla that are more sensitive to environmental perturbations, including *Bacteroidetes*, *Actinobacteria*, and *Verrucomicrobia*, along with corresponding reductions in the genera *Bifidobacterium* and *Akkermansia* (Liao et al., 2025). The expansion of acid-producing *Lactobacillus* likely suppresses the growth of competing taxa, including obligate anaerobes such as *Clostridium*, which exhibit a transient increase on day 1 followed by complete disappearance by day 3 (Liao et al., 2025). These observations from murine models are consistent with findings from human high-altitude studies, which similarly report early reductions in microbial diversity accompanied by shifts in community composition (Karl et al., 2018).

2.2. Long-Term Hypoxia and Chronic Adaptation

With prolonged exposure to hypoxia, the gut microbiota demonstrates a remarkable capacity for adaptation. In the same murine model, by day 12 of hypoxic exposure, the reduced microbial diversity and richness observed during short-term exposure were reversed, returning to levels comparable to those of normoxic controls (Liao et al., 2025). This recovery suggests a restructuring and stabilization of the microbial community into a new, hypoxia-adapted state. While the dominant phyla (e.g., *Firmicutes* and *Bacteroidetes*) return

to near-baseline levels, certain taxa exhibit persistent changes. For example, the relative abundance of *Staphylococcus* shows a steady increase in the stomach, whereas *Stomatobaculum* consistently increases in the small intestine over the 12-day exposure period, indicating that these taxa may play key roles in long-term hypoxic adaptation (Liao et al., 2025).

These adaptive changes are also evident in human populations residing permanently at high altitudes. Tibetan populations, for instance, exhibit a gut microbiota composition distinct from that of low-altitude populations, often characterized by a higher abundance of short-chain fatty acid (SCFA)–producing genera such as *Ruminococcus*. This microbial configuration may represent an adaptive strategy to enhance energy extraction from fiber-rich diets under challenging environmental conditions (Han et al., 2021; Li & Zhao, 2015). Collectively, these observations suggest that long-term hypoxia drives the selection of a microbial community that is not only tolerant of low-oxygen environments but also functionally advantageous to the host.

3. Functional Consequences of the Hypoxia-Microbiota Axis

The interplay between hypoxia and the gut microbiota extends beyond mere compositional changes, with profound functional consequences for host physiology, particularly in the regulation of mucosal barrier function and immunity.

3.1. Enhancement of the Intestinal Barrier

The hypoxia-HIF-1 signaling axis, often initiated and reinforced by microbiota-derived SCFAs, is critical for maintaining intestinal barrier integrity. HIF-1 activation in IECs upregulates the expression of key barrier-protective genes (Pral et al., 2021). These include mucins (e.g., MUC2, MUC3), which form the protective mucus layer that physically separates luminal bacteria from the epithelium, and components of tight junctions (e.g., claudin-1, occludin), which seal the paracellular space between IECs. Furthermore, HIF-1 drives the expression of antimicrobial peptides (AMPs), such as defensins and cathelicidins, which control microbial populations at the epithelial surface. The stabilization of HIF-1 by butyrate has been shown to protect mice from *C. difficile* infection by enhancing these barrier functions, reducing intestinal permeability and bacterial translocation (Fachi et al., 2019). Therefore, the microbiota-hypoxia-HIF-1 axis forms a robust, multi-layered defense system for the gut.

3.2. Regulation of Mucosal Immunity

The hypoxia-microbiota axis also plays a significant role in orchestrating mucosal immune responses. Type 3 innate lymphoid cells (ILC3s) are a critical immune cell population in the gut that contributes to homeostasis and defense against pathogens. ILC3s are regulated by signals from the environment, including SCFAs. Butyrate and other SCFAs can promote the production of the cytokine Interleukin-22 (IL-22) from ILC3s (Yang et al., 2020). This effect is mediated, at least in part, through the stabilization of HIF-1α in the immune cells. IL-22 is a key cytokine that acts directly on IECs to promote their proliferation, survival, and production of AMPs, thereby contributing to epithelial repair and barrier integrity (Pral et al., 2021). This illustrates how microbial metabolites, acting through a hypoxia-sensing pathway, can directly modulate immune cell function to protect the host.

4. Implications for Human Disease

Given its central role in gut homeostasis, dysregulation of the hypoxia-microbiota axis is increasingly implicated in various diseases.

Inflammatory Bowel Disease (IBD): IBD is characterized by chronic inflammation of the gut, which is paradoxically a highly hypoxic environment due to high oxygen consumption by infiltrating immune cells. The mechanisms described above, where the HIF-1 axis promotes barrier function, are considered protective. Therapeutic strategies aimed at stabilizing HIF-1 (e.g., using PHD inhibitors) are being explored as a means to enhance mucosal healing in IBD (Pral et al., 2021).

Neonatal Necrotizing Enterocolitis (NEC): NEC is a devastating disease of premature infants characterized by intestinal inflammation and necrosis, with hypoxia being a major etiological factor. The immature gut of preterm infants has an unstable microbial community. Studies show that prior to NEC diagnosis, there is often a decrease in microbial diversity and a bloom of *Proteobacteria*, coupled with a reduction in protective anaerobic bacteria like *Clostridium* (Morrow et al., 2013). This dysbiosis, in a gut already compromised by hypoxic insults, likely contributes to the breakdown of the mucosal barrier and uncontrolled inflammation seen in NEC (Han et al., 2021).

Cancer: The tumor microenvironment is classically hypoxic. As discussed in liver cancer, hypoxia-driven HIF-1 activation promotes tumor growth and metastasis. The "gut-liver axis"

means that intestinal dysbiosis, which is common in liver disease, can lead to the translocation of bacterial products like lipopolysaccharide (LPS) to the liver, promoting inflammation and exacerbating the hypoxic and pro-cancerous environment (Han et al., 2021).

COVID-19: Severe COVID-19 is associated with systemic hypoxia due to lung damage. Patients with severe disease exhibit significant gut microbiota dysbiosis, with a depletion of beneficial commensals and an increase in opportunistic pathogens (Han et al., 2021). This disruption of the gut-lung axis may compromise immune responses and predispose patients to the life-threatening secondary bacterial infections that are a common cause of mortality in COVID-19.

5. Conclusion and Future Directions

The relationship between hypoxia and the gut microbiota is a dynamic and deeply integrated system fundamental to intestinal health. A state of physiological hypoxia, actively maintained by host and microbial metabolism, stabilizes the master regulator HIF-1. This signaling cascade, in turn, fortifies the mucosal barrier and modulates immune responses, creating a homeostatic environment that favors a symbiotic microbiota. Hypoxia, as an environmental pressure, also directly shapes microbial community structure, driving a process of adaptation from acute dysbiosis to a stable, hypoxia-tolerant ecosystem.

While significant progress has been made, many questions remain. The precise roles of other hypoxia-sensing pathways (e.g., HIF-2, HIF-3) in the gut are still being elucidated (Pral et al., 2021). The functional consequences of the specific, persistent microbial changes seen in long-term hypoxia, such as the increase in *Staphylococcus*, are unknown and warrant further investigation (Liao et al., 2025). Moreover, how external factors like diet, medication, and daily circadian rhythms interact with the hypoxia-microbiota axis is an important area for future research. A deeper understanding of this complex interplay will be crucial for developing novel therapeutics that can target this axis to treat a wide range of human diseases linked to gut dysbiosis and inflammation.

Conflict of Interest

The authors declare that they have no known competing financial interests in the work reported in this paper.

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Mechanistic Insights into Arsenic-Induced Neurodegeneration in the Mammalian Brain

Arpita Chakraborty [1], Krishnendu Karmakar [2], Krishti Bakshi [2], Nimai Chandra Saha[3], Palash Kumar Pal[1*]

[1] *Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Kolkata- 700121*

[2] *Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Kolkata- 700121*

[3] *Dean of Science and Director, School of Life Sciences, Swami Vivekananda University, Kolkata- 700121*

[*]*Corresponding author*: palashkp@svu.ac.in

Abstract

Arsenic, a heavy metalloid, has been considered as a global threat regarding our health and lives. Many countries like Bangladesh and India are facing adverse effects of arsenic induced toxicity. It is mostly found in diverse natural sources like soil, volcanic eruptions, rock, ground water etc. The most dangerous forms of arsenic that are injurious to our health include arsenite and arsenate. In addition, exposure imposed by anthropological activities such as growing industrialization, agricultural runoffs, etc. has enhanced the chance of arsenic contamination to the environment. Arsenic may enter the body of nay mammal through drinking water, air or food. Thus, it has been evident to promote damages to several vital organs including skin, lungs, kidney and brain. Being n pivotal mammalian organ, brain has been considered as one of the susceptible organs for arsenic induced toxicity where it may induce neurotoxicity and neurodegenerative diseases like Alzheimer and Parkinson's disease. Available evidences suggested the involvement of increasing oxidative stress, DNA damage and apoptosis as the preliminary pathways that helps arsenic to exert its toxic effects in brain. Property of arsenic to cross the blood brain barrier facilitates it induced detrimental alterations in the mammalian brain tissue. Thus, it is high time to explore the underlying molecular mechanism of arsenic mediated toxicity for the better understanding of its pathogenesis in the brain cells. In such regards, the present review attempts to gather the experimental evidences observed in diverse mammalian models keeping kin focus on its entry mechanism, association and modulation of cellular signaling molecules associated with the neurodegeneration. Such practice will help to develop effective remedial measures for its removal and to lessen its neurotoxic effects in the brain of any mammalian species.

Keywords: Arsenic, Toxicity, Heavy metal, Neurotoxicity, Neurodegeneration, Brain.

1. Introduction

Arsenic, a naturally occurring element, is a notorious environmental toxicant that affects millions of people worldwide (Naujokas et al., 2013). The primary route of human exposure is through the consumption of arsenic-contaminated groundwater, a problem especially prevalent in regions like Bangladesh, India, and parts of the United States (Smith et al., 2000; Argos et al., 2010). While the association between arsenic exposure and various cancers is well-established, its detrimental effects on the central nervous system (CNS) have garnered significant attention in recent years (Vahidnia et al., 2007; Tyler and Allan, 2014).

Neurotoxicity induced by arsenic can manifest as a range of neurological and cognitive dysfunctions, including learning and memory deficits, mood disorders, and developmental alterations (Wasserman et al., 2004; Tolins et al., 2014). Chronic exposure, even at low concentrations, is increasingly being recognized as a risk factor for the development of debilitating neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD) (Coon et al., 2006; O'Bryant et al., 2011). The vulnerability of the brain to arsenic is compounded by the metalloid's ability to cross the blood-brain barrier (BBB) and accumulate in critical brain regions (Zheng et al., 2003).

The pathophysiological mechanisms underlying arsenic-induced neurodegeneration are complex and multifactorial. A growing body of evidence points to oxidative stress as a central player, triggering a cascade of downstream events including mitochondrial dysfunction, DNA damage, and programmed cell death (apoptosis) (Chou et al., 2004; Flora et al., 2011). Furthermore, arsenic exposure has been shown to provoke a persistent neuroinflammatory response, contributing to the progressive loss of neuronal structure and function (Wu et al., 2012).

This review aims to provide a comprehensive summary of the current understanding of the mechanisms through which arsenic exerts its neurotoxic effects. We will discuss the sources of exposure, the metabolic fate of arsenic in the body, and its transport into the brain. The primary focus will be on the molecular pathways disrupted by arsenic, leading to neuronal damage and the potential for neurodegeneration. By synthesizing the existing literature, we hope to illuminate the gravity of arsenic neurotoxicity and underscore the urgent need for further research into protective and therapeutic strategies.

2. Sources and Metabolism of Arsenic

Arsenic exists in the environment in both organic and inorganic forms. The inorganic forms, arsenite (As-III) and arsenate (As-V), are more toxic and are the predominant species found in contaminated water (Hughes, 2002). Human exposure occurs through various sources, including natural geological formations, industrial processes such as mining and smelting, agricultural pesticides, and wood preservatives (IARC, 2012). However, the most significant source of exposure for large populations remains naturally contaminated drinking water from underground aquifers (Nordstrom, 2002).

Following ingestion, inorganic arsenic is readily absorbed from the gastrointestinal tract and undergoes a complex metabolic process primarily in the liver. This process involves a series of reduction and oxidative methylation reactions, converting the more toxic inorganic forms into less acutely toxic, but still harmful, methylated metabolites such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Thomas et al., 2001; Vahter, 2002). These metabolites are then distributed throughout the body via the bloodstream and are eventually excreted, mainly in the urine (Buchet et al., 1981; Devesa et al., 2001). However, a portion of the arsenic and its metabolites can accumulate in various tissues, including the brain (Devesa et al., 2001). The efficiency of this methylation process varies among individuals and can influence susceptibility to arsenic toxicity (Chung et al., 2002).

3. Molecular Mechanisms of Arsenic Neurotoxicity

The neurotoxic effects of arsenic are mediated by a complex interplay of molecular events that disrupt neuronal homeostasis and lead to cell death. The ability of arsenic to cross the BBB allows it to directly impact the CNS.

3.1. Oxidative stress and mitochondrial dysfunction

Oxidative stress is considered a primary mechanism of arsenic toxicity (Flora et al., 2011). Arsenic, particularly the trivalent form (As-III), has a high affinity for sulfhydryl groups, leading to the inactivation of critical antioxidant enzymes like glutathione reductase and catalase (Shi et al., 1994). This enzymatic inhibition, coupled with the direct generation of reactive oxygen species (ROS) during arsenic metabolism, leads to a state of severe oxidative stress within neuronal cells (Jomova et al., 2011).

The brain is particularly vulnerable to oxidative damage due to its high oxygen consumption rate and lipid-rich composition. ROS can inflict widespread damage on cellular components, including lipids (lipid peroxidation), proteins, and nucleic acids, impairing their

function (Halliwell, 2007). Mitochondria, the cellular powerhouses, are major targets of arsenic-induced oxidative stress. Arsenic can uncouple oxidative phosphorylation, inhibit key enzymes of the electron transport chain, and induce the opening of the mitochondrial permeability transition pore (mPTP), leading to mitochondrial dysfunction, energy failure, and the release of pro-apoptotic factors (Bernardi et al., 2009; Tyler and Allan, 2014).

3.2. Apoptosis and neuroinflammation

The culmination of oxidative stress and mitochondrial dysfunction often leads to the activation of apoptotic pathways. Arsenic has been shown to induce apoptosis in various neuronal cell types by modulating the expression of Bcl-2 family proteins, activating caspases, and triggering DNA fragmentation (Han et al., 2003; Chou et al., 2004).

In addition to directly killing neurons, arsenic can trigger a chronic neuroinflammatory response. It activates microglia and astrocytes, the resident immune cells of the CNS, leading to the release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-á), interleukin-1-beta (IL-1â), and interleukin-6 (IL-6) (Wu et al., 2012; Block and Hong, 2005). This sustained inflammatory environment further exacerbates neuronal damage and contributes to the progressive nature of neurodegeneration.

4. Link To Neurodegenerative Diseases

The pathological hallmarks of arsenic neurotoxicity, including oxidative stress, mitochondrial dysfunction, apoptosis, and neuroinflammation, are also central to the pathogenesis of major neurodegenerative diseases. Epidemiological and experimental studies have begun to draw a link between chronic arsenic exposure and an increased risk of AD and PD (Coon et al., 2006; O'Bryant et al., 2011).

In the context of AD, arsenic has been shown to promote the aggregation of amyloid-beta (Aâ) peptides and the hyperphosphorylation of tau protein, two key pathological features of the disease (Luo et al., 2009). Similarly, in PD models, arsenic exposure has been demonstrated to induce the loss of dopaminergic neurons in the substantia nigra, a hallmark of the disease, and promote the aggregation of á-synuclein (Lee et al., 2008; Kumar et al., 2010).

6. Conclusion

Arsenic neurotoxicity represents a formidable public health challenge. The mechanisms underlying its detrimental effects on the brain are complex and interwoven, with oxidative stress playing a pivotal role. The link between chronic arsenic exposure and an increased risk for devastating neurodegenerative diseases highlights the urgent need for action. Future research should focus on elucidating the precise molecular targets of arsenic in the brain, identifying susceptible populations, and developing effective and accessible strategies for both prevention and treatment. Public health initiatives aimed at providing arsenic-free drinking water remain the most critical step in mitigating this global health crisis.

Conflict of Interest

The authors declare that they have no known financial or personal conflicts of interest that could have influenced the work presented in this paper.

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Biologics Development and Challenges: Focus on Biosimilars

Ananyo Mazumdar[1], Suranjana Sarkar[2], Priyajit Banerjee[1]*

[1]*Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore*

[2]*Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore*

**Corresponding author: priyajitb@svu.ac.in*

Abstract

The global pharmaceutical industry is experiencing a transformative shift driven by biosimilars. It is highly similar biologic products to reference biologics produced by different manufacturers. Encompassing diverse therapies such as hormones (e.g., insulin, growth hormone), vaccines, erythropoietin (EPO), monoclonal antibodies (mAbs), antibody-drug conjugates (ADCs), and bispecific antibodies (BsAbs), biologics have revolutionized treatment for complex diseases. As patents of biologics expire, biosimilars promise enhanced patient access and substantial healthcare cost reductions, particularly in underserved regions. Yet, their development faces multifaceted challenges including rigorous regulatory approval, demonstration of quality, potency, efficacy, immunogenicity, and safety equivalence, alongside market adoption barriers. This chapter provides an in-depth exploration of biosimilar development pipelines, approval pathways, benefits, and persistent hurdles to guide future integration into healthcare systems.

Keywords: Biologics, Biopharmaceuticals, Biosimilars, Drug Designing, Drug Modification

1. Introduction

Biologics represent the pinnacle of modern pharmacotherapy, comprising large, complex molecules derived from living organisms that target intricate disease pathways unattainable by small-molecule drugs (Leader, Baca, & Golan, 2008). The term "biologics" broadly includes recombinant proteins, hormones, cytokines, vaccines, blood products, and advanced antibody-based constructs like mAbs, ADCs, and BsAbs. Unlike chemically synthesized small molecules, biologics exhibit inherent heterogeneity due to post-translational modifications (PTMs) such as glycosylation, which critically influence their pharmacokinetics (PK), pharmacodynamics (PD), efficacy, and immunogenicity (Sarvepalli et al., 2025).

The advent of biosimilars—defined by the World Health Organization (WHO) as "biotherapeutic products which are similar in terms of quality, safety and efficacy to an already licensed reference biotherapeutic product"—marks a pivotal evolution (Schellekens et al., 2016). Triggered by patent expirations on high-revenue "blockbuster" biologics (e.g., adalimumab/Humira exceeding $20 billion annually), biosimilars enable generic-like competition in the biologics space. Projections indicate over $100 billion in global sales

potential by 2030, with the U.S. market alone poised for $30-50 billion in savings through 2025 (McCamish et al., 2012).

However, biosimilar development diverges fundamentally from small-molecule generics. Lacking access to originators' proprietary cell lines, expression systems, and manufacturing processes, developers must employ reverse engineering via advanced analytics to achieve "comparability" (Monga et al., 2025). Regulatory bodies like the European Medicines Agency (EMA), U.S. Food and Drug Administration (FDA), and WHO demand a "totality-of-the-evidence" approach encompassing analytical, nonclinical, and clinical data (Kozlowski, Woodcock, Midthun, & Behrman Sherman, 2011). Challenges span technical (e.g., matching glycosylation profiles), regulatory (e.g., immunogenicity risk assessment), and commercial (e.g., prescriber hesitancy) domains. This chapter dissects these elements systematically (McCamish & Woollett, 2012).

2. Diversity of Biologic Therapies

Biologics' structural complexity underpins their therapeutic versatility but complicates replication (Vulto & Jaquez, 2017). Hormones like insulin (molecular weight ~5,808 Da) and human growth hormone (~22 kDa) exemplify simpler recombinant proteins produced in bacterial or yeast systems. Insulin biosimilars, approved since 2015, demonstrate feasibility for less complex molecules. Vaccines, comprising attenuated pathogens or subunit antigens, face unique stability and potency challenges.

EPO (~30 kDa glycoprotein) treats anaemia in chronic kidney disease and oncology, with biosimilars entering markets post-2007 EMA approvals (Jelkmann, 2013). mAbs (~150 kDa), the dominant class, target cell surface receptors in oncology (e.g., trastuzumab/Herceptin) and immunology (e.g., infliximab/Remicade). ADCs fuse mAbs with cytotoxic payloads via labile linkers, demanding precise drug-antibody ratios (DAR) (Beck, Goetsch, Dumontet, & Corvaïa, 2017). BsAbs, binding dual antigens (e.g., blinatumomab/CD19-CD3), enable T-cell redirection but introduce mispairing risks during assembly.

Table 1: This table illustrates escalating complexity from left to right, correlating with development timelines and costs.

Biologic Type	Examples	MW (kDa)	Production Host	Primary Indications	Biosimilar Status
Hormones	Insulin, hGH	5.8-22	E. coli/Yeast	Diabetes, Growth deficiency	Approved (e.g., Semglee)
Cytokines	EPO, G-CSF	18-30	CHO cells	Anemia, Neutropenia	Multiple approvals
mAbs	Rituximab, Adalimumab	145-150	CHO/NS0	Cancer, RA, Psoriasis	>50 global
ADCs	Trastuzumab emtansine	150+	CHO	Breast cancer	Emerging
BsAbs	Amivantamab	150+	CHO	NSCLC	Preclinical-heavy

1. Global Market Landscape and Patent Cliff

The biologics market, valued at $400+ billion in 2025, anticipates a "patent cliff

" with 118 major expirations by 2034, including Humira (2023 U.S.), Stelara (2025), and Soliris (2026) (Song et al., 2025). Europe leads with 100+ biosimilars approved since 2006, achieving 70% market penetration for rituximab. The U.S., delayed by litigation, saw uptake surge post-2022 with 40+ approvals. Biosimilars deliver 20-50% price erosion, yielding $54 billion U.S. savings (2017-2026 estimate). In low-middle income countries, uptake enhances equitable access, e.g., EPO biosimilars in India reducing costs 60%. Yet, U.S. penetration lags at <5% for adalimumab due to rebate traps and patent thickets.

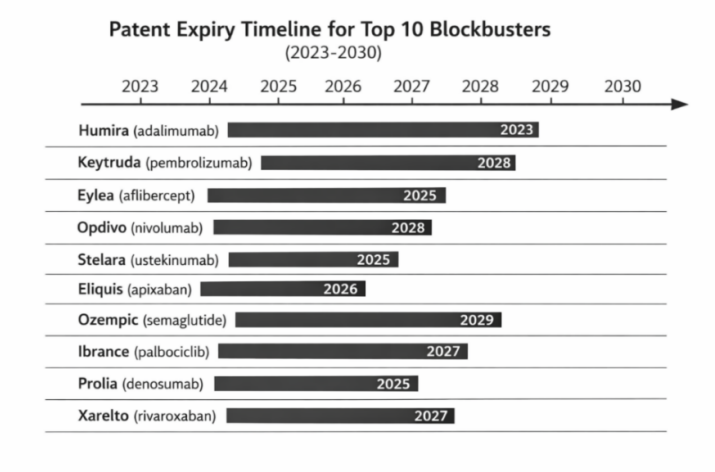

Figure 1: Patent expiry timeline for top 10 blockbusters, 2023-2030.

2. Biosimilar Development Pipeline

Development spans 7-10 years and $100-300 million, versus $2-3 billion for originators. Phase I: Target selection and host optimization (CHO cells preferred for human-like glycosylation). Reverse engineering dissects reference product via mass spectrometry (MS), NMR, and circular dichroism for primary/secondary/tertiary structure (Berkowitz, Engen, Mazzeo, & Jones, 2012).

Upstream: Cell line development (GS or DHFR amplification), fed-batch perfusion (yields 5-10 g/L). Downstream: Protein A capture, ion-exchange, hydrophobic interaction chromatography; viral inactivation. Analytics target critical quality attributes (CQAs): purity (>98%), potency (cell-based assays), glycan profiling (UPLC-MS). Formulation stabilizes against aggregation (e.g., polysorbate surfactants). Stability per ICH Q5C ensures 24-36 months shelf-life.

3. Regulatory Approval Pathways

EMA's centralized procedure mandates analytical similarity (e.g., 20+ CQAs), functional bioassays, and reduced clinical trials (PK/PD confirmatory) (Kurki et al., 2017). FDA's 351(k) BPCIA mirrors this, with optional interchangeability via switching/non-inferiority studies. WHO's "similar biotherapeutic products" (SBPs) aids emerging markets.

Table 2: Clinical requirements scale inversely with analytical confidence: high similarity may waive large efficacy trials, relying on PD endpoints.

Agency	Analytical	Non-Clinical	Clinical	Interchangeable?	Reference
EMA	Comprehensive (MS, SPR)	PK/PD in animals	PK + 1 confirmatory	No (but substitutable)	European Medicines Agency. (2014). Guideline on similar biological medicinal products.
FDA	Totality-of-evidence	In vitro/in vivo	PK/PD + 1 efficacy if needed	Yes, with switching data	U.S. Food and Drug Administration. (2015). Scientific considerations in demonstrating biosimilarity to a reference product.
WHO	Risk-based	Case-by-case	Scaled to complexity	National discretion	World Health Organization. (2022). Guidelines on evaluation of biosimilars.

4. Technical and Quality Challenges

Matching originator heterogeneity is paramount. Glycosylation variants (e.g., afucosylated mAbs enhancing ADCC) demand identical profiles; deviations risk immunogenicity. Aggregates (>1%) trigger complement activation (Liu, 2015).

Immunogenicity arises from anti-drug antibodies (ADAs), mitigated by deimmunization algorithms and post-approval pharmacovigilance (ICH S9). Potency assays (e.g., cAMP for EPO) must exceed 90% relative potency.

Table 3: Regulatory Framework for Biosimilars

Challenge	Impact	Assessment Tools	Mitigation	References
Glycosylation drift	Efficacy loss, clearance	N-glycan mapping (HILIC-MS)	Process controls, clones	Hossler, P., et al. (2009)
Immunogenicity	Neutralization, hypersensitivity	ECD-ELISA, SPR	Sequence optimization	Shankar, G., et al. (2008)
Purity/Impurities	Safety (e.g., HCP)	SEC, iCE	Orthogonal purification	Wang, X., et al. (2009)
Stability	Aggregation	DSC, DLS	Formulation excipients	Wang, W. (2005)

5. Market Adoption Barriers and Strategies

Despite equivalence, U.S. uptake <20% versus Europe's 60%. Barriers: "Black-box" rebates favoring originators; no pharmacist substitution (except 5 states); prescriber education gaps (70% unaware of approval rigor).

Strategies: Payer policies mandating non-medical switching; real-world evidence (RWE) registries; global naming conventions (INN + suffix). Cost savings amplify adherence, e.g., 15% uptake in RA boosting persistence 20%.

8. Benefits, Clinical Evidence, and Future Directions

Biosimilars match originator efficacy/safety: meta-analyses show biosimilar: mAb response ratios 0.97-1.03 (Botteri, Krendyukov, & Curigliano, 2018). Savings fund novel therapies; e.g., filgrastim biosimilars saved $7B globally.

Future: Next-gen biosimilars (hypersimilars with improvements); AI-driven analytics; continuous manufacturing. Harmonized regs and trust-building will unlock $230B opportunity by 2034.

9. Conclusion

The arrival of biologics has reshaped how we treat disease, allowing us to tackle targets that traditional drugs simply couldn't touch. From standard hormones to sophisticated designs like antibody-drug conjugates (ADCs) and bispecific antibodies, these macromolecules are incredibly powerful. However, the very complexity that makes them work so well is also what makes them so difficult to create (Leader, Baca, & Golan, 2008; Vulto & Jaquez, 2017). Brand-name biologics are becoming too expensive for healthcare systems to keep up with, which is why biosimilars are stepping into the spotlight. With a wave of major patents set to expire between now and 2034—often called the "patent cliff"—the global market is about to see a huge shift toward more accessible, lower-cost options (Song et al., 2025).

Developing a biosimilar is a far more complex challenge than creating a generic pill. Since developers don't have the original manufacturer's recipe—including their specific cell lines or exact purification steps—they have to work backward to reconstruct the drug. This requires exhaustive reverse engineering using high-tech tools like mass spectrometry and NMR spectroscopy to ensure every detail, from the protein's shape to its biological activity, matches the original perfectly (Berkowitz, Engen, Mazzeo, & Jones, 2012).

In summary, biosimilars occupy a uniquely complex position at the intersection of advanced molecular biology, precision manufacturing, and translational medicine. Regulatory harmonization across ICH, EMA, FDA, and WHO frameworks—particularly for compatibility and substitution—will be critical to translating scientific equivalence into clinical and commercial confidence (Kurki et al., 2017; McCamish & Woollett, 2012).

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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Effects of thermal stress and Pollutants on Fish Physiology: A Combined Stressor Approach

Mayisha Sultana[1], Priyankar Pal[1]*

[1]*Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata 700121*

[1*]*Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata 700121*

[*]*Corresponding author*: priyankarp@svu.ac.in

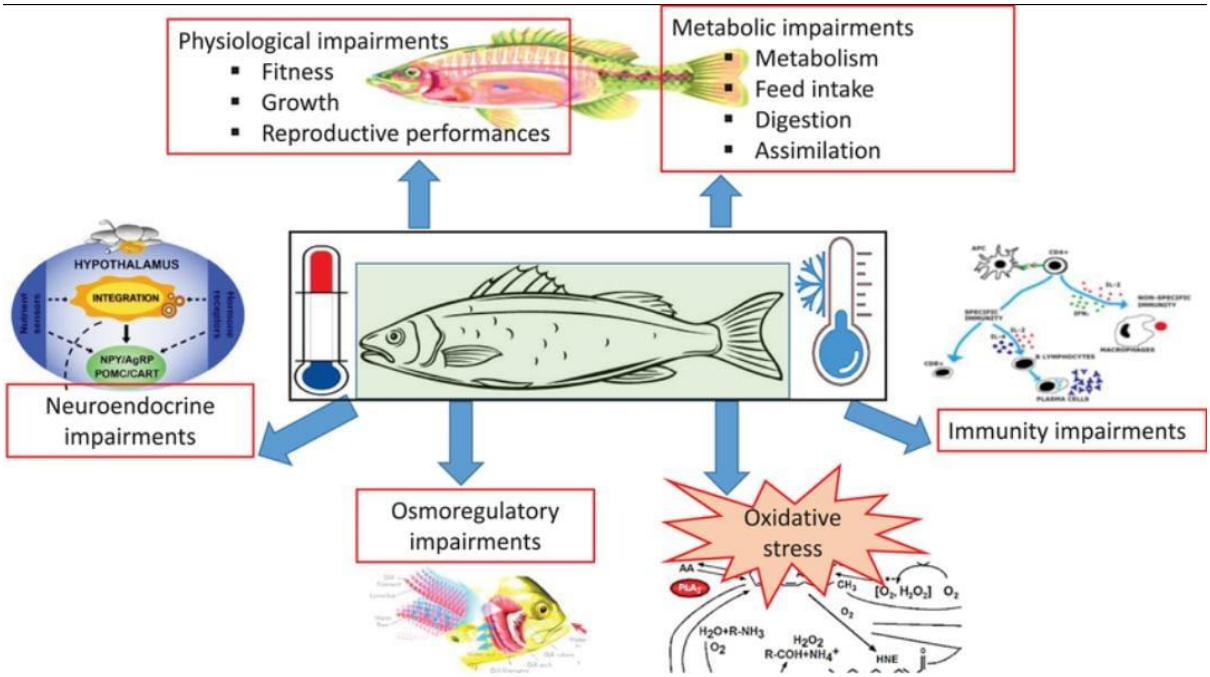
Abstract

Global climate change and the increase of anthropogenic pollution present major challenges for aquatic ecosystems. Fish live in dynamic water conditions where they face many biotic and abiotic stresses. Among them, two main causes of physiological disturbance are high temperatures (thermal stress) and chemical pollutants (such as heavy metals and organic toxins). Recent studies highlight that simultaneous exposure can have a non-additive impact on fish physiology and fitness, despite the fact that each stressor has been thoroughly examined separately. Fish are affected by the physiological, biochemical, and molecular responses that are changed by thermal stress, and also, fish at similar levels are affected by pollutant exposure. The interaction of pollutants and thermal stress affects fish performance, energetics, resilience, and population-level outcomes. . We highlight key mechanistic pathways like oxidative stress, endocrine disruption, energy budget imbalance, gene expression, and tissue damage in the context of climate change and pollution-intensive aquatic systems, but identifying important knowledge gaps and making recommendations for future research and management approaches are more crucial. Rising temperatures can increase metabolic rates, reduce oxygen availability, weaken defence mechanisms, and enhance the toxicity of pollutants. Thus, studying these stressors in isolation is no longer sufficient. Understanding their combined effects is essential to predict ecological risks, fish health, fisheries productivity, and biodiversity conservation. So, to find a combined-stressor strategy to better anticipate fish outcomes in increasingly stressed aquatic ecosystems by viewing pollution and thermal stress is necessary.

Keywords*:* Anthropogenic, Physiological, Pollutant, Toxicity, Aquatic ecosystems

1. Introduction

Fish and other aquatic ectotherms are completely associated to their habitat's chemical and physical conditions. Two of the most familiar human impacts on aquatic environments are differences caused in water temperature and pollution levels (Smith & Jones, 2021). Fish are related to thermal strain due to increasing temperature patterns which are brought by both localized warming (such as wastewater discharges) and global climate change (Lee et al., 2020). Increased vulnerability to the contaminants, such as pesticides, modern medications, and noxious metals, simultaneously throws off physiological balance (Garcia & Patel, 2019). Thermal stress and chemical pollution they both together are very fascinating since their high temperatures can increase the metabolic rates, impair biotransformation, and they can increase the toxicity of pollutants (Wang & Zhou, 2022). Upcoming fish reactions to predictable environmental changes requires an understanding of how these stressors interact with each other and harm the aquatic life (Robinson, 2023). Thus, the evidence regarding heat stress, pollution stress, and their combined effects on fish physiology is exaggerated in this study. Aquatic ectotherms are particularly sensitive to the contemporary dual threat of rising temperatures and chemical runoff because they are inextricably linked to the physical and chemical integrity of their habitat (Adams, 2002). Fish are driven to their physiological breaking points by localized industrial wastes and changes in the global environment, but the presence of pesticides, heavy metals, and medications produces a lethal combination (Schindler, 2006). A fish's metabolism is accelerated by higher temperatures, forcing them to consume more contaminated water and perhaps making some toxins far more deadly (Nikaido & Dickson, 2012). This study emphasizes that we can no longer consider heat or pollution separately; rather, we must acknowledge that their combined effects provide a compounding stress that poses a threat to fish population stability and the resilience of entire aquatic ecosystems (Heugens, 2001).



. Figure 1: Temperature stress impacts on fish

2. Thermal Stress and Fish Physiology

2.1 Temperature as a Master Regulator

In the world of aquatic biology, temperature isn't just an environmental factor it is the master regulator that dictates the pace of life. Because fish are ectothermic (cold-blooded), their internal body temperature mirrors their surroundings, directly controlling the speed of every chemical reaction within their bodies (Schmidt-Nielsen, 1997).

Key Physiological Impacts:

Metabolic Demands: As waters warm, a fish’s engine runs faster, requiring significantly more oxygen and energy just to maintain basic survival (Clarke & Johnston, 1999).

Respiratory Efficiency: Higher temperatures reduce the water's ability to hold dissolved oxygen, forcing fish to breathe harder even as their aerobic capacity hits a ceiling (Pörtner & Knapp, 2007).

Biochemical Catalysis: Essential enzymes function within narrow thermal windows; if the water is too cold, reactions stall, and if it is too hot, proteins begin to denature (Hochachka & Somero, 2002).

Nutritional Processing: While warmth can initially speed up digestion and appetite, extreme heat can shut down the gut's ability to process nutrients effectively (Jobling, 1994).

Life Cycle Milestones: From the rate of embryonic development to the hormonal triggers required for spawning, temperature governs the timing of growth and reproduction (Pankhurst & Mounfort, 1995).

Disease Resistance: Thermal shifts can suppress the immune system, making fish more susceptible to pathogens and parasites that thrive in warmer conditions (Harvell et al., 2002).

2.2 Immediate Responses to heat stress

When a fish is suddenly hit with a heatwave we see changes in them, its body immediately shifts into an emergency survival mode that is both intense and exhausting. This “red alert” begins with a massive surge of stress hormones like cortisol and adrenaline, which act as a chemical alarm system to mobilize every bit of available energy (Barton, 2002). Simultaneously, the fish’s metabolism kicks into high gear, causing a desperate spike in oxygen demand just as the warming water is losing its ability to hold oxygen (Pörtner & Knapp, 2007). At the microscopic level, the body deploys "Heat Shock Proteins" to act as molecular bodyguards, preventing vital proteins from melting or losing their shape (Iwama et al., 1998). However, this metabolic racing also produces "biological rust” reactive oxygen species that can damage cells and DNA (Lushchak, 2011). If the heat doesn't subside, the fish loses its ability to balance internal salts and fluids (Gonzalez, 2012). While these responses are lifesaving in the short term, they are so taxing that they eventually lead to total physiological exhaustion (Feder & Hofmann, 1999).

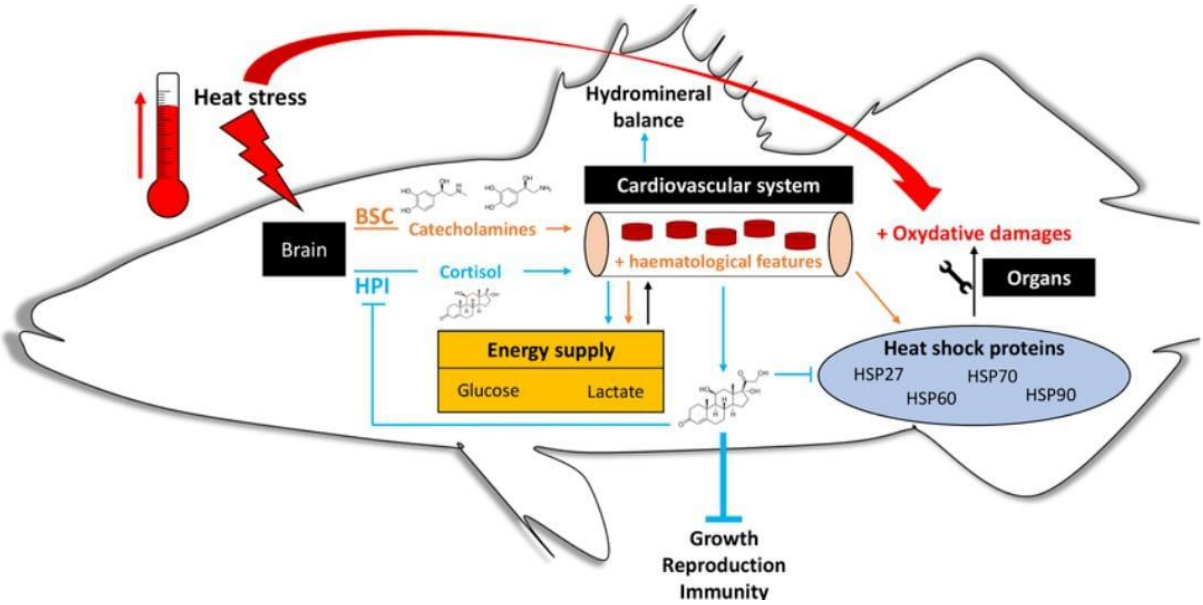


Figure 2: Stress response on acute exposure to a sudden elevation in temperature

2.3 Energetic consequences and performance trade-offs

The fish must essentially pay a significant heat tax in order to sustain its heartbeat due to thermal stress. Because heat acts as a gas pedal for metabolism, the fish's internal engine starts to accelerate, burning down energy sources like lipids (fats) and glycogen (sugars) at an unsustainable rate (Gillooly et al., 2001). The discrepancy between the amount of energy needed for a fish to stay still and the highest amount of energy it can produce is referred to as "metabolic scope" (Fry, 1947). As the temperature rises, that difference decreases (Pörtner & Knapp, 2007). Eventually, the fish uses so much energy for maintenance just to survive that it has no energy left over for anything else (Clarke & Johnston, 1999).

3. Pollutant Stress: Heavy Metals and Other Contaminants

3.1 Overview of pollutant types and exposure pathways

Heavy metals like lead and mercury, as well as contemporary medications and pesticides, are among the invisible invasion of chemical stresses that aquatic habitats are currently experiencing (Schindler, 2006). Fish operate like biological sponges because they are always submerged in their environment, absorbing these toxins through their skin, gills, and diet (Wood et al., 2012). Bioaccumulation, in which these persistent chemicals accumulate in the fish's tissues over time more quickly than they can be eliminated, is the true threat (Meyer et al., 2013). This poisonous accumulation weakens the circulation, damages important organs like the liver, and even targets the DNA of the fish (Baccarelli & Bollati, 2009). In the end, these pollutants take away the fish's natural immunity, rendering them vulnerable to illness and unable to sustain the fundamental internal chemistry needed to exist in a world that is becoming more and more contaminated (De Smet et al., 1998).

3.2 Physiological and molecular mechanisms of pollutant toxicity

General mechanistic themes include:

ROS generation: metals catalyse redox reactions, generate free radicals, deplete antioxidants (GSH, SOD, CAT, GPx) (Livingstone, 2001; Valente et al., 2012).

Bioaccumulation and tissue-specific damage: gills, liver, kidney often show lesions, necrosis, hyperplasia, pigment deposition, altered function (Hinton et al., 1992; Au, 2004).

Endocrine disruption: pollutants can alter hormone levels, inhibit reproduction, impair development (Colborn et al., 1993; Kloas et al., 2009).

Hemato-biochemical alterations: changes in RBC/WBC counts, hemoglobin, hematocrit, enzyme biomarkers (e.g., alkaline phosphatase, ALT, AST) and metabolic disruption (protein/lipid/glycogen metabolism) (Larsson et al., 1985; Almeida et al., 2002).

3.3 Sublethal effects, biomarkers and ecological significance

Sublethal pollutant exposure may not cause immediate mortality but can impair growth, reproduction, immune competence, making fish vulnerable to disease and predation (Adams, 2002; Scott & Slade, 2003). Biomarkers such as antioxidant enzyme activities, metallothionein levels, DNA damage, tissue histopathology are used for monitoring (Peakall, 1992; Van der Oost et al., 2003). From a population perspective, such impairments can reduce recruitment success and alter community structures (Källman & Newman, 2012; Segner, 2007).

4. Combined Stressors: Thermal Stress and Pollutants

4.1 Need for investigating interaction

Single-stressor studies dominate the literature, but real-world aquatic environments present stressors in isolation (Heugens et al., 2001). The interaction between warming and chemical contamination is of particular concern: temperature may affect contaminant toxicity (e.g., by altering bioavailability, uptake kinetics, detoxification capacity) and fish metabolic/state (Nikaido & Dickson, 2012). A modelling study on invertebrates presented a Dynamic Energy Budget (DEB) framework to predict combined effects of chemical, temperature and food stressors (Kimmel & Stark, 2013).

4.2 Evidence of interaction effects in fish

Although fewer in number than single-stressor studies, some investigations demonstrate how temperature modulates pollutant stress: A study on juvenile hybrid yellow catfish exposed to heat stress (35 °C vs 28 °C) showed increased RBC, WBC, HGB, HCT, elevated antioxidant enzyme activity and disturbed energy reserves (Li et al., 2018). Though pollutant exposure was not combined in that study, it illustrates how thermal stress alone intensifies physiological load. A recent study on hexavalent chromium (Cr VI) exposure combined with

elevated temperature showed increased pollutant toxicity under higher temperature conditions indicating an interactive effect (Sappal et al., 2019).

4.3 Mechanistic pathways of interaction

Oxidative stress: Both thermal stress and pollutants generate ROS; combined exposure may overwhelm antioxidant capacity faster (Lushchak, 2011).

Energy budget compromise: Thermal stress increases maintenance costs; pollutants impose detoxification and repair costs; combined demands may reduce energy for growth/reproduction/immune defence (Sokolova et al., 2012).

Impaired detoxification: Elevated temperature may alter enzyme kinetics of detoxification systems (e.g., cytochrome P450, metallothioneins), or change membrane permeability, leading to increased pollutant uptake (Heugens et al., 2001).

Endocrine and immune system cross-talk: Stress axis activation (cortisol) by thermal stress may down-regulate immune and detox pathways, making fish more susceptible to pollutant effect (Tort, 2011).

Tissue damage synergy: Thermal stress may compromise tissue integrity (e.g., gill epithelium, gut barrier) allowing greater pollutant penetration; pollutants may impair heat shock responses and acclimation ability (Iwama et al., 1998).

4.4 Impacts on fish performance and population

There is reduced growth and condition factor due to diverted energy (Pörtner & Knapp, 2007). The altered behaviour (reduced feeding, impaired predator avoidance) (Scott & Slade, 2003). Thereafter decreased reproductive output via endocrine or gonadal damage (Kloas et al., 2009). Therefore the increased mortality or reduced recruitment, altering population dynamics (Källman & Newman, 2012).

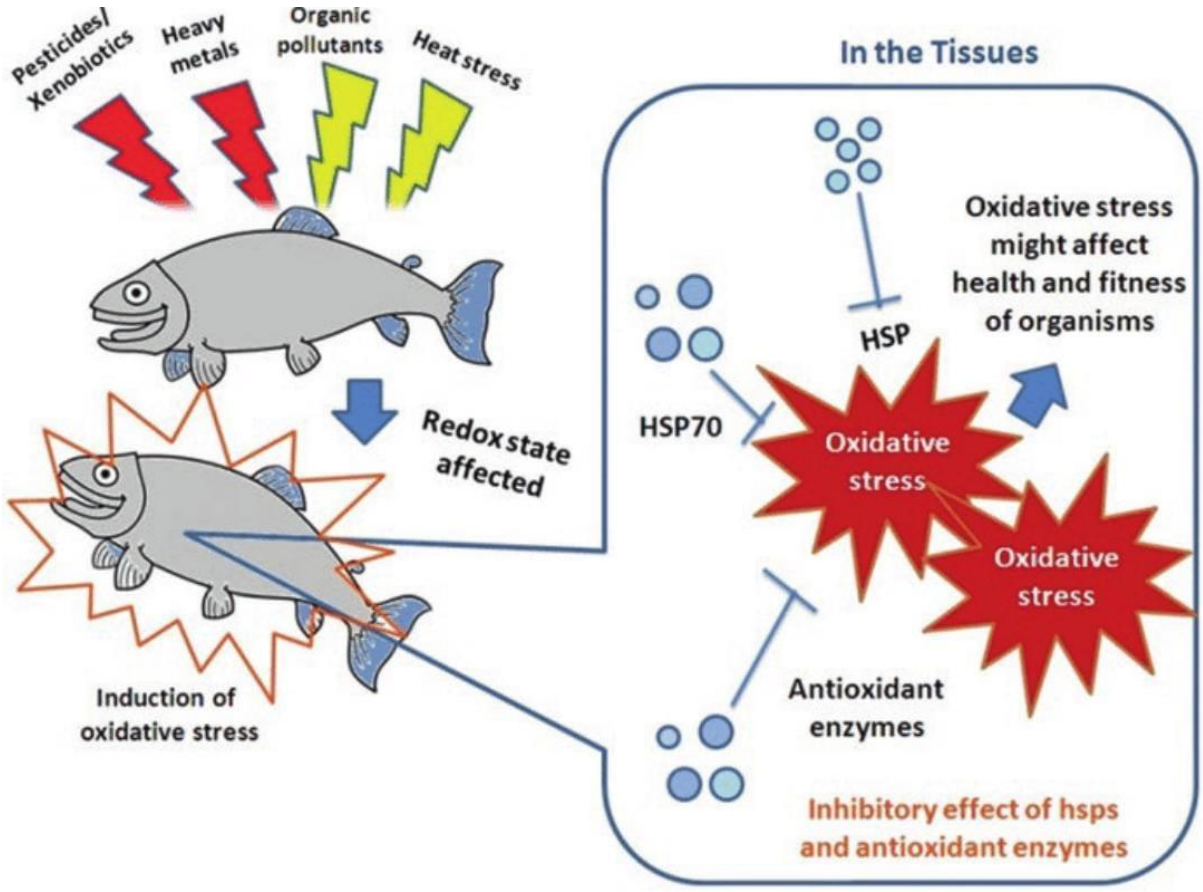


Figure 3: Induction of oxidative stress in fish and inhibitory effect of heat shock proteins

5. Biomarkers and Monitoring Approaches

The multi-level impact of combined stressors, biomarker suites spanning molecular also the cellular organismal scales are needed (Van der Oost et al., 2003). Some recommendations include:

Firstly the molecular biomarkers: HSP70/HSP90 expression, metallothionein induction, detox genes (e.g., cytochrome P450), oxidative stress gene transcripts (Iwama et al., 1998; Regoli & Giuliani, 2014).

Also the biochemical/enzymatic biomarkers: SOD, CAT, GPx, GST, GSH/GSSG ratios, lipid peroxidation (MDA), enzyme activities for digestion and metabolism (Livingstone, 2001; Valente et al., 2012).

Therefore the Hematological and immunological parameters: RBC/WBC count, hemoglobin, hematocrit, cortisol levels, lysozyme activity, immune cell function (Tort, 2011; Scott & Slade, 2003).

6. Knowledge Gaps

The limited empirical studies on combined exposures: The literature on fish specifically exposed to both elevated temperature and pollutant load is still small (Heugens et al., 2001; Nikaido & Dickson, 2012).

The life-stage and species sensitivity: Different species and life stages (larvae, juveniles, adults) vary in sensitivity; understanding these differences under combined stressors is critical (Kloas et al., 2009; Segner, 2007).

The recovery and acclimation potential: How fish recover from combined stressor exposures and whether acclimation can mitigate effects remains underexplored (Sokolova et al., 2012).

The energetic modelling and predictive frameworks: Applying bioenergetic/DEB models to fish (as has been done for invertebrates) to predict interactive effects of stressors would advance predictive capacity (Kimmel & Stark, 2013).

The field validation and ecological scaling: Laboratory findings need to be validated in natural or semi-natural systems where multiple stressors (pollution, warming, hypoxia, competition) co-occur (Adams, 2002).

The management and mitigation strategies: Research should link mechanistic understanding to mitigation: e.g., pollutant load reductions, habitat restoration, thermal refuge creation, and aquaculture best-practices (Schindler, 2006).

The Omics and system-level integration: Use of transcriptomics, proteomics and metabolomics to unravel comprehensive pathways of combined stress, and linking molecular changes to organismal outcomes (Regoli & Giuliani, 2014).

7. Conclusion

The rising temperatures and chemical pollution are creating a double-jeopardy for the fish survival, which is a perfect storm for aquatic life. These problems operate as a deadly partnership in the wild, so studying them separately is no longer sufficient. As a biological accelerator, heat causes a fish's metabolism to redline, depleting the energy reserves it sorely needs to develop, procreate, and maintain its health. Chemical pollutants like heavy metals and pesticides enter to complete the damage at the precise period when the fish is most worn out by this heat tax, causing tissue damage, immune system collapse, and cellular damage that the fish cannot afford to heal. There is a survival of entire populations and ecosystems is

at stake due to this synergy, not just individual fish. In this time climate change is fast, our strategy must change if we are to preserve our fisheries, aquatic biodiversity and aquatic lives. We must transition to an integrated model of research that integrates sophisticated energy modeling with real-time biomarker monitoring. Before these cumulative pressures reach a point of no return, conservationists and politicians can start constructing true resilience for our undersea habitat by comprehending how these stressors interact.

8. Future Prospective

Multi-stressor science will become the gold standard as we enter a new era. The next ten years will be devoted to anticipating the precise ways in which an increase in a particular river will increase the toxicity of nearby agricultural runoff, rather than merely asking how hot the water is or how many chemicals are in it. "Precision conservation," which uses AI-driven models and real-time genetic sensors to alert us when a fish population is nearing a physiological tipping point before a huge die-off ever starts, is probably going to become more popular. Furthermore, the way we manage wild fisheries and aquaculture will need to change in the future. In order to provide fish with a recovery area, we might witness the establishment of key cool-water refuges where pollution is strictly prohibited or the development of thermal-resilient breeding programs. The link between lab research and policy ultimately determines our potential success. We can develop a management approach that is as dynamic and adaptable as the ecosystems we are attempting to preserve by approaching the health of the ocean as a complex, linked patient rather than as a collection of discrete issues.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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COX-2 inhibitors vs traditional NSAIDs: A comprehensive review

Sanjukta Banerjee[1], Sucheta Bhowmik[1], Rupesh Dutta Banik[1]*

[1]*Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal-700121, India*

**Corresponding author:* rupeshduttabanik99@gmail.com

Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most used medications for managing pain, fever, and inflammation. Their therapeutic effects arise mainly from inhibition of cyclooxygenase (COX) enzymes, which are essential for prostaglandin synthesis. Conventional NSAIDs inhibit both COX-1 and COX-2 isoenzymes, providing effective symptom relief but frequently causing gastrointestinal and renal complications due to the suppression of protective prostaglandins. The recognition of COX-2 as the inflammation-inducible form led to the development of selective COX-2 inhibitors, intended to preserve the anti-inflammatory efficacy while reducing COX-1–related toxicity. Comparative analyses demonstrate that COX-2 inhibitors significantly lower the risk of gastrointestinal injury and bleeding; however, they may elevate cardiovascular risk by disturbing the physiological balance between prostacyclin and thromboxane A_2, thus promoting thrombosis. Both traditional and selective NSAIDs can still produce hepatic toxicity and hypersensitivity reactions, though these are less common. Despite the therapeutic advantages of COX-2 selectivity, clinical use requires careful consideration of individual cardiovascular risk factors. Overall, while COX-2 inhibitors and traditional NSAIDs act via similar mechanisms, their differing safety profiles highlight the need for an individualized approach that balances efficacy with gastrointestinal and cardiovascular safety.

Keywords: Non-steroidal anti-inflammatory drugs (NSAIDs), COX-2 inhibitors; Cyclooxygenase inhibition, Prostaglandin synthesis, Drug safety, Pharmacological comparison.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) represent one of the most widely prescribed classes of medications globally, utilized extensively for the management of pain, inflammation, and fever across diverse clinical settings (Sostres, Gargallo, Arroyo, & Lanas, 2010). These pharmacological agents have become indispensable in modern therapeutic practice, with millions of individuals worldwide relying on them for both acute symptom relief and chronic disease management. The prevalence of NSAID use reflects their efficacy in treating conditions ranging from minor headaches and musculoskeletal pain to more severe inflammatory disorders such as rheumatoid arthritis and osteoarthritis.

The primary mechanism underlying the therapeutic efficacy of NSAIDs involves the inhibition of the cyclooxygenase (COX) enzyme system, which catalyzes the conversion of arachidonic acid into prostaglandins and thromboxanes—critical mediators of inflammation, pain transmission, hemostasis, and various physiological protective mechanisms (Vane & Botting, 1998). The discovery of this mechanism by Sir John Vane in the early 1970s revolutionized our understanding of anti-inflammatory drug action and earned him the Nobel Prize in Physiology or Medicine. This breakthrough laid the foundation for subsequent research that identified two distinct isoforms of the COX enzyme, each with unique physiological roles and tissue distribution patterns.

Two principal isoforms of the COX enzyme have been characterized: COX-1, a constitutive enzyme that mediates physiological functions such as gastric mucosal protection, renal hemodynamics, and platelet aggregation, and COX-2, an inducible isoform expressed predominantly at sites of inflammation in response to inflammatory stimuli, growth factors, and cytokines (Brune & Patrignani, 2015). The COX-1 enzyme is constitutively expressed in most tissues and maintains homeostatic functions, including the production of prostaglandins that protect the gastric mucosa from acid-induced damage, regulate renal blood flow and sodium excretion, and facilitate platelet function through thromboxane A_2 synthesis. In contrast, COX-2 expression is typically minimal under normal physiological conditions but becomes rapidly upregulated during inflammatory processes, tissue injury, and cellular stress responses (Vane, Bakhle, & Botting, 1998).

Traditional NSAIDs, including widely used agents such as ibuprofen, naproxen, diclofenac, and indomethacin, exert their therapeutic effects through non-selective inhibition of both COX-1 and COX-2 isoforms. While this dual inhibition provides effective relief from pain and inflammation by reducing prostaglandin synthesis at inflammatory sites, it simultaneously compromises the protective mechanisms mediated by COX-1-derived prostaglandins in the gastrointestinal tract and kidneys (Vane & Botting, 1998). The blockade of COX-1-mediated prostaglandin synthesis disrupts the gastric mucosal barrier, decreases bicarbonate and mucus secretion, reduces mucosal blood flow, and impairs epithelial repair mechanisms, thereby predisposing patients to a spectrum of adverse gastrointestinal events including dyspepsia, gastritis, peptic ulceration, and potentially life-threatening gastrointestinal bleeding and perforation (Schoen & Vender, 1989).

The significant morbidity and mortality associated with NSAID-induced gastrointestinal toxicity prompted intensive research efforts to develop safer alternatives that could maintain anti-inflammatory efficacy while minimizing adverse effects. This led to the rational development of selective COX-2 inhibitors (coxibs) in the late 1990s, with celecoxib and rofecoxib being among the first agents to receive regulatory approval (Geis, 1999; Simon et al., 1999). These selective inhibitors were designed with the explicit goal of preferentially targeting the COX-2 isoform involved in inflammation while sparing COX-1-mediated protective functions in the gastrointestinal tract and platelets. Initial clinical trials and early post-marketing surveillance data suggested that COX-2 inhibitors indeed offered superior gastrointestinal safety compared to traditional NSAIDs, generating considerable enthusiasm within the medical community and among patients suffering from chronic pain and inflammatory conditions.

However, subsequent long-term clinical studies and comprehensive safety analyses revealed an unexpected association between COX-2 selective inhibition and increased cardiovascular risk, particularly with certain agents such as rofecoxib, which was voluntarily withdrawn from the market in 2004 (Bacchi, Palumbo, Sponta, & Coppolino, 2012). This cardiovascular risk has been attributed to a disruption in the delicate physiological balance between vasodilatory prostacyclin (PGI_2), which is primarily COX-2-derived in vascular endothelium, and prothrombotic thromboxane A_2 (TXA_2), which is predominantly COX-1-derived in platelets. Selective COX-2 inhibition reduces prostacyclin synthesis while leaving thromboxane production intact, potentially creating a prothrombotic state that favors platelet

aggregation, vasoconstriction, and increased blood pressure (Patrignani, Tacconelli, Bruno, Sostres, & Lanas, 2011).

Furthermore, both traditional NSAIDs and COX-2 selective inhibitors can produce hepatic toxicity, ranging from mild transient elevations in liver enzymes to severe hepatocellular injury and, in rare cases, acute liver failure requiring transplantation (Elsevier, 2006). Hypersensitivity reactions, including bronchospasm, urticaria, angioedema, and severe cutaneous adverse reactions, also represent important safety concerns with both drug classes, occurring through immunological and non-immunological mechanisms. Additionally, NSAID use during pregnancy poses specific risks, including premature closure of the fetal ductus arteriosus, oligohydramnios, and potential delays in labor and parturition (Patrignani et al., 2011).

Given the widespread clinical utilization of NSAIDs across virtually all medical specialties and the evolving understanding of their complex risk-benefit profiles, it has become essential to critically evaluate the pharmacological distinctions, therapeutic efficacy, and safety outcomes that differentiate traditional NSAIDs from selective COX-2 inhibitors. Healthcare providers must navigate an increasingly complex landscape of evidence when selecting appropriate NSAID therapy for individual patients, considering factors such as the indication for treatment, duration of therapy, concomitant medications, and patient-specific risk factors including age, renal function, cardiovascular disease history, and gastrointestinal risk profile.

This comprehensive review aims to synthesize current evidence from preclinical studies, randomized controlled trials, observational cohort studies, meta-analyses, and real-world clinical experience regarding the mechanisms of action, therapeutic applications, and adverse effect profiles of traditional NSAIDs and COX-2 inhibitors. Particular emphasis will be placed on comparing gastrointestinal and cardiovascular safety outcomes between these drug classes, as these represent the most clinically significant determinants of overall safety and tolerability (Steinmeyer, 2000). Through an integrated appraisal of experimental mechanistic data and clinical outcome evidence, this review seeks to provide a balanced perspective that can inform optimized, evidence-based, individualized NSAID prescribing practices in contemporary clinical medicine.

2. Mechanism of action

2.1 The cyclooxygenase pathway

The cyclooxygenase enzymes occupy a central position in the arachidonic acid cascade, serving as the rate-limiting step in the biosynthesis of prostanoids, which include prostaglandins and thromboxanes (Smith, DeWitt, & Garavito, 2000). When cellular membranes are disrupted by various stimuli—including mechanical injury, inflammatory mediators, growth factors, or immune responses—phospholipase A_2 is activated to cleave arachidonic acid from membrane phospholipids. This liberated arachidonic acid then serves as the substrate for COX enzymes, which catalyze two sequential reactions: a cyclooxygenase reaction that converts arachidonic acid to prostaglandin G_2 (PGG_2), followed by a peroxidase reaction that reduces PGG_2 to prostaglandin H_2 (PGH_2) (Vane et al., 1998). Prostaglandin H_2 serves as the common precursor for various tissue-specific prostanoids, including prostaglandin E_2 (PGE_2), prostaglandin D_2 (PGD_2), prostaglandin $F_2\alpha$ ($PGF_2\alpha$), prostacyclin (PGI_2), and thromboxane A_2 (TXA_2), each synthesized by distinct downstream enzymes and exerting diverse physiological and pathophysiological effects through specific prostanoid receptors.

2.2 Traditional NSAIDs: Non-selective COX inhibition

Traditional NSAIDs exert their pharmacological effects through competitive or time-dependent irreversible inhibition of both COX-1 and COX-2 isoenzymes, thereby reducing the synthesis of all downstream prostanoids in a relatively indiscriminate manner (Díaz-González & Sánchez-Madrid, 2015). The inhibition of COX-1 results in decreased production of prostaglandins that normally fulfill crucial protective and homeostatic roles throughout the body. In the gastrointestinal tract, COX-1-derived prostaglandins, particularly PGE_2 and PGI_2, maintain mucosal integrity through multiple mechanisms: stimulation of mucus and bicarbonate secretion, enhancement of mucosal blood flow, promotion of epithelial cell proliferation and migration during healing, and inhibition of gastric acid secretion (Wallace & Chin, 1997; Silen & Ito, 1985). Consequently, COX-1 inhibition compromises these protective mechanisms, rendering the gastric mucosa vulnerable to damage from acidic gastric contents and pepsin.

In the renal system, COX-1-derived prostaglandins help maintain renal blood flow, glomerular filtration rate, and sodium and water homeostasis, particularly under conditions of

volume depletion or decreased renal perfusion (Vane & Botting, 1998). Inhibition of renal COX-1 can therefore precipitate sodium retention, edema formation, hypertension, and in susceptible individuals, acute kidney injury or exacerbation of chronic kidney disease. Furthermore, in platelets where COX-1 is the predominant isoform, inhibition reduces thromboxane A_2 synthesis, resulting in decreased platelet aggregation and prolonged bleeding time—an effect that is therapeutically exploited with low-dose aspirin for cardiovascular prophylaxis but represents an adverse effect with other NSAIDs when bleeding complications occur.

The inhibition of COX-2 by traditional NSAIDs accounts for their primary therapeutic benefits (Sostres et al., 2010). At sites of inflammation, COX-2 expression is dramatically upregulated in response to pro-inflammatory cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and various growth factors. The resulting increased production of prostaglandins, particularly PGE_2, mediates multiple components of the inflammatory response including vasodilation, increased vascular permeability, sensitization of peripheral nociceptors to painful stimuli, and fever generation through effects on hypothalamic thermoregulatory centers. By inhibiting COX-2 and reducing prostaglandin synthesis at inflammatory sites, traditional NSAIDs effectively alleviate pain, reduce inflammation, and lower fever (Brune & Patrignani, 2015).

However, this non-selective inhibition creates an inherent therapeutic dilemma: achieving adequate COX-2 inhibition for anti-inflammatory efficacy necessitates simultaneous COX-1 blockade, with its attendant risks of gastrointestinal and renal toxicity. This pharmacological limitation has been estimated to cause tens of thousands of hospitalizations and thousands of deaths annually from NSAID-related gastrointestinal complications in the United States alone, predominantly affecting elderly patients and those with other risk factors (Sostres et al., 2010).

2.3 COX-2 inhibitors: Selective mechanism

Selective COX-2 inhibitors were rationally designed based on structural differences between the active sites of COX-1 and COX-2 isoforms (Smith et al., 2000). The COX-2 enzyme possesses a larger and more accessible active site compared to COX-1, featuring a side pocket that can accommodate bulkier substituents on inhibitor molecules. By incorporating specific structural features—such as sulfonamide or sulfone groups—into their molecular architecture, coxibs achieve preferential binding to and inhibition of COX-2 while exhibiting

substantially reduced affinity for COX-1 (Brune & Patrignani, 2015). The degree of selectivity varies among different coxibs, with some agents demonstrating several hundred-fold greater selectivity for COX-2 over COX-1 in biochemical assays.

This selective inhibition strategy was designed to maintain the therapeutic anti-inflammatory, analgesic, and antipyretic effects derived from COX-2 blockade while preserving COX-1-mediated physiological functions, particularly the synthesis of gastroprotective prostaglandins in the stomach and thromboxane A_2 in platelets (Geis, 1999). Multiple clinical trials have confirmed that COX-2 inhibitors provide anti-inflammatory and analgesic efficacy comparable to traditional NSAIDs for conditions such as rheumatoid arthritis, osteoarthritis, and acute pain, while demonstrating significantly reduced rates of endoscopically confirmed gastric and duodenal ulcers and clinically significant upper gastrointestinal complications including bleeding, perforation, and obstruction (Simon et al., 1999).

However, the preservation of COX-1-dependent thromboxane A_2 synthesis in platelets, combined with suppression of COX-2-dependent prostacyclin production in vascular endothelium, creates a potentially dangerous imbalance in the hemostatic equilibrium (Bacchi et al., 2012). Prostacyclin functions as a potent vasodilator, inhibitor of platelet aggregation, and suppressor of vascular smooth muscle proliferation, thereby exerting atheroprotective and antithrombotic effects. Thromboxane A_2, conversely, promotes platelet aggregation, vasoconstriction, and vascular smooth muscle proliferation. Under normal physiological conditions, these opposing forces maintain a delicate balance. Selective COX-2 inhibition tips this balance toward a prothrombotic state characterized by unopposed thromboxane activity, potentially increasing the risk of arterial thrombotic events including myocardial infarction, ischemic stroke, and peripheral arterial thrombosis (Patrignani et al., 2011).

Table 1: Comparative mechanisms of traditional NSAIDs and COX-2 inhibitors

Feature	Traditional NSAIDs	COX-2 inhibitors
COX-1 Inhibition	Yes (non-selective)	Minimal to none
COX-2 Inhibition	Yes (non-selective)	Yes (selective)
Gastric Prostaglandin Synthesis	Significantly reduced	Relatively preserved
Platelet Thromboxane A_2	Reduced (anti-platelet effect)	Preserved (normal platelet function)
Vascular Prostacyclin	Reduced	Significantly reduced
Anti-inflammatory Effect	Present	Present
Analgesic Effect	Present	Present
Antipyretic Effect	Present	Present

3. Therapeutic applications

Both traditional NSAIDs and COX-2 inhibitors share similar therapeutic indications, with the choice between them primarily guided by individual patient risk profiles rather than differences in efficacy. Common clinical applications include management of osteoarthritis and rheumatoid arthritis, where these agents help control pain and inflammation, though they do not modify disease progression (Steinmeyer, 2000). In acute musculoskeletal injuries and postoperative pain, both drug classes provide effective analgesia. For acute gouty arthritis and other acute inflammatory conditions, NSAIDs represent first-line pharmacological interventions. Additionally, certain NSAIDs, particularly indomethacin, have specific applications in conditions such as pericarditis and for inducing closure of patent ductus arteriosus in neonates (Brune & Patrignani, 2015).

The selection between traditional NSAIDs and COX-2 inhibitors increasingly depends on careful assessment of individual patient characteristics, including gastrointestinal risk factors (history of peptic ulcer disease, gastrointestinal bleeding, advanced age, concurrent anticoagulant or corticosteroid use), cardiovascular risk factors (history of cardiovascular disease, hypertension, hyperlipidemia, diabetes), renal function status, and concomitant medication profiles (Patrignani et al., 2011). For patients at high gastrointestinal risk but low

cardiovascular risk, COX-2 inhibitors may be preferred, potentially with additional gastroprotective strategies such as proton pump inhibitors. Conversely, for patients with elevated cardiovascular risk, traditional NSAIDs may be more appropriate, with consideration of concurrent low-dose aspirin for cardiovascular protection, though this combination may partially negate the gastrointestinal benefits and requires gastroprotective co-therapy.

4. Adverse effects profile

4.1 Gastrointestinal toxicity

Gastrointestinal complications represent the most common and clinically significant adverse effects associated with traditional NSAID use, affecting both the upper and lower gastrointestinal tract (Sostres et al., 2010). Upper gastrointestinal toxicity manifests across a spectrum of severity, ranging from mild dyspeptic symptoms such as epigastric pain, nausea, and heartburn—experienced by up to 60% of chronic NSAID users—to more serious complications including gastric and duodenal erosions, ulcers, bleeding, perforation, and gastric outlet obstruction. Epidemiological studies indicate that chronic NSAID users face a 3- to 5-fold increased risk of serious upper gastrointestinal complications compared to non-users, with risk factors including advanced age (particularly >65 years), history of peptic ulcer disease or gastrointestinal bleeding, concurrent use of corticosteroids or anticoagulants, Helicobacter pylori infection, high-dose or multiple NSAID use, and alcohol consumption (Schoen & Vender, 1989).

The pathophysiology of NSAID-induced gastropathy involves both topical irritant effects and systemic mechanisms related to prostaglandin depletion (Wallace & Chin, 1997). Topically, NSAIDs are weak acids that can directly damage the gastric epithelium upon contact, causing mucosal injury, disruption of the epithelial barrier, and back-diffusion of hydrogen ions. Systemically, COX-1 inhibition reduces synthesis of cytoprotective prostaglandins PGE_2 and PGI_2, leading to decreased mucus and bicarbonate secretion, reduced mucosal blood flow, impaired epithelial cell proliferation and repair, and increased susceptibility to acid-pepsin injury (Silen & Ito, 1985). The combination of reduced mucosal defense mechanisms and maintained aggressive factors creates an imbalance that facilitates ulcer formation and complications.

Lower gastrointestinal toxicity, increasingly recognized as a significant problem with NSAID use, includes small intestinal and colonic ulceration, bleeding, perforation, stricture formation, and protein-losing enteropathy (Sostres et al., 2010). Unlike upper gastrointestinal injury, lower gastrointestinal toxicity appears to occur with similar frequency with both traditional NSAIDs and COX-2 inhibitors, suggesting mechanisms beyond simple COX-1 inhibition, possibly involving enterohepatic recirculation of NSAIDs, bacterial translocation, and local topical effects.

COX-2 selective inhibitors have consistently demonstrated superior gastrointestinal safety compared to traditional NSAIDs in multiple large-scale clinical trials (Simon et al., 1999). The CLASS (Celecoxib Long-term Arthritis Safety Study) and other pivotal trials showed approximately 50-60% reductions in symptomatic ulcers and serious upper gastrointestinal complications with celecoxib compared to ibuprofen or diclofenac. However, this gastrointestinal benefit is substantially attenuated when low-dose aspirin is used concomitantly, as aspirin irreversibly acetylates and inhibits platelet COX-1, eliminating the selectivity advantage and increasing bleeding risk (Patrignani et al., 2011).

4.2 Cardiovascular toxicity

The cardiovascular safety of NSAIDs, both traditional and selective, has emerged as a major concern following the withdrawal of rofecoxib and subsequent meta-analyses revealing increased cardiovascular risk with various NSAIDs (Bacchi et al., 2012). The mechanisms underlying NSAID-associated cardiovascular toxicity are multifactorial and involve disruption of the prostacyclin-thromboxane balance, elevation of blood pressure, promotion of fluid retention, acceleration of atherosclerosis, and direct effects on myocardial function and remodeling.

Selective COX-2 inhibitors suppress endothelial prostacyclin synthesis while sparing platelet thromboxane production, creating a prothrombotic milieu that favors platelet aggregation, vasoconstriction, and thrombosis (Patrignani et al., 2011). This imbalance has been implicated in increased rates of myocardial infarction, ischemic stroke, and cardiovascular death observed with rofecoxib and, to a lesser degree, with other coxibs. Traditional NSAIDs also carry cardiovascular risks, though the profile may differ based on their relative COX-2 selectivity and effects on platelet function. Some traditional NSAIDs, particularly diclofenac, exhibit relatively high COX-2 selectivity and have been associated with cardiovascular risk comparable to coxibs (Bacchi et al., 2012).

Additionally, all NSAIDs can elevate blood pressure through multiple mechanisms including sodium and water retention secondary to reduced renal prostaglandin synthesis, increased peripheral vascular resistance, and interference with antihypertensive medication efficacy (Vane & Botting, 1998). This hypertensive effect, though typically modest (averaging 3-5 mmHg increase in mean arterial pressure), can be clinically significant in hypertensive patients and may contribute to cardiovascular event risk. Furthermore, NSAIDs can precipitate or exacerbate heart failure in susceptible individuals through fluid retention and direct effects on myocardial contractility.

Current evidence suggests that naproxen may have the most favorable cardiovascular profile among traditional NSAIDs, possibly due to its relatively prolonged antiplatelet effect resembling low-dose aspirin, though it still carries some cardiovascular risk (Patrignani et al., 2011). Celecoxib at approved doses (≤200 mg daily) appears to have cardiovascular risk similar to some traditional NSAIDs, whereas higher doses and other coxibs may confer greater risk.

Table 2: Comparative adverse effects of traditional NSAIDs and COX-2 inhibitors

Adverse effect category	**Traditional NSAIDs**	**COX-2 inhibitors**	**Primary mechanism**
Upper GI Ulcers/Bleeding	High risk	Reduced risk (50-60% lower)	COX-1 inhibition → reduced gastric prostaglandins
Lower GI Complications	Moderate risk	Similar risk	Topical effects, enterohepatic circulation
Cardiovascular Events	Moderate risk (variable by agent)	Moderate to high risk	PGI_2/TXA_2 imbalance, hypertension
Hypertension	Common	Common	Renal prostaglandin inhibition
Renal Dysfunction	Common	Similar risk	Reduced renal prostaglandins
Fluid	Common	Common	Sodium retention

Adverse effect category	Traditional NSAIDs	COX-2 inhibitors	Primary mechanism
Retention/Edema			
Hepatotoxicity	Uncommon	Uncommon	Idiosyncratic, metabolic stress
Platelet Dysfunction	Present (beneficial for thrombosis, risk for bleeding)	Minimal	Platelet COX-1 inhibition
Hypersensitivity	Uncommon	Uncommon	Leukotriene shunting, immune mechanisms

4.3 Renal toxicity

Both traditional NSAIDs and COX-2 inhibitors can adversely affect renal function through inhibition of prostaglandin synthesis, as both COX-1 and COX-2 contribute to renal prostanoid production (Vane & Botting, 1998). Under normal physiological conditions, renal prostaglandins, particularly PGE_2 and PGI_2, help maintain renal blood flow and glomerular filtration rate, promote natriuresis and diuresis, and modulate renin release. These effects become particularly important under conditions of reduced renal perfusion, such as volume depletion, heart failure, cirrhosis with ascites, or chronic kidney disease, where prostaglandins serve a compensatory vasodilatory role to preserve renal function.

NSAID-induced renal toxicity encompasses several distinct clinical syndromes (Bacchi et al., 2012). Acute kidney injury, the most common renal complication, typically manifests as prerenal azotemia due to renal vasoconstriction and reduced glomerular filtration. This is usually reversible upon NSAID discontinuation but can progress to acute tubular necrosis if prolonged. Sodium and water retention occurs frequently, leading to peripheral edema, weight gain, hypertension, and potential exacerbation of heart failure. Hyperkalemia may develop due to decreased renal potassium excretion, particularly in patients with underlying renal impairment or those taking concurrent medications affecting potassium homeostasis (angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, potassium-sparing diuretics).

Less commonly, NSAIDs can cause acute interstitial nephritis, an immunologically mediated hypersensitivity reaction characterized by fever, rash, eosinophilia, and acute kidney injury with pyuria and eosinophiluria. Chronic NSAID use has been associated with analgesic nephropathy, chronic interstitial nephritis, and papillary necrosis, though these complications are now less frequent with modern NSAIDs than with historical analgesic combinations containing phenacetin (Vane & Botting, 1998).

COX-2 inhibitors do not appear to offer significant renal safety advantages over traditional NSAIDs, as COX-2 plays important roles in renal prostaglandin synthesis, particularly in the macula densa, medullary interstitial cells, and podocytes (Brune & Patrignani, 2015). Patients at highest risk for NSAID-induced renal toxicity include the elderly, those with pre-existing chronic kidney disease, volume-depleted states, heart failure, cirrhosis, concurrent use of diuretics, ACE inhibitors, or angiotensin receptor blockers, and those receiving contrast agents.

4.4 Hepatotoxicity

Hepatotoxicity associated with NSAID use ranges from asymptomatic transient elevations in serum aminotransferases, occurring in up to 15% of chronic users, to rare but serious hepatocellular injury, cholestatic hepatitis, mixed hepatocellular-cholestatic patterns, and fulminant hepatic failure (Elsevier, 2006). The mechanism of NSAID-induced liver injury is not fully understood but likely involves both direct toxic effects related to reactive metabolite formation and idiosyncratic hypersensitivity reactions with immune-mediated hepatocyte damage.

Most cases of NSAID hepatotoxicity are mild and reversible upon drug discontinuation, with liver enzyme elevations typically returning to normal within several weeks (Patrignani et al., 2011). However, severe hepatotoxicity, though rare (estimated incidence 1-10 per 100,000 users), can progress rapidly to acute liver failure requiring transplantation or resulting in death. Diclofenac has been particularly associated with hepatotoxicity, prompting regulatory warnings and recommendations for periodic liver function monitoring in some jurisdictions. Risk factors for NSAID-induced hepatotoxicity include advanced age, female gender, high doses, prolonged duration of therapy, concurrent hepatotoxic medications, and underlying liver disease.

4.5 Hypersensitivity reactions

Hypersensitivity reactions to NSAIDs encompass a diverse spectrum of clinical manifestations ranging from mild cutaneous reactions to severe systemic responses (Patrignani et al., 2011). These reactions can be classified into immunological (drug-specific, mediated by IgE antibodies or T cells) and non-immunological (cross-reactive, related to COX inhibition) mechanisms. Respiratory hypersensitivity, particularly bronchospasm and exacerbation of asthma, occurs in approximately 10-20% of adult asthmatics who use NSAIDs, a condition termed aspirin-exacerbated respiratory disease (AERD) or Samter's triad when associated with chronic rhinosinusitis and nasal polyposis.

The mechanism of NSAID-induced bronchospasm involves shunting of arachidonic acid metabolism toward the lipoxygenase pathway when COX enzymes are inhibited, resulting in increased production of cysteinyl leukotrienes (LTC_4, LTD_4, LTE_4), which are potent bronchoconstrictors and pro-inflammatory mediators (Sostres et al., 2010). This represents a pharmacological cross-reactive response, meaning patients sensitive to one NSAID will typically react to other NSAIDs but may tolerate COX-2 selective inhibitors, which appear to cause less leukotriene shunting.

Cutaneous hypersensitivity reactions include urticaria, angioedema, maculopapular rashes, fixed drug eruptions, and rarely, severe cutaneous adverse reactions such as Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug reaction with eosinophilia and systemic symptoms (DRESS syndrome). Anaphylaxis to NSAIDs, though uncommon, can occur through IgE-mediated mechanisms, particularly with selective sensitivity to specific NSAIDs while other NSAIDs are tolerated.

4.6 Central nervous system effects

Central nervous system adverse effects associated with NSAID use include headache, dizziness, drowsiness, confusion, cognitive impairment, tinnitus, and rarely, aseptic meningitis (Steinmeyer, 2000). These effects are generally more common with indomethacin, which has greater central nervous system penetration, but can occur with other NSAIDs, particularly in elderly patients or those with predisposing factors. The mechanisms underlying CNS effects involve inhibition of central prostaglandin synthesis, which modulates neurotransmission, cerebral blood flow, and various neuronal functions (Brune &

Patrignani, 2015). Most CNS adverse effects are dose-related and reversible with dose reduction or drug discontinuation.

4.7 Effects in pregnancy

NSAID use during pregnancy poses significant risks to both the mother and fetus, with effects varying depending on the timing of exposure during gestation (Patrignani et al., 2011). During the first trimester, NSAID use has been associated with increased risk of spontaneous abortion in some studies, though the evidence remains controversial and confounded by the underlying conditions for which NSAIDs are prescribed. In the second trimester, concerns include potential teratogenic effects, though most evidence suggests that NSAIDs are not major human teratogens at therapeutic doses.

The most serious risks occur with NSAID exposure during the third trimester, particularly after 30 weeks of gestation (Patrignani et al., 2011). Prostaglandins play critical roles in maintaining patency of the fetal ductus arteriosus, a vascular shunt that directs blood flow away from the non-functioning fetal lungs. Inhibition of prostaglandin synthesis by NSAIDs can cause premature constriction or closure of the ductus arteriosus in utero, leading to pulmonary hypertension, right ventricular dysfunction, and tricuspid regurgitation. This complication can occur within days of NSAID exposure and may result in persistent pulmonary hypertension of the newborn (PPHN), a life-threatening condition requiring intensive neonatal care.

Additionally, NSAIDs can reduce fetal renal function by inhibiting prostaglandin-mediated renal blood flow, potentially causing oligohydramnios (reduced amniotic fluid) due to decreased fetal urine production. Oligohydramnios can lead to pulmonary hypoplasia, skeletal deformities, and fetal growth restriction. Furthermore, NSAID use near term may inhibit uterine contractions and prolong labor by suppressing prostaglandin-mediated myometrial contractility, potentially increasing the risk of postpartum hemorrhage due to uterine atony. Based on these risks, regulatory agencies have issued warnings against NSAID use after 20 weeks of gestation, and most guidelines recommend avoiding NSAIDs throughout pregnancy unless the benefits clearly outweigh the risks and no safer alternatives are available.

4.8 Hematologic effects

The hematologic effects of NSAIDs primarily involve alterations in platelet function and coagulation (Vane & Botting, 1998). Traditional NSAIDs inhibit platelet COX-1, thereby reducing thromboxane A_2 synthesis and impairing platelet aggregation. This results in prolonged bleeding time, which typically returns to normal within several days after drug discontinuation for most NSAIDs as new platelets are generated. Aspirin is unique among NSAIDs in causing irreversible platelet inhibition through covalent acetylation of COX-1, requiring 7-10 days for complete recovery of normal platelet function as the entire platelet pool is replaced.

While the antiplatelet effect of aspirin is therapeutically beneficial for cardiovascular prophylaxis, the antiplatelet effects of other traditional NSAIDs represent an adverse effect that increases bleeding risk, particularly in patients with underlying coagulation disorders, thrombocytopenia, concurrent anticoagulant therapy, or those undergoing surgical procedures (Patrignani et al., 2011). Preoperative discontinuation of NSAIDs is generally recommended to minimize perioperative bleeding complications. In contrast, COX-2 selective inhibitors do not significantly affect platelet function or bleeding time, as platelet COX-1 remains uninhibited, which may represent an advantage in patients requiring antiinflammatory or analgesic therapy who have bleeding risks or are undergoing surgery.

Rarely, NSAIDs have been associated with other hematologic abnormalities including thrombocytopenia, neutropenia, agranulocytosis, and aplastic anemia, though these complications are extremely uncommon and typically idiosyncratic in nature (Bacchi et al., 2012). The mechanisms underlying these rare hematologic toxicities likely involve immune-mediated destruction of blood cells or direct toxic effects on bone marrow progenitor cells.

Table 3: Organ system-specific adverse effects and risk factors

Organ System	Major Adverse Effects	High-Risk Patient Populations	Risk Reduction Strategies
Gastrointestinal	Ulcers, bleeding, perforation	Age >65 years, prior GI event, H. pylori infection, anticoagulants, corticosteroids	COX-2 inhibitors, PPI co-therapy, H. pylori eradication, lowest effective dose

Organ System	Major Adverse Effects	High-Risk Patient Populations	Risk Reduction Strategies
Cardiovascular	MI, stroke, hypertension, heart failure	Prior CV disease, hypertension, diabetes, hyperlipidemia	Use naproxen or lowest CV-risk NSAID, control BP, limit duration, avoid in post-MI period
Renal	AKI, fluid retention, hyperkalemia	CKD, elderly, volume depletion, heart failure, cirrhosis, diuretics, ACE-I/ARB use	Monitor renal function, ensure adequate hydration, avoid in severe CKD, limit duration
Hepatic	Transaminase elevation, hepatitis	Elderly, high doses, prolonged use, pre-existing liver disease	Monitor LFTs periodically, avoid in active liver disease, use lowest effective dose
Hematologic	Bleeding, platelet dysfunction	Anticoagulant use, coagulopathies, thrombocytopenia, perioperative period	Consider COX-2 inhibitors, discontinue before surgery, avoid with anticoagulants
Respiratory	Bronchospasm, asthma exacerbation	Aspirin-sensitive asthma, nasal polyps, chronic rhinosinusitis	Consider COX-2 inhibitors (may be tolerated), use alternative analgesics
Pregnancy	Ductus arteriosus closure, oligohydramnios, labor prolongation	All pregnant women, especially third trimester	Avoid after 20 weeks gestation, use alternative analgesics (acetaminophen)

5. Comparative clinical efficacy

Numerous randomized controlled trials and systematic reviews have evaluated the comparative efficacy of traditional NSAIDs and COX-2 inhibitors across various clinical conditions, consistently demonstrating therapeutic equivalence for most indications (Simon et al., 1999; Geis, 1999). In the management of osteoarthritis, both drug classes provide comparable reductions in joint pain, improvement in physical function, and overall symptom

control. Large-scale clinical trials such as the CLASS trial comparing celecoxib to diclofenac and ibuprofen, and the TARGET trial comparing lumiracoxib to naproxen and ibuprofen, found no significant differences in efficacy outcomes for arthritis symptom relief.

Similarly, in rheumatoid arthritis, COX-2 inhibitors have demonstrated efficacy equivalent to traditional NSAIDs in reducing joint pain, morning stiffness, and inflammatory markers, though neither class modifies the underlying disease progression or prevents joint destruction, necessitating concurrent disease-modifying antirheumatic drugs (DMARDs) for comprehensive disease management (Brune & Patrignani, 2015). For acute pain conditions including postoperative pain, dental pain, musculoskeletal injuries, and dysmenorrhea, both traditional NSAIDs and COX-2 inhibitors provide effective analgesia with comparable onset and duration of action (Steinmeyer, 2000).

The primary clinical distinction between traditional NSAIDs and COX-2 inhibitors lies not in efficacy but in their differing adverse effect profiles, particularly regarding gastrointestinal and cardiovascular safety (Patrignani et al., 2011). This recognition has led to a paradigm shift in NSAID selection, with the choice increasingly guided by individualized risk assessment rather than efficacy considerations. Clinical practice guidelines now emphasize the importance of weighing gastrointestinal risk factors (history of peptic ulcer or GI bleeding, advanced age, concurrent anticoagulants or corticosteroids, H. pylori infection) against cardiovascular risk factors (established cardiovascular disease, multiple cardiovascular risk factors, history of thrombotic events) when selecting between traditional NSAIDs and COX-2 inhibitors.

For patients at high gastrointestinal risk but low cardiovascular risk, COX-2 inhibitors represent a rational choice, potentially combined with proton pump inhibitor (PPI) co-therapy for additional gastroprotection in very high-risk patients (Sostres et al., 2010). Conversely, for patients at high cardiovascular risk, traditional NSAIDs such as naproxen may be preferred due to their more favorable cardiovascular profile, though gastroprotective strategies with PPIs should be strongly considered. In patients with both high gastrointestinal and high cardiovascular risk, the use of any NSAID becomes problematic, and alternative analgesic strategies including acetaminophen, topical NSAIDs, or adjunctive therapies should be explored.

6. Risk stratification and patient selection

Optimal NSAID prescribing requires systematic assessment of individual patient risk factors to inform personalized treatment decisions (Patrignani et al., 2011). Several validated risk stratification tools and algorithms have been developed to assist clinicians in this process. The gastrointestinal risk assessment should evaluate established risk factors including age greater than 65 years (particularly >75 years), prior history of uncomplicated peptic ulcer disease or previous gastrointestinal bleeding, concurrent use of oral corticosteroids or anticoagulants, high-dose or multiple NSAID use, serious comorbid medical conditions, and Helicobacter pylori infection status.

Patients can be categorized into low, moderate, or high gastrointestinal risk groups based on these factors (Sostres et al., 2010). Low-risk patients (no risk factors) may use traditional NSAIDs without additional gastroprotective measures, though the lowest effective dose for the shortest necessary duration should always be employed. Moderate-risk patients (one to two risk factors) warrant consideration of COX-2 inhibitors or traditional NSAIDs with PPI co-therapy. High-risk patients (multiple risk factors or history of complicated ulcer) should receive COX-2 inhibitors with mandatory PPI co-therapy, and alternative analgesic strategies should be strongly considered.

Cardiovascular risk stratification should assess established cardiovascular disease (prior myocardial infarction, stroke, peripheral arterial disease, coronary revascularization), cardiovascular risk factors (hypertension, diabetes mellitus, hyperlipidemia, smoking, family history), and the Framingham or other validated cardiovascular risk scores (Bacchi et al., 2012). Patients with established cardiovascular disease or multiple risk factors should preferentially receive naproxen if NSAID therapy is necessary, as it appears to have the most favorable cardiovascular profile among available NSAIDs, though it still carries some risk. COX-2 inhibitors and diclofenac should be avoided in high cardiovascular risk patients when possible. All NSAID use in the immediate post-myocardial infarction period (within 1-2 weeks) should be avoided due to particularly elevated risk during this vulnerable period.

Renal risk assessment should evaluate baseline kidney function, age, volume status, concomitant medications affecting renal hemodynamics (diuretics, ACE inhibitors, angiotensin receptor blockers), and conditions predisposing to decreased renal perfusion (heart failure, cirrhosis) (Vane & Botting, 1998). In patients with chronic kidney disease stage 3 or higher (estimated glomerular filtration rate <60 mL/min/1.73m²), NSAIDs should

be used with extreme caution if at all, with close monitoring of renal function and electrolytes. Alternative analgesic approaches should be strongly considered in this population.

Table 4: Risk-based NSAID selection algorithm

Risk category	GI risk	CV risk	Recommended approach
Low-Low	Low	Low	Traditional NSAID at lowest effective dose for shortest duration
High-Low	High	Low	COX-2 inhibitor + PPI or Traditional NSAID + PPI
Low-High	Low	High	Naproxen (lowest effective dose) + PPI for gastroprotection
High-High	High	High	Avoid NSAIDs if possible; If necessary: Naproxen + PPI with extreme caution; Consider alternative analgesics (acetaminophen, topical NSAIDs, adjunctive therapies)
Renal Dysfunction	Any	Any	Avoid NSAIDs; Use alternative analgesics; If NSAIDs necessary, monitor closely

7. Drug interactions and special considerations

NSAIDs, both traditional and selective, participate in numerous clinically significant drug interactions that can alter efficacy, increase toxicity, or affect the pharmacokinetics of concurrent medications (Brune & Patrignani, 2015). Pharmacodynamic interactions include the synergistic gastrointestinal toxicity observed when NSAIDs are combined with corticosteroids, increasing ulcer and bleeding risk approximately 4-fold compared to NSAIDs alone. Concurrent use of anticoagulants (warfarin, direct oral anticoagulants) or antiplatelet agents (clopidogrel, ticagrelor) with NSAIDs substantially elevates bleeding risk through additive effects on hemostasis and potential direct mucosal injury.

The combination of NSAIDs with selective serotonin reuptake inhibitors (SSRIs) or serotonin-norepinephrine reuptake inhibitors (SNRIs) increases upper gastrointestinal bleeding risk approximately 3- to 4-fold, likely due to impaired platelet function caused by reduced platelet serotonin uptake (Patrignani et al., 2011). NSAIDs can reduce the efficacy of several antihypertensive medications including ACE inhibitors, angiotensin receptor

blockers, beta-blockers, and diuretics, potentially requiring dose adjustments to maintain blood pressure control. The combination of NSAIDs with ACE inhibitors or angiotensin receptor blockers in the presence of diuretics creates a "triple whammy" effect that dramatically increases acute kidney injury risk, particularly in volume-depleted or elderly patients.

NSAIDs can increase serum lithium levels by reducing renal lithium clearance, potentially precipitating lithium toxicity (Bacchi et al., 2012). They may also increase methotrexate concentrations by reducing renal clearance, particularly at high methotrexate doses, raising concerns for methotrexate toxicity. Concurrent use of aspirin with other NSAIDs can reduce the cardioprotective effects of aspirin through competitive inhibition at the platelet COX-1 active site, an interaction that varies depending on the specific NSAID and timing of administration. Ibuprofen, when taken before aspirin, can block aspirin's antiplatelet effect, whereas naproxen and COX-2 inhibitors do not appear to significantly interfere with aspirin's cardioprotective activity.

Special populations requiring particular consideration for NSAID use include the elderly, in whom pharmacokinetic changes (reduced renal and hepatic clearance), pharmacodynamic alterations (increased sensitivity to adverse effects), and increased prevalence of comorbidities and polypharmacy substantially elevate risk for both gastrointestinal and cardiovascular complications (Sostres et al., 2010). Pediatric NSAID use requires attention to appropriate weight-based dosing, avoidance during viral illnesses (particularly varicella and influenza) due to potential Reye syndrome risk with aspirin, and recognition that children generally tolerate NSAIDs well with lower rates of gastrointestinal toxicity compared to adults.

Patients with renal impairment require dose adjustments, extended dosing intervals, or avoidance of NSAIDs depending on the severity of kidney dysfunction (Vane & Botting, 1998). Those with hepatic impairment face increased risk of hepatotoxicity and may experience altered drug metabolism, necessitating cautious use with appropriate monitoring. Patients with inflammatory bowel disease (Crohn's disease, ulcerative colitis) may experience disease exacerbations with NSAID use, though the evidence is conflicting and some patients tolerate NSAIDs without problems.

8. Alternative and adjunctive strategies

Given the significant limitations and risks associated with both traditional NSAIDs and COX-2 inhibitors, exploration of alternative and adjunctive analgesic and antiinflammatory strategies represents an important component of comprehensive pain management (Steinmeyer, 2000). Acetaminophen (paracetamol) provides effective analgesia and antipyresis without the gastrointestinal or cardiovascular risks associated with NSAIDs, making it a valuable first-line agent for mild to moderate pain, particularly in patients with contraindications to NSAID use. However, acetaminophen lacks significant antiinflammatory activity and carries hepatotoxicity risk at excessive doses (>4 grams daily) or in patients with underlying liver disease or chronic alcohol use.

Topical NSAIDs formulated as gels, creams, or patches deliver medication directly to affected peripheral sites while minimizing systemic exposure and reducing the risk of systemic adverse effects including gastrointestinal, cardiovascular, and renal toxicity (Brune & Patrignani, 2015). Clinical trials have demonstrated efficacy comparable to oral NSAIDs for localized conditions such as osteoarthritis of the knee or hand, soft tissue injuries, and tendinitis, with substantially lower plasma concentrations and reduced systemic adverse event rates. Topical NSAIDs represent an particularly attractive option for elderly patients or those at high risk for systemic NSAID complications.

Opioid analgesics provide potent pain relief for moderate to severe pain but carry their own significant risks including respiratory depression, sedation, constipation, nausea, cognitive impairment, tolerance, physical dependence, and addiction potential (Patrignani et al., 2011). The current opioid epidemic has highlighted the dangers of liberal opioid prescribing, leading to more restrictive guidelines and emphasis on multimodal analgesia incorporating non-opioid approaches. Opioids should generally be reserved for severe acute pain, cancer pain, or palliative care situations, with careful patient selection, informed consent discussion of risks, and close monitoring.

Adjunctive medications including antidepressants (tricyclics, SNRIs, duloxetine) and anticonvulsants (gabapentin, pregabalin) play important roles in managing neuropathic pain and chronic pain conditions, often allowing reduction in NSAID or opioid requirements through multimodal analgesia approaches (Steinmeyer, 2000). Intra-articular corticosteroid injections provide temporary relief for inflammatory arthritis and can bridge patients through

acute flares while minimizing systemic medication exposure. Hyaluronic acid injections for knee osteoarthritis remain controversial but may offer benefit in selected patients.

Non-pharmacological interventions including physical therapy, exercise, weight loss, heat or cold application, transcutaneous electrical nerve stimulation (TENS), acupuncture, cognitive-behavioral therapy, and mindfulness-based stress reduction represent important components of comprehensive pain management that can reduce reliance on NSAIDs and other analgesics (Brune & Patrignani, 2015). Patient education regarding the risks and benefits of different treatment options, realistic expectations about pain relief, and active patient participation in treatment decisions contribute to improved outcomes and satisfaction.

9. Future directions and novel approaches

Ongoing research continues to explore strategies for developing safer anti-inflammatory and analgesic agents that preserve therapeutic efficacy while minimizing adverse effects (Steinmeyer, 2000). Novel COX-2 inhibitors with improved selectivity profiles and additional mechanisms of action are under investigation. Dual inhibitors targeting both COX and lipoxygenase pathways aim to provide broader anti-inflammatory effects while potentially reducing leukotriene-mediated toxicity. Nitric oxide-donating NSAIDs (NO-NSAIDs) and hydrogen sulfide-releasing NSAIDs (H_2S-NSAIDs) incorporate gasotransmitter-releasing moieties designed to enhance gastroprotection, improve vascular function, and reduce cardiovascular risk while maintaining anti-inflammatory efficacy (Patrignani et al., 2011).

Selective inhibitors of downstream prostaglandin synthases or specific prostanoid receptors represent another promising approach, potentially allowing more targeted modulation of specific prostanoid pathways while avoiding the broad effects of COX inhibition (Brune & Patrignani, 2015). For example, selective microsomal prostaglandin E synthase-1 (mPGES-1) inhibitors would selectively reduce PGE_2 production without affecting other prostanoids, theoretically preserving cardiovascular and gastrointestinal protective mechanisms. Similarly, selective antagonists of specific prostanoid receptors (EP receptors for PGE_2, TP receptors for thromboxane) could provide targeted effects without global prostaglandin suppression.

Personalized medicine approaches incorporating pharmacogenomic data to identify patients at higher risk for adverse effects or those likely to experience superior efficacy with specific agents represent an exciting frontier (Bacchi et al., 2012). Genetic polymorphisms affecting

drug metabolism (CYP2C9 variants), prostaglandin synthesis and signaling, and inflammatory mediator pathways may help guide individualized NSAID selection and dosing. Integration of clinical risk factors, biomarkers, imaging data, and genomic information into predictive algorithms could enable truly personalized anti-inflammatory therapy.

Development of improved drug delivery systems including nanoparticle formulations, sustained-release preparations, and targeted delivery approaches aim to maximize therapeutic concentrations at sites of inflammation while minimizing systemic exposure and toxicity (Steinmeyer, 2000). Advances in understanding the molecular mechanisms of pain, inflammation, and tissue repair continue to identify novel therapeutic targets beyond the COX pathway, potentially leading to entirely new classes of anti-inflammatory and analgesic agents with superior safety profiles.

10. Conclusion

Non-steroidal anti-inflammatory drugs remain essential therapeutic agents for managing pain, inflammation, and fever across diverse clinical contexts. Traditional NSAIDs provide effective symptom relief through non-selective inhibition of both COX-1 and COX-2 enzymes but frequently cause gastrointestinal and renal complications due to disruption of protective prostaglandin synthesis. Selective COX-2 inhibitors were developed to preserve anti-inflammatory efficacy while reducing COX-1-related toxicity, and they have successfully demonstrated superior gastrointestinal safety in clinical trials. However, the emergence of cardiovascular safety concerns associated with COX-2 selectivity has complicated the risk-benefit calculus and necessitated careful patient selection based on individualized risk assessment. Both drug classes share similar therapeutic efficacy for most indications, with the primary clinical distinction lying in their differing adverse effect profiles rather than effectiveness. Optimal NSAID use requires systematic evaluation of gastrointestinal, cardiovascular, and renal risk factors, selection of the most appropriate agent and dose for each individual patient, implementation of risk reduction strategies including gastroprotective co-therapy when indicated, use of the lowest effective dose for the shortest necessary duration, and consideration of alternative analgesic approaches in high-risk patients. Continued research focused on developing novel agents with improved selectivity, alternative mechanisms of action, enhanced drug delivery systems, and personalized medicine approaches hold promise for advancing the field beyond current limitations and

providing safer, more effective options for patients requiring anti-inflammatory and analgesic therapy.

Conflict of Interest

The authors declare that they have no known competing financial interests in the work reported in this paper.

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Targeting the *Purinome*: An Integrated In-Silico and In Vitro Strategy for Discovering Novel Adenosine Deaminase Inhibitors from Marine-Derived Fungi

Archita Dhara[1], Arpita Das[1], Samudra Pal[1]

[1]*Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, 700121, West Bengal, India*

**Corresponding author: iamsamudra94@gmail.com*

Abstract

Adenosine deaminase (ADA), a pivotal enzyme in purine metabolism, plays a critical role in the development and function of the lymphoid system. Its dysregulation is directly linked to immunodeficiency, while its inhibition constitutes a key therapeutic strategy for certain hematological malignancies and autoimmune diseases. Current clinically used ADA inhibitors, such as pentostatin, are potent but are associated with significant dose-limiting toxicities, creating a pressing need for novel inhibitors with improved therapeutic profiles. This review posits that marine-derived fungi, an underexplored source of immense chemical diversity, represent a promising frontier for the discovery of next-generation ADA inhibitors. We advocate for an integrated discovery pipeline that synergistically combines computational (*in silico*) and experimental (*in vitro*) methodologies to accelerate this process. We first provide a comprehensive overview of ADA's biochemical function, its role in the "purinome," and its validation as a therapeutic target in oncology and immunology. The review then systematically details a modern, integrated workflow: beginning with the *in silico* virtual screening of marine fungal natural product libraries against the crystal structure of human ADA to identify high-priority candidates, followed by the parallel *in vitro* screening of marine fungal extracts for ADA inhibitory activity. We discuss how bioassay-guided fractionation, informed by computational predictions, can rapidly lead to the isolation of novel active compounds. By synthesizing the principles of this convergent approach and evaluating the current technological landscape, we highlight a rational and efficient path from marine biodiversity to potent, selective, and potentially less toxic clinical candidates, capable of modulating the purinome for therapeutic benefit.

Keywords: Adenosine Deaminase (ADA) Inhibitors, Marine Fungi, Drug Discovery, In Silico, Virtual Screening, Molecular Docking, Natural Products, Purinome.

1. Introduction

The purine metabolic network, or "*purinome*," comprises a complex and exquisitely regulated web of enzymes and transporters responsible for the synthesis, interconversion, and degradation of purine nucleotides. These molecules are not only the fundamental building blocks of DNA and RNA but also serve as the universal currency of cellular energy (ATP, GTP) and as critical signaling molecules (adenosine, cyclic AMP). Given their central role in cellular proliferation, bioenergetics, and signaling, the enzymes of the purinome have emerged as highly attractive targets for therapeutic intervention, particularly in the fields of oncology and immunology (Yin et al., 2018).

Among the key enzymes in this network is Adenosine Deaminase (ADA; E.C. 3.5.4.4). ADA catalyzes the irreversible hydrolytic deamination of adenosine and 2'-deoxyadenosine to inosine and 2'-deoxyinosine, respectively. This function is a critical step in the purine salvage pathway, preventing the accumulation of adenosine and its metabolites (Cristalli et al., 2001). The profound physiological importance of ADA is most dramatically illustrated by the consequences of its genetic deficiency. The absence of functional ADA leads to the systemic accumulation of deoxyadenosine, which is subsequently phosphorylated to dATP. Lymphocytes are uniquely sensitive to high levels of dATP, which inhibits the enzyme ribonucleotide reductase, thereby starving the cells of the balanced pool of deoxynucleotides required for DNA synthesis and repair. This selective lymphotoxicity leads to apoptosis and results in Severe Combined Immunodeficiency (SCID), a devastating condition characterized by a near-complete loss of functional B and T lymphocytes (Hershfield, 2017).

The targeted lymphotoxicity caused by ADA deficiency provides a powerful therapeutic rationale for its inhibition. By pharmacologically blocking ADA, one can mimic this effect to selectively eliminate malignant lymphoid cells. This strategy has proven successful in the treatment of certain hematological cancers, particularly Hairy Cell Leukemia and Acute Lymphoblastic Leukemia (ALL), with the potent transition-state inhibitor pentostatin (2'-deoxycoformycin) (Grever et al., 2011). However, the clinical use of pentostatin is hampered

by significant toxicities, including profound myelosuppression, neurotoxicity, and immunosuppression, which limit its therapeutic window.

This clinical need drives the search for novel ADA inhibitors with improved efficacy, selectivity, and safety profiles. Natural products have historically been a rich source of enzyme inhibitors, but conventional terrestrial sources are yielding diminishing returns. This review champions a two-pronged strategy to accelerate the discovery of next-generation ADA inhibitors. The first prong is to explore a new and chemically diverse biological resource: marine-derived fungi. The unique environmental pressures of the marine biome have fostered the evolution of novel secondary metabolic pathways, offering the potential for new chemical scaffolds. The second prong is to employ a modern, integrated discovery workflow that synergistically combines the predictive power of computational (*in silico*) modeling with the empirical validation of experimental (*in vitro*) screening. By using virtual screening to mine the chemical space of marine fungi and guiding experimental efforts, we can dramatically accelerate the path from marine biodiversity to novel clinical candidates targeting the purinome.

2. Literature Search Methodology

This review was synthesized from a comprehensive search of the scientific literature focusing on adenosine deaminase (ADA) as a therapeutic target and the integrated discovery of its inhibitors, with a specific emphasis on marine natural products. The literature search was conducted across the major scientific databases PubMed, Scopus, Web of Science, and Google Scholar, and included publications up to December 2025.

The search strategy was designed to be multi-faceted. Primary keywords included "Adenosine Deaminase," "ADA inhibitors," "purine metabolism," "drug discovery," "Severe Combined Immunodeficiency," and "Acute Lymphoblastic Leukemia." These terms were combined with keywords related to the source of compounds, such as "natural products," "marine fungi," "marine natural products," "*Aspergillus*," and "*Penicillium*." A third layer of search terms focused on the discovery methodology, including "in silico," "virtual screening," "molecular docking," "structure-based drug design," "in vitro screening," and "bioassay-guided fractionation." An example of a combined query is ("Adenosine Deaminase" OR "ADA inhibitor") AND ("marine fungi" OR "natural products") AND ("virtual screening" OR "molecular docking").

Inclusion criteria for selected articles were: (1) original research papers, detailed reviews, and methodological articles published in English; (2) studies detailing the biochemical function of ADA and its role in disease; (3) research on the discovery, characterization, and clinical application of ADA inhibitors; (4) studies describing the use of integrated *in silico* and *in vitro* drug discovery workflows; and (5) publications on the chemical diversity of marine fungal metabolites. Priority was given to literature from the last decade to focus on recent advancements in drug discovery technologies, while seminal papers on ADA deficiency and the development of pentostatin were included for their foundational importance.

Exclusion criteria included: (1) studies on purine metabolism not directly related to ADA; (2) research on immunodeficiencies or cancers not linked to ADA; (3) articles in languages other than English; and (4) conference abstracts or patents lacking sufficient scientific detail.

The initial search yielded over 1,600 articles. A screening of titles and abstracts narrowed this pool to approximately 350 articles for full-text review. From this group, around 115 publications that were most relevant to the central theme of discovering novel ADA inhibitors from marine sources using an integrated approach were selected for detailed analysis and synthesis in this review.

3. Adenosine Deaminase: A Critical Therapeutic Target

3.1. Biochemical Function and Physiological Role

Adenosine deaminase is a cytosolic enzyme that is ubiquitously expressed in mammalian tissues, with the highest levels found in lymphoid tissues such as the thymus, spleen, and lymph nodes. Its primary function is to maintain the intracellular and extracellular concentrations of adenosine and deoxyadenosine within a narrow physiological range. By converting these substrates to inosine and deoxyinosine, ADA directs them towards the purine degradation pathway, ultimately leading to the formation of uric acid (Benveniste & Cohen, 1989).

The critical importance of this function is most evident in lymphocytes. These cells have high levels of the kinases that phosphorylate deoxyadenosine to its triphosphate form, dATP. In the absence of ADA, dATP accumulates to toxic levels. dATP is a potent allosteric inhibitor of ribonucleotide reductase, the enzyme responsible for converting all four ribonucleotides into their deoxyribonucleotide counterparts (dATP, dCTP, dGTP, dTTP) (Wilson et al., 1979). The inhibition of this enzyme starves the cell of the balanced pool of deoxynucleotides

required for DNA replication and repair, triggering an apoptotic cascade that leads to the death of the lymphocyte. This exquisite sensitivity of lymphocytes to ADA deficiency underpins its entire therapeutic rationale.

3.2. ADA in Disease

Immunology (SCID): Autosomal recessive ADA deficiency accounts for approximately 15% of all cases of Severe Combined Immunodeficiency. Infants born with this condition lack functional T- and B-lymphocytes, leaving them profoundly susceptible to opportunistic infections. This "bubble boy" disease was the first genetic disorder for which enzyme replacement therapy (with bovine ADA) was developed and was also the target of the first human gene therapy trials, highlighting its historical and clinical significance (Hershfield, 2017).

Oncology: The selective lymphotoxicity of dATP accumulation is exploited in cancer therapy. Certain hematological malignancies, particularly those of lymphoid origin like Acute Lymphoblastic Leukemia (ALL), T-cell lymphomas, and Hairy Cell Leukemia, exhibit high levels of ADA activity. Inhibition of ADA in these cancer cells leads to the same toxic accumulation of dATP and subsequent apoptosis that occurs in congenital ADA deficiency. This makes ADA an ideal and validated target for anti-leukemic drugs (Smyth et al., 1978).

Inflammation and Autoimmunity: Extracellular adenosine, whose levels are controlled by ADA and other ectoenzymes, is a potent signaling molecule that generally has anti-inflammatory and immunosuppressive effects through its interaction with adenosine receptors (A1, A2A, A2B, A3) on immune cells (Antonioli et al., 2013). By inhibiting ADA, one can increase the local concentration of adenosine, which can suppress inflammatory responses. This provides a rationale for developing ADA inhibitors for the treatment of autoimmune and chronic inflammatory diseases like rheumatoid arthritis and inflammatory bowel disease.

4. Existing ADA Inhibitors and Their Limitations

The most successful ADA inhibitors are transition-state analogues. These molecules are designed to mimic the tetrahedral transition state of the substrate as it is being attacked by the enzyme, causing them to bind to the active site with extremely high affinity (typically in the picomolar range).

The first and most important clinical ADA inhibitor is **pentostatin (2'-deoxycoformycin)**. Originally isolated from the bacterium *Streptomyces antibioticus*, pentostatin is a powerful, quasi-irreversible inhibitor of ADA (Agarwal et al., 1977). It received FDA approval in 1991 for the treatment of Hairy Cell Leukemia refractory to standard therapy, where it produces high rates of complete and durable remission. It is also used in other lymphoid malignancies.

Despite its efficacy, pentostatin's clinical utility is significantly constrained by its toxicity profile. Because it inhibits a fundamental metabolic enzyme, its effects are not limited to cancer cells. The major dose-limiting toxicities include (Grever et al., 2011):

Profound Immunosuppression: By killing both malignant and healthy lymphocytes, pentostatin leads to severe and prolonged T- and B-cell lymphopenia, increasing the risk of life-threatening opportunistic infections.

Myelosuppression: Inhibition of other hematopoietic precursor cells can lead to neutropenia and thrombocytopenia.

Neurotoxicity: At higher doses, central nervous system toxicity, including lethargy, coma, and seizures, can occur.

Renal and Hepatic Toxicity: The drug can also cause kidney and liver damage.

These severe side effects highlight the critical need for new ADA inhibitors with an improved therapeutic index—either through greater selectivity, different pharmacokinetic properties, or a novel chemical scaffold with a different off-target profile.

5. Marine-Derived Fungi: A Frontier for Novel Chemical Scaffolds

The search for new drug leads is increasingly turning to untapped biological resources. Marine fungi, including those living as free-living saprophytes, endophytes within marine algae, or symbionts within invertebrates like sponges and corals, represent a vast and chemically creative reservoir (Rateb & Ebel, 2011).

The rationale for exploring this niche is compelling. The marine environment imposes unique selective pressures—high salinity, extreme pressure, low temperatures, and intense competition for space—that have driven the evolution of novel secondary metabolic pathways. This has resulted in the production of natural products with chemical scaffolds and functionalities that are rare or absent in their terrestrial counterparts. A notable feature is the frequent incorporation of halogen atoms (chlorine and bromine) into the molecular structure,

which can dramatically alter bioactivity (Gribble, 2015). Marine fungi have already yielded a plethora of compounds with potent anti-cancer, anti-inflammatory, and antimicrobial activities, including novel alkaloids, polyketides, and terpenoids (Bugni & Ireland, 2004). This proven track record of chemical novelty makes them an ideal source to search for new ADA inhibitors that are structurally distinct from the classic purine analogues.

6. The Integrated Discovery Pipeline: *In Silico* Meets *In Vitro*

To efficiently mine the chemical space of marine fungi for ADA inhibitors, a modern, integrated workflow that synergizes computational and experimental approaches is essential. This strategy uses the predictive power of *in silico* methods to guide and focus the resource-intensive experimental work.

6.1. *In Silico* Front-Loading: The Computational Sieve

This phase uses the known 3D structure of human ADA to virtually screen for potential binders.

1. **Target Preparation:** The high-resolution crystal structure of human ADA, complexed with various ligands, is readily available from the Protein Data Bank (PDB). This structure is prepared for docking by adding hydrogen atoms, assigning charges, and defining the active site pocket.

2. **Virtual Library Generation:** A comprehensive virtual database of all known natural products isolated from marine fungi is compiled from literature sources and databases like the MarinLit database. The 3D structures of these compounds are generated and optimized.

3. **Virtual Screening (VS) via Molecular Docking:** A molecular docking program (e.g., AutoDock, Glide, GOLD) is used to systematically "dock" each compound from the virtual library into the active site of the ADA structure. The program calculates a docking score for each compound, which estimates its binding affinity (Forli et al., 2016).

4. **Hit Prioritization:** Compounds are ranked by their docking scores. The top-ranking virtual hits are then visually inspected to analyze their binding mode. Do they form hydrogen bonds with key active site residues like His238 or Glu217? Do they have good shape complementarity? This analysis, combined with *in silico* ADMET prediction models to filter out compounds with likely toxicity or poor drug-like properties, generates a final, prioritized list of a few hundred high-potential candidates.

6.2. *In Vitro* Validation: The Experimental Proof

This phase runs in parallel to the *in-silico* work and provides the essential experimental validation.

1. **Extract Library and Enzymatic Screening:** Obtain crude extracts from a library of cultured marine fungal isolates. These extracts are then screened for their ability to inhibit ADA activity *in vitro*. A common method is a spectrophotometric assay that measures the decrease in absorbance at 265 nm as adenosine is converted to inosine (Giusti, 1974). Extracts that show significant inhibition at a low concentration are deemed "active."

2. **Bioassay-Guided Fractionation and Isolation:** The most active extracts are subjected to a process of iterative separation and testing. The crude extract is fractionated using High-Performance Liquid Chromatography (HPLC). Each fraction is then re-tested in the ADA inhibition assay. The active fraction is subjected to further, higher-resolution chromatography, and this cycle is repeated until a single, pure active compound is isolated. Its structure is then definitively determined by MS and NMR analysis.

6.3. Synergy: Closing the Loop

The power of the integrated approach lies in connecting these two workflows. The prioritized list of virtual hits from the *in-silico* screen serves as a valuable guide during the experimental phase. When an active extract is being fractionated, LC-MS can be used to rapidly check if any of the predicted virtual hits are present in the active fractions. If a predicted high-scoring compound is found to be a major component of a highly active fraction, it becomes a top priority for isolation. This synergy dramatically accelerates the often-laborious process of identifying the active principle from a complex natural product extract.

7. Experimental Evidence and Case Studies

Upon successful isolation, a novel compound must be rigorously characterized. This involves determining its IC_{50} value (the concentration required to inhibit 50% of ADA activity), performing enzyme kinetic studies to determine its mode of inhibition (e.g., competitive, non-competitive, or uncompetitive), and assessing its selectivity by testing it against other related enzymes in the purinome.

The therapeutic potential is then assessed in cell-based assays. For an anti-cancer indication, the compound would be tested on a panel of lymphoid cancer cell lines (e.g., Jurkat T-cell

leukemia cells) to determine its ability to induce apoptosis or inhibit proliferation. The results would be compared to those for pentostatin.

While specific examples of marine fungal ADA inhibitors discovered through this exact pipeline are still emerging, this integrated strategy has been successfully applied to discover inhibitors for numerous other therapeutic targets. For instance, this approach has been used to identify novel inhibitors of enzymes like protein tyrosine phosphatase 1B (PTP1B, a diabetes target) and indoleamine 2,3-dioxygenase (IDO, an immuno-oncology target) from various natural product sources (Lupien et al., 2015). The principles are directly translatable to the search for ADA inhibitors.

8. Clinical and Translational Relevance

The discovery of a novel class of ADA inhibitors from marine fungi would have significant translational implications. A compound with a different chemical scaffold from the purine analogues could exhibit:

An Improved Safety Profile: It might have greater selectivity for ADA over other enzymes or a different off-target activity profile, potentially avoiding the severe neurotoxicity or myelosuppression associated with pentostatin.

Novel Pharmacokinetics: A new scaffold could have better oral bioavailability, stability, or tissue distribution.

Activity Against Resistant Strains: In the unlikely event that resistance to pentostatin emerges, a structurally distinct inhibitor would likely still be effective.

However, the path to the clinic is long and fraught with challenges, particularly for marine natural products. The "supply problem"—obtaining a sustainable, large-scale supply of the compound for preclinical toxicology and human trials—is a major hurdle. If the producing fungus is rare or difficult to culture, this can halt development.

9. Current Limitations and Knowledge Gaps

In Silico Accuracy: The predictive accuracy of docking scoring functions remains a key limitation, often leading to the mis-ranking of potential hits. The static nature of most virtual screens, which ignore protein flexibility, is another source of error.

Cultivation and Expression: A large proportion of marine fungi are difficult to culture in the lab. Furthermore, the biosynthetic gene cluster for a desired compound may be "silent" and not expressed under standard laboratory conditions.

Bioavailability: Many complex natural products have poor physicochemical properties (e.g., low solubility, high molecular weight) that lead to poor oral bioavailability, limiting their potential as drugs.

Target Specificity: While ADA is a well-validated target, the downstream effects of modulating adenosine levels can be complex and context-dependent, which can complicate development for inflammatory or autoimmune indications.

10. Future Directions

The future of this field will focus on leveraging new technologies to overcome these limitations.

AI and Machine Learning: Deep learning is revolutionizing *in silico* discovery. AI-based models are being developed for more accurate prediction of binding affinity, ADMET properties, and even for the *de novo* design of novel inhibitor structures inspired by natural product motifs (Schneider, 2018).

Genomic Mining and Synthetic Biology: To bypass cultivation and supply issues, the genomes of marine fungi can be sequenced and "milled" for promising biosynthetic gene clusters (BGCs). These BGCs can then be cloned and heterologously expressed in a robust, easily cultured host like *Aspergillus niger* or yeast, which can be fermented on an industrial scale to produce the compound (Harvey et al., 2015).

Cryo-Electron Microscopy (Cryo-EM): Recent advances in cryo-EM are allowing for the determination of high-resolution structures of enzymes, like ADA, in complex with their inhibitors. This structural information is invaluable for understanding binding mechanisms and guiding the rational, structure-based optimization of lead compounds.

Phenotypic Discovery: An alternative approach is to use high-content imaging to screen for compounds that induce a desired cellular phenotype (e.g., apoptosis in leukemia cells). If a hit is found, target deconvolution techniques (like thermal proteome profiling or affinity chromatography) can then be used to identify its molecular target, which may or may not be ADA.

11. Conclusion

Adenosine deaminase is a clinically validated and highly compelling therapeutic target for specific cancers and immunological disorders. The significant toxicities of existing inhibitors create a clear medical need for novel agents with improved therapeutic profiles. Marine-derived fungi, with their proven record of producing unique and bioactive chemical scaffolds, represent a rich and underexplored resource for this discovery effort. The historical challenges of natural product discovery—the slow, laborious process of isolation and identification—can now be dramatically accelerated by a modern, integrated strategy. By synergistically combining the vast screening capacity of *in silico* molecular modeling with the empirical power of *in vitro* enzymatic assays and bioassay-guided fractionation, we can navigate the chemical space of marine fungi with unprecedented speed and precision. This convergent pipeline, further enhanced by advances in AI and synthetic biology, provides a rational and efficient roadmap to translate the chemical treasures of the deep sea into the next generation of life-saving medicines targeting the purinome.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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Lipase producing bacteria and protein designing of lipase

Puja Dutta[1], Semanti Ghosh[2]*

[1]Department of Microbiology, School of Life Science, Swami Vivekananda University,Barrackpore, West Bengal-700121, India.

[2]Department of Biotechnology, School of Life Science, Swami Vivekananda University, Barrackpore, West Bengal-700121, India.

**Corresponding author: semantig@svu.ac.in*

Abstract

Lipases are multidimensional biocatalysts with far-reaching industrial and biotechnological uses, including food processing and pharmaceuticals, biodiesel production and environmental cleanup. Bacteria are also an interesting source of lipases because they exhibit high growth rates, their genomes can be manipulated genetically, and they secret lipases with a wide range of catalytic activities. Bacterial lipases include a variety of species including *Pseudomonas, Bacillus, Burkholderia,* and *Staphylococcus* which have been actively pursued as large-scale enzyme sources. Nevertheless, the inherent characteristics of lipases, including poor thermostability, solvent tolerance, and substrate selectivity tend to limit their practical application in industry. Submerged fermentation is the main producer of bacterial lipases, which are mostly extracellular. Hydrophobic interaction chromatography is the most widely used method to purify the enzyme, although reverse micellar and aqueous two-phase systems are also used more recently. The majority of lipases are active over a broad pH and temperature range, but alkaline bacterial lipases exist in greater numbers. Lipases are serine hydrolases and are very stable in an organic solvent. In addition to these, a few lipases are chemo-, regio and enantioselective. The most recent innovation in lipase studies is the production of new and better lipases via the application of molecular methods, including directed evolution and the study of natural communities via the metagenomic approach.

Developments in protein engineering have allowed the creation of superior lipases that can have custom functions. Molecular docking, molecular dynamics simulations, rational design, and machine learning-based predictions are computational methods that can be used to predict structure-function interactions and find useful mutations. In a complementary fashion, experimental methods, including site-directed mutagenesis, error-prone PCR, DNA shuffling,

and high-throughput screening, have been used to isolate new lipase variants with improved activity, stability and enantioselectivity. Combining computational modeling and experimental validation helps in the faster discovery of strong biocatalysts with minimal time and cost.

Keywords: Biocatalyst, Enantioselectivity, Lipase, Protein engineering, Rational design *Staphylococcus*.

1. Introduction

1.1 Importance of Lipases:

Lipases can be utilized in other forms of industries, which has been previously one of the most significant types of biocatalysts with biotechnological uses (Jaeger & Eggert, 2002). Lipases have the most commercially significant role in the detergent industry and secondly in food and beverage industry. In food technology, lipases have a number of applications, including in flavour-development, baked goods, the production of dairy products, the production of butter and milk substitutes, meat and fish processing, animal feed, and many others (Aravindan et al., 2007). The other significant application of these enzymes is that they can be used as biocatalysts in the resolution of the racemic mixtures to form pure enantiomers that are used in the pharmaceutical industry (Sharma et al., 2001). One of these seminal cases, which caused more stringent regulations and more intense pressure towards enantiomerically pure compounds, is the Thalidomide tragedy of the late 1950s (Blaser, 2013). Though biocatalysts are also used in the chemical synthesis processes through industrial processes, the enzymes applied in these processes can be regarded as specialty enzymes because they are only needed in small amounts and are of higher value in comparison with industrial enzymes (Freedonia Group, 2014). An increased use of lipases in the production of alternative renewable and environmentally friendly fuel, namely, biodiesel, has acquired increased significance in the recent years (Ghaly et al., 2010). The enzymatic transesterification can replace the most commonly industrially applied process-the alkaline transesterification because it has a number of strengths over the latter, including the ability of both the free fatty acid and triglyceride to undergo the reaction process without a washing step that follows (Fjerbaek et al., 2009).

Less than a decade ago Jaeger and Eggert posed the question: What is it that makes lipases so attractive? We now rephrase such a question to be are lipases still attractive? In an attempt to answer this question, the paper under discussion explores the technological growth of four

strategic sectors of industry at various levels of development and technological advancements. This work provides a review of the R&D activity in these two industries in a period of thirty years by studying the scientific and technological indicators of the activity through publications and patents respectively. Last but not the least, cumulative patent data are applied in the lifecycle analysis of such technologies and in technologies forecasting. (Sarmah et al., 2018)

The trend of manufacturing industries has shifted to the growing alarm on climate change as well as environmental concerns.been slowly moving towards development of alternative greener, safe and sustainable processes. One of such initiatives is the enzyme catalysis which is used to catalyze the reaction process at near ambient conditions and with greater specificity. The industrial examples of enzyme are numerous. catalysis has been instrumental in intensification of processes. The most popular biocatalysts with have lipases. excellent uses in the advertisement of a variety of biochemical processes in the industry. They have been identified to be effective in catalyzing many processes that are important to the food, pharmaceutical, leather, cosmetic, detergent, medical diagnostics,dairy, beverage, fatty acid and paper industries.

1.2 Bacterial lipases as industrial enzymes:

Bacterial lipases are carboxyl ester hydrolases able to catalyse breakdown of long-chain triglycerides at the oil-water interface resulting in the free fatty acids and glycerol. Due to their exceptional catalytic broadness, stability and specificity to the substrate, bacterial lipases have acquired significant significance as biocatalysts in industries (Gupta et al., 2004). The enzymes are commonly generated by bacterial genera that include Pseudomonas, Bacillus, Burkholderia, Staphylococcus, and Achromobacter (Hasan et al., 2006). One major benefit of bacterial lipases relative to plant and animal lipases is their ability to withstand extreme physicochemical environments such as very low and high temperatures, diverse pH and the presence of organic solvents. Moreover, the cost-effective large-scale manufacturing, quick development, and genetic manipulation is also feasible in bacterial systems, which allows enzyme yield improvement and optimization of functionality to be carried out via recombinant DNA technology and protein engineering (Jaeger & Eggert, 2002; Treichel et al., 2010). The use of bacterial lipases is widely used in industries. They are used in food industry in flavour enhancement, ripening of cheese, fat modification and synthesis of structured lipids (Sharma et al., 2001). The alkaline and thermostable lipases are used in the

detergent sector so that the lipid-based stains can be removed easily under the conditions of low temperatures washing (Hasan et al., 2006). Moreover, bacterial lipases are also important in biodiesel production because they catalyze transesterification reactions and they are environmentally sustainable as compared to the use of chemical catalysts (Gupta et al., 2004). The growing focus on the green chemistry and sustainable industrial processes has also increased the pressure on the use of bacterial lipases. On-going enhancement of strain, fermentation technology and strategies of enzyme immobilization are likely to increase their industrial utility, which further strengthens bacterial lipases as an indispensable instrument in the present-day biotechnology. (Treichel et al., 2010).

1.3 Need for protein design:

The native bacterial lipases usually have intrinsic drawbacks including low thermostability, low activity in organic solvents, and substrate specificity that limit their large scale industrial use. Enzymes used in biotechnological processes can be subjected to severe conditions, which require them to be active at high temperature, at high and low pH, and with non-aqueous conditions, e.g. biodiesel production as well as pharmaceutical synthesis. Nevertheless, under such conditions, the denaturation of wild-type lipases and its loss of catalytic activity often occur (Gupta et al., 2004). One way out of these limitations has therefore been the design and engineering of proteins through rational design, site-directed mutagenesis and directed evolution. The protein design increases the stability of enzymes, their catalytic efficiency, solvent tolerance and selectivity in enzymes by altering certain amino acid residues to improve them to be more applicable in industries. In turn, protein-designed lipases are important to enhance the efficiency, sustainability, and the economic viability of the industrial biocatalysis by enhancing enantioselectivity and resistance to denaturing agents and by aiding the capability to work at extended temperatures and in non-aqueous conditions. This is especially valuable in the use of these in biodiesel production, pharmaceutical applications, food processing, and detergent applications (Schmid et al., 2001). Moreover, the engineered lipases are also more compatible with the immobilization methods enhancing enzyme re-usability and stability of operation.

2. Lipase Producing Bacteria

There are numerous species of bacteria that synthesize lipases that decompose esters of glycerol with long-chain fatty acids (preferably). They produce action at the interface formed

between a hydrophobic lipid substrate in an aqueous medium. One of the properties of is its characteristic. lipase is referred to as interfacial activation, which implies that a sharp rise of the lipase activity is observed when the substrate begins to form an emulsion, and thus offering to the enzyme an interfacial area. The kinetics of a lipase reaction, as a result, are not obeyed by the classical model of Michaelis Menten. Bacterial lipases can hydrolyze a triacylglycerol completely with only a few exceptions substrate though some preference has been seen towards primary ester bonds. There are many lipase assay techniques.

2.1 Major bacterial genera:

Various genera of bacteria are known to be proficient extracellular lipase producers of great industrial value. Pseudomonas species are the most common ones with the highest lipase production, wide substrate affinity, and organic solvent stability, which makes them suitable in biocatalysis and biodiesel generation (Jaeger and Eggert, 2002). Another significant group is Bacillus species that are treasured in terms of thermostable and alkaline lipases, which find a lot of application in food processing and detergents (Gupta et al., 2015). Burkholderia spp. lipases are highly enantioselective and they are typically used in the synthesis of pharmaceuticals. Besides, Staphylococcus species secrete lipases whose esterification and transesterification properties are good and other genera consisting of Acinetobacter, Serratia and Proteus have also reported as potential sources of industrially useful lipases (Hasan et al., 2006).

2.2 Screening and production methods:

The isolates of the dominating bacteria in the nutrient agar plate were obtained and filtered lipolytic activity. Directly observed by lipolysis is alterations in the structure of the substrate like tributyrin and triolein that are mechanically emulsified in different growth media and poured into a petri dish. The bacterial isolates were screened in regard to lipolytic activity on tributyrin (1%, w/v) agar plates, agar (2%, w/v) in Luria–Bertani medium (Fakhreddine et al., 1998). Lipase production is denoted by the establishment of distinct halos about the developed colonies on tributyrin- based agar plates. (Jaeger et al., 1994, Kim et al., 2001, Ertuğrul et al., 2007)

Many microorganisms and higher eukaryotes produce lipases. The majority of commercially useful lipases are of microbial sources. Microorganisms of lipase have been identified in a wide variety such habitats as industrial wastes, vegetable oil processing factories, dairies,

oiled up soil, oily oilseeds, rotting food, hot springs, coal tips and compost heaps (Sztajer et al., 1988, Wang et al., 1995). Lipase producing the microorganisms are bacteria, fungi, yeasts and actinomyces. Microorganisms that produce lipases have been identified to be diverse industrial wastes, vegetable oil-processing plants, and others dairies, soil that has oil on it, oilseeds, and rotting food, piles of compost, coal tips, hot springs (Wang et al., 1995). The colonies on tributyrin agar plates are regarded as positive colonies of lipase enzyme production in terms of halos around them. Isolation and identification of such colonies was done through phenotypic characterization through morphological, biochemical and physiological characters as per Bergeys Manual of Systematic Bacteriology (Sneath, 1986). Biochemical identification of the isolate with greater lipolytic activity was done both by 16s r RNA sequencing and by biochemical methods.

3. Production and Purification of Lipases

Lipases (triacylglycerol hydrolases) are enzymes that are secreted by mammals, plants, fungi and bacteria and catalyze the breakdown of triacylglycerols into glycerol and fatty acids. The selectivity of lipases has been utilized in the last few years in emulsifiers, pharmaceutical, cosmetic, flavors, fragrances, and the pretreatment of lipid-rich wastewater, bioremediation of oils and biodiesel production. The review provides data that have been gathered within the past 10 years on the sources of lipases besides developments in the production, purification methods and the principal uses in the industry.

3.1 Fermentation methods:

The production of bacterial lipases is mainly done through submerged fermentation (SmF) and solid-state fermentation (SSF). The most common way of producing lipases industrial is submerged fermentation because it is easy to control the process, such as pH, temperature, aeration and agitation. SmF permits the effective nutrient supply and is applicable in the large-scale enzyme production by means of bioreactors. Conversely, solid-state fermentation uses solid feeds, which are low moisture, including agro-industrial residues, and is said to be cost-effective with elevated enzyme yields in some instances. The benefits of SSF include lower energy and wastewater production, as well as improved stability of enzymes, which is why it is an appealing alternative to lipase production (Pandey et al., 2000; Bharathi and Rajalakshmi, 2019). The choice of the suitable fermentation technique will be based on the strain of microbes, availability of substrates and the desired industrial use.

3.2 Purification Strategies:

The purification of lipase is an extremely sensitive procedure that should be carefully performed to maintain the bioactive form of the lipase. Various purification techniques have been used to purify lipases to the level of homogeneity in various microbial sources. The purification strategies could be categorized into two broad groups namely (Jaeger & Reetz, 1998) classical purification techniques and (Kumar et al., 2012) modern purification techniques. The classical methods tend to be non-specific, tedious, and multistage and the level of purity attained is not sufficient. Conversely, the current purification methods are non-stressful, selective, large-scale and able to attain high purity levels. The advancements in purification methods have also led to a diversity of options in the design of exceptionally focused purification methods of various microbial lipases. The isolation of the lipases to a degree of homogeneity aided in the sequence of the amino acids that was further combined with the 3D structure of the given amino acids that helped in the better understanding of the characteristic unique features of the lipases when applied in various reactions.

4. Recent Trends and Targets in Lipase Engineering

Lipases are highly active with high stability, and therefore, they have a broad spectrum of desirable appli. cations. The genetic modification and enhancement of the is being put into the limelight. scaling-up procedure to produce lipases in an efficient and cost-effective way. Initially, the The engineering of enzymes was done by using Rational protein design (RD) approach, which is grounded on the principles determined by the structural analysis of enzymes and mechanis. tic evidence and the methods of molecular biology including site-directed mutagenesis. (SDM) (Illanes et al., 2012, Soni, 2022). Application of RD needs the specific identification of the residues. in charge of the contact between the substrate and the enzyme, and specificity, stability, and information as to the structure of the enzymes. RD is structured on deterministic basis. nation and structural functional relationships whereas molecular dynamics (MD) predicts. potential mutations that can be made to enhance lipase enantioselectivity. Recently, MD and The thermostability of lipases is being enhanced with the use of RDsimulations, but without going down. their activity (Illanes et al., 2012, Soni, 2022). Computational methods and site-directed are used. mutagenesis or directed evolution (DE) methods can be potent methods. The directed evolution (DE) method is rooted on error prone polymerase chain. reaction (ep-PCR) and DNA shuffling whereas it does not demand

the geometric properties of lipase (Hwang et al., 2014). In DE, randomly mutated products undergo a DE process by subjecting them to imposing. The selection pressure and are then examined to bring out the improved features. The main steps in DE involve random mutagenesis to form libraries of a parent gene, insertion and transfection into a viable host, and screening of the mutant library generated. There have other goals in lipase engineering to enhance aggregate properties, including thermostability, catalytic activity, and solven tolerance (Hwang et al., 2014, Illanes et al., 2012).

4.1 Thermo-Stability:

Enzymes used include lipases of thermophilic microorganisms, including Thermomyces lanuginose, Bacillus subtilis and Rhizopus orzae have been reported to be highly heat tolerant (90◦C) (de Miguel Bouzas et al., 2006, Castilla et al., 2022). The effect of long-term temperature on the performance of the lipases is negative though. and therefore are not able to match industrial standards. Directed evolution and rational design (RD). To increase thermostability of lipase, (DE) are employed (Wang et al., 2020) cloned the RCL Rhizopus chinesis gene and was expressed in Pichia pastoris. The lipase r27RCL had excellent results in the food and feed sector as well as it was not widely used because of its low. thermostability. They estimated the free energy to be improved with RD (FoldX). lipase thermostability, and a small pool of 19 mutated residues were found to have 30 single-point mutations mutations with reasonable free energy values was chosen from the 293 residues of lipase and tested in gene expression, purification of enzyme and thermostability, output. in 4 mutations, which were denoted as S142A, S250Y, Q239F, and D217V. The variation (m31) was produced through the combination of these 4 mutations and had a 5 C higher optimum lower temperature when compared to the wild type. The model used to analyze the structure of the protein was that of a protein and describe molecular conformation change, which demonstrate that the higher hydrophobic. The ability of m31 was mainly due to stacking force within specific secondary structures. improved thermostability. They also carried out MD simulation to explore the mutant 1-thermostability mechanism, and three of four of the positive mutations were verified in the thermally adaptable regions. These results have a strong implication that combination should be put into use of free energy-based RD simulations and MD simulations to enhance lipase thermostability would be a highly advantageous policy. (Yu et al., 2012) have demonstrated that the thermostability of lipase of Rhizopus improved chinensis Two ep-PCR and two rounds of shuffling the DNA using a DE

strategy. More recently, in order to enhance thermostability of lipase of Pseudomonas Alcaligenes (PaL). On the same note, (Yu et al., 2012) introduced a new method called combined rational evaluation in thermostability engineering (CREATE). The hydrolysis of racemic menthol propionate to synthesize L-menthol, which is one of the most common flavoring compounds in the food, cosmetic, and The PaL lipase catalyzes the activation of the pharmaceutical industries. However, L-menthol has low thermostability which renders its use at higher temperatures hard and restricts its industrial applications. The CREATE strategy forecasted a pool of using three approaches. possible stabilizing mutations: thermophilic orthologous sequence comparisons, the FireProt servers, and Protein Repair One-stop Shop (PROSS) application. Then, based on a reasonable assessment of the location in the 3D structure, free energy change, flexibility change, and distance to the active center, mutations that have a high potential to improve. stability were found. The authors prepared 36 single-mutant derivatives and evaluated them in terms of catalytic performance and thermal stability it was discovered that 4 single-variants were more thermostable. than wild-type PaL. The optimal 4M form displayed a half-life that was 15-fold higher at 50°C a melting temperature (Tm) value increased by 14°C over the wild type, and all. combinations of the 4 mutations that were feasible were made to improve further on the stability It was discovered that the CREATE approach was useful in the direction of mutation selection the possibility to be extrapolated to other enzymes (Yu et al., 2022). As a variety of possible amino acids positions are involved in likely mutations, numerous structural aspects are involved in lipase thermostability. DE is in most instances a more efficient method of searching potential mutations than RD

4.2 Catalytic Activity:

Lipases do not have desirable characteristics needed in industries processes as normal the catalytic activity of natural lipases is highest at the 30-50°C temperature. range, but as temperature increases, the rate of lipase reaction is low and the reduced one catalytic functionality in the primary process, which makes it time-consuming and more costly reaction. The thermostability of the lipase is increased which results in a higher tolerance of the lipase to high working temperature sand is one of the main objectives of lipase engineering. (Ma et al., 2022) expression of lipase by the thermophilic bacterium *Thermomicrobium roseum* DSM5159 (TrLip) because of its greater thermostability and its great solvent resistance. However, the catalytic long-chain fatty acid showed reduced activity

of the lipase (TrLip) has a very strong influence on its usefulness in the industry. Therefore, sequence and structural information naturally evolved trends in lipase reconstruction of the ancestral order later on are utilized and this increased the catalytic activity and affinity greatly with longer chains, keeping at the same time the optimum pH and thermostability of the lipase. Therefore, the improved catalytic potential and stability of TrLip can be used in the food and chemical industries. (Xu et al., 2021) identified using new amino acid ionic liquids as chemical modifiers theoretical and experimental data of the enhanced catalytic behavior of lipase B. of Candida antarcta produced through chemical modification with ionic liquids of amino acids. They came to the conclusion that the catalytic activity of altered Candida antarctina lipase B (CALB) was enhanced under different temperatures and pH and that it had high thermostability and resisted organic solvents were enhanced. The structural stability and improved in the modified CALBhad improved increased catalysis of the substrate (Xu et al., 2021, Tian et al., 2022). Moreover, introduced semi-rational DE strategy and N-glycosylation in order to enhance the methanol tolerance and catalytic activity of *Rhizomucor miehei* lipase in one step in a commercial manner biodiesel synthesis.

4.3 Solvent Tolerance:

It has been established that protein physical state depends on its hydration state. characteristics and their rate of reactions It is not clear however the way this affects enzyme kinetics (Peng et al., 2020). The significance of the water activity in the enzyme activity determination has been recognized. Organic media Hydrolase-catalyzed esterification reactions are in organic media often used as a method of enzyme activity. In these, the use of lipases is common types of reactions. The influence of the water activity on the rate of lipases reaction has been studied (Zulkeflee et al., 2022). The findings indicated that lipases of various sources respond to an increase affects water activity in various ways. The Rhizopus arrhizus lipases show optimum activity in the presence of low water activities, whereas lipases in the Pseudomonas species have activity which rises with water activity. Candida rugosa lipases have intermediate profiles that are more extended (Zulkeflee et al., 2022, Wehtje and Adlercreutz, 1997). The synthetic reactions may be deactivated by lipase as a result of change in temperature certain to interfaces, and chemical denaturants, typically found in the case of esterification reaction systems either as substrates or products. Lipase deactivation may also take place as a result of physical modifications of the enzyme structure or chemical modifications like breaking of disulfide bonds age. The activity of lipase is

subject to the tolerance in various solvent systems as well as stability is a requirement to its use in several industrial processes. A lipase's application potential can be assessed in varied solvent systems, and plans are taken to do so engineer lipase to withstand the activities of organic solvents. The use of lipase in organic media have a number of strengths that include higher activity, greater solubility of the substrate, and processing downstream convenience. N-glycosylation was reported to be taking place on the by (Tian et al., 2022) enhancement of the methanol tolerance of the lipase in Rhizomucor miehei and showed that the mutant N267 had a 64% activity following incubation with 50% methanol after 8 h incubation. The formation of newhydrogen bonds were responsible to this elevated methanol tolerance of N267 (Tian et al., 2022). Methods include genetic recombination, amino acid modification and immobilization onto support material are used continuously to increase the thermostability and microbial lipase solvent tolerance (Fatima et al., 2021). Industrial processing is promoted through recombinant lipases. And the heterogeneous expression of lipase can be regarded as a positive approach towards improving the overall reaction In lipase genetic recombination technique, the desirable gene is cloned down onto a vector and was then transferred to the host to heterologously express lipase with targeted characteristics. Genetic modification is however not a certainty that will bring about the successful heterologous carrying lipase gene (Ismail et al., 2021, Yan et al., 2017). To carry out the recombinant heterologous expression of lipases, A number of microbial host strains are utilized (bacteria, fungi, and yeast) and there are many expression vectors that showed large differences in every host system of lipase production. Heterologous lipase gene is most commonly used in Escherichia coli. The most frequently used ones are expression (Chen et al., 2021, Fitri & Illavi, 2022), and Pichia pastoris and Saccharomyces cerevisiae expressed lipase genes in used eukaryotic host strains. The right strain has to be chosen to obtain maximum recombinant lipase (Duman-Özdamar & Binay, 2021). Past research indicated that in the case of host selection, an appropriate vector to produce lipase is necessary. The characteristics of each of the vectors, in particular, its promoter can determine the degree of lipase production. The immobilization methods are also used to enhance the ability of the lipase to withstand the increased temperatures in the presence of organic solvents in other industrial applications since the process offers mechanical stability and also enables the lipase to be reused (Ismail et al., 2021, Yan et al., 2017). Moreover, the cost of making thermostable and solvent-tolerant lipases is high and this is the major setback in industry. Scientists are looking at the application of protein engineering method to develop a viable low-cost

production process of these lipases. The solvent tolerability and thermostability will enhance the durability and reuse of lipases in most industrial procedures. Likewise, there must be a strong immobilization. These characteristics will also be enhanced through mechanism.

5. Properties and Applications

Bacterial lipases have a wide range of biochemical characteristics which predispose them to be useful in industries. These enzymes usually have a general substrate specificity and can catalyze hydrolysis, esterification and transesterification reactions. A large number of bacterial lipases have a broad pH (neutral to alkaline) range of activity and are highly thermostable and can therefore be used in severe processing environments (Hasan et al., 2006). Furthermore, a number of lipases are active in organic solvents, metal ions and surfactants necessary to biocatalyze in the non-aqueous medium (Jaeger & Eggert, 2002).

Lipases are used widely in different industries due to these properties. They are applied in the food industry in enhancing their flavor, modifying their fat, and also in processing dairy products. Lipases are used in the pharmaceutical and fine chemical industry in enantioselective synthesis of optically pure products. The lipases are also effective in the production of bio-diesel because they catalyze the transesterification reactions because it is Green as opposed to the use of chemical catalysts. Also, lipases can be used in detergents, bioremediation, and wastewater treatment, which demonstrates their significance in the environment and industry (Gupta et al., 2015).

5.1 Stability and catalytic properties:

Bacterial lipases have incredible catalytic versatility, thus being able to catalyze hydrolysis, esterification, and transesterification reactions with wide substrate specificity. Their catalytic activity is mainly regulated by a conserved catalytic triad (Ser-His-Asp/Glu) and a mobile lid domain, which regulates the accessibility of substrates and the interfacial activation. A good number of these bacterial lipases are highly regio- and enantioselective, which makes them ideal in stereospecific synthesis during pharmaceutical and fine chemical manufacturing.

Stability-wise, bacterial lipases can be tolerant to a great number of pH levels, high temperatures, and organic solvents. Thermostable lipases, especially of the Bacillus and Pseudomonas species, are active at high temperature levels and pH, which would be critical in industrial applications like the production of detergents and biodiesel (Gupta et al., 2015). Another application is that solvent-stable lipases retain catalytic power even in non-aqueous

mediums allowing its use in organic synthesis reactions and transesterification reactions (Hasan et al., 2006). Such stability and catalytic properties render bacterial lipases very attractive protein engineering targets in a bid to improve industrial operation further.

5.2 Industrial and environmental applications:

The identification of large catalytic efficiency, stability, and versatility of substrate have made bacterial lipases very popular in the industrial industries. Lipases are also used as flavor enhancers, fat modifiers, and in the ripening of cheese as catalysts in the hydrolysis of triglycerides in the food industry (Hasan et al., 2006). Alkaline lipases and thermostable lipases are applicable in detergent industry in the removal of lipid based stain in harsh washing conditions to enhance efficiency of washing (Jaeger & Eggert, 2002). Lipases are also very important in any pharmaceutical and fine chemical industry because they find application in enantioselective production of optically pure substances and drug intermediates. Moreover, lipases are also biocatalysts that are friendly to the environment since they can carry out the transesterification reaction as alternative biocatalysts to the chemical catalysts used in making biodiesel (Gupta et al., 2015).

Lipases have a great use in environmental remedies in the sense that they break down oil spills, grease, and lipid-contaminated areas of polluted soils and waters. They also find application in treatment of waste water to decompose fats, oils, as well as grease (FOG) and thus avert blockage of pipes thus improving effectiveness of treatment (Hasan et al., 2006). Lipases have been useful in ensuring that industries are environmentally friendly and sustainable due to their biodegradability, specificity and their being environmentally benign.

6. Protein Designing of Lipase

Lipase protein designing is an influential method to eliminate the weakness of the native enzyme, as well as to make it more favorable to the industrial. Natural lipases are generally not quite stable at extreme temperatures, pH, or organic solvents, limiting their use at large scale. Rational design, site-directed mutagenesis, and directed evolution are the protein engineering approaches that are used to enhance the most important properties, including thermostability, solvent tolerance, catalytic performance, and substrate specificity. Rational design and directed evolution protein engineering techniques allow the accurate alteration of amino acid residues on substrate binding, catalytic activity, and structural stability. These have applied to enhancing lipase performance and have mainly been used to enhance stability

and catalytic performance in biodiesel production and other industrial uses Bassegoda et al. (2012). Therefore, designing of proteins is important towards designing of powerful, efficient and application-specific lipases to suit contemporary industrial needs.

6.1 Need for protein design:

Despite their popularity as biocatalysts, numerous native enzymes still possess a series of disadvantages, i.e. low thermostability, lack of organic solvent tolerance, secondary substrate range and low catalytic power at industrial temperatures. These are the disadvantages that limit their use in large-scale processes, such as biodiesel production, detergent formulations and pharmaceutical synthesis. Designing of proteins has thus become a necessity to design lipases with improved functional characteristics that could be applied in tough operational conditions Bassegoda et al. (2012). Rational design and directed evolution protein engineering methods allow the specific modification of amino acid residues that play a role in substrate binding, catalysis and structural stability. These have been effectively implemented to enhance the work of lipases especially in terms of stability and catalytic reaction in the production of biodiesel and other industrial uses Bornscheuer and Pohl (2001). Protein designing is therefore a very important aspect in coming up with robust, efficient, and application specific lipases to satisfy the modern industry needs.

6.2 Rational design and directed evolution:

Two of the most common methods of performance enhancement of lipase to be used in an industry are rational protein design and directed evolution. Rational design uses extensive information on the structure of enzymes, active site architecture and sequence function relationships to implement targeted amino acid replacements to increase catalytic efficiency, substrate specificity or stability. It has been demonstrated that the interfacial activation and solvent tolerance of lipases are enhanced by structural changes of the active site and lid domain. Rational design of protein structure and function is quickly becoming an important method of general testing in protein chemistry (Bryson et al., 1995). To form a protein or an active site, it is necessary to have all interaction required. The design approach is thus one method of experimenting with the bounds of completeness of understanding. Moreover, in the event that the experiments are put forward in a forward-building manner, (so that the simplest possible designs are attempted first, with adding more complex interaction on top of them, until the required outcome occurs) then perhaps one can find a minimal set of

components. The main element of the design approach is the so-called design cycle where theory and experiment interchange. The initial step would be the creation of a molecular model, which is the rules of protein structure and functionality and an algorithm to apply these rules. This is then succeeded by experimental construction and study of the characteristics of the designed protein. In case of the failure or the partial success of the experiment the next round of the design cycle is initiated where more complexity is added, the rules and parameters are tightened or the rules and parameters are altered. Such a design cycle is described in the article by Dahiyat and Mayo (Dahiyat & Mayo, 1997) published in the latest issue of these Proceedings. A computer design algorithm was used to produce sequences that were predicted to repack the interior of a small protein using various different sets of parameters that defined the interactions between the proteins during packing, thus providing a direct experimental relationship between the design parameters and the properties of the resulting proteins. It is the most recent of a sequence of such endeavors, where objective computational methods that have been formulated to form protein structure or to determine function are actually being experimentally tested. The final aim of these processes is the creation of a complete protein design process (Dahiyat & Mayo, 1996). The initial rational design methods employed qualitative principles of protein frameworks employed through checking (Richardson et al., 1992). These experiments showed that it can be made to design sequences de novo, which can assume specified structures (Bryson et al., 1995, Hecht et al., 1990). Moreover, they showed that, through a progressive design approach [or the so-called hierachic design (Bryson et al., 1995)] where complexity is added in more and more steps, additional information about the principles of protein structure and functionality can be obtained. Among the significant discoveries of these experiments was the fact that it is very easy to get globally correct folds. But the local particulars were only very hard to arrive at with accuracy. The interior of such designed proteins is highly disordered, not in the sense of the highly packed unique structure of natural systems. Global correctness of such designs seemingly was achieved through incorporation of the right binary pattern of hydrophobic and hydrophilic residues that gave the geometry specification of the interior and exterior of the protein so that the hydrophobic effect can take action (Cordes et al., 1996, Beasley & Hecht, 1997). The problem of designing well-ordered cores can be regarded as specificity. Disordered core side chains do not take one, specific structure, but instead, a large number of alternative structures of approximately equal energy.

By contrast, directed evolution does not demand any advance knowledge of the structure, and is modeled after natural evolution by producing large mutant libraries via random mutagenesis or DNA recombination, and screening those variants that are better adapted. This strategy has been effectively used to improve the lipase thermostability, enantioselectivity and activity in harsh conditions (Bornscheuer & Pohl, 2001). Rational design in combination with directed evolution has been especially useful in producing robust lipases to produce biodiesel and other industrial biocatalytic reactions (Hwang et al., 2014).

7. Future Prospects:

It has been predicted that future studies of bacterial lipases would involve the production of more stable, efficient and economical enzymes in the industries and the environment. Further protein engineering, and especially the combination of rational design with directed evolution, will allow the specific reorganization of lipase structure to enhance thermostability, solubility and substrate specificity. Design of enzymes guided by structure will also be enabled more by the growing number of high-resolution protein structures and computational modeling tools.

The future of lipases with selective catalytic functions is likely to be revealed by the use of metagenomics and functional screening of uncultured microbial communities. Moreover, the progress in recombinant expression systems and fermentation will be used to increase production of lipase on a large scale. The future direction of machine learning and artificial intelligence regarding the enzyme prediction and optimization is promising. In general, such advancements will increase the industrial applicability of engineered bacterial lipases in sustainable bioprocess, such as in the production of biodiesel, waste management, and green chemistry.

8. Conclusion:

Bacterial lipases are a significant category of biocatalysts with extensive industrial and environmental uses because of their great catalytic efficacy, ubiquity, and simplicity of production. Developments in screening, fermentation and purification methods have greatly enhanced the yield and activity of lipase. Nevertheless, constraints with respect to the stability and substrate specificity remain as a constraint to their wider industrial application. The use of protein designing methods, such as rational design and directed evolution, has developed as a useful plan to resolve these problems. Computational tools, metagenomics and

advanced bioprocessing methods are all likely to be integrated and used to produce robust and custom lipases to improve their application in sustainable and green biotechnology.

Conflict of Interest

The authors declare that they have no known competing financial interests in the work reported in this paper.

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MicroRNA-Mediated Gene Silencing as a Therapeutic Strategy Against Nipah Virus: Computational Predictions and Molecular Mechanisms

Sibashish Baksi*

Department of Biotechnology, School of Biotechnology, Swami Vivekananda University, Barrackpore, Kolkata -700121

**Corresponding author: sibashishb@svu.ac.in*

Abstract

Nipah virus (NiV) represents a critical biosafety level-4 pathogen with mortality rates exceeding 75% and no approved therapeutics or vaccines. This chapter synthesizes recent computational and bioinformatics approaches investigating microRNA (miRNA)-mediated gene silencing as a potential antiviral strategy against NiV. We examine three complementary approaches: (1) prediction of viral-encoded miRNAs targeting human genes involved in encephalitis pathogenesis, (2) design of synthetic small interfering RNAs (siRNAs) targeting conserved regions of the nucleocapsid protein gene, and (3) identification of human cellular miRNAs capable of silencing viral genes through sequence complementarity. Computational analyses revealed 18-47 mature miRNAs encoded by different NiV strains (Malaysian and Bangladesh variants) targeting 458-1881 human genes involved in neurological and respiratory pathways. Conversely, ten universal siRNAs were designed against the nucleocapsid gene with favourable thermodynamic properties, while 123 human miRNAs demonstrated complementary binding sites across nine NiV genes. Gene ontology analysis identified critical host targets including TLR3, TJP1, NOTCH2, and GRIA3 associated with immune response, blood-brain barrier integrity, and encephalitis. The divergent miRNA profiles between NiV-M and NiV-B variants correlate with distinct clinical manifestations, with Bangladesh strains uniquely targeting respiratory pathway genes. These findings establish a molecular framework for RNA-based therapeutic development and provide insights into strain-specific pathogenesis mechanisms.

Keywords: Encephalitis; Gene silencing; MicroRNA; Nipah virus; RNA interference

1. Introduction

Nipah virus (NiV), first identified during a 1998 outbreak in Malaysia, belongs to the Henipavirus genus within the Paramyxoviridae family and represents one of the most lethal zoonotic pathogens known to medical science (Chua et al., 2000). The virus possesses a non-segmented, negative-sense, single-stranded RNA genome approximately 18.2 kb in length, encoding six primary genes (N, P, M, F, G, and L) that produce nine functional proteins through alternative transcriptional mechanisms (Harcourt et al., 2005). Fruit bats of the Pteropus genus serve as natural reservoirs, with transmission occurring through multiple routes including contaminated food products, intermediate animal hosts such as pigs, and direct human-to-human contact (Luby et al., 2009).

The clinical manifestations of NiV infection are severe and multifaceted, characterized by acute encephalitis with mortality rates ranging from 40% in Malaysian outbreaks to over 75% in Bangladesh and Indian cases (Goh et al., 2000). Patients typically present with influenza-like symptoms progressing to neurological complications including drowsiness, disorientation, seizures, and coma. The Bangladesh variant additionally manifests acute respiratory distress syndrome, significantly contributing to increased fatality rates and enhanced human-to-human transmission potential (Mire et al., 2016). Long-term sequelae include persistent neurological deficits, cognitive impairment, and recurrent encephalitis episodes in survivors.

Despite recognition by the World Health Organization as a priority pathogen requiring urgent research attention, no licensed vaccines or specific antiviral therapeutics currently exist for NiV infection (Singh et al., 2019). Experimental approaches including monoclonal antibody therapy and vaccine candidates have shown promise in preclinical studies but face substantial challenges in clinical translation due to limited outbreak data, biosafety level-4 containment requirements, and difficulties conducting large-scale trials. This therapeutic void necessitates innovative approaches to combat this emerging infectious threat.

2. MicroRNA Biology and Antiviral Mechanisms

MicroRNAs are evolutionarily conserved, small non-coding RNA molecules typically 21-25 nucleotides in length that function as post-transcriptional regulators of gene expression (Ambros, 2003). The canonical miRNA biogenesis pathway involves transcription of primary miRNA transcripts (pri-miRNAs), nuclear processing by Drosha to generate precursor miRNAs (pre-miRNAs), cytoplasmic export, and final maturation by Dicer to produce

functional miRNA duplexes. One strand, termed the guide strand, incorporates into the RNA-induced silencing complex (RISC) containing Argonaute (AGO) proteins, particularly AGO2, which possesses endonuclease activity essential for target cleavage (Meister et al., 2004).

Target recognition operates primarily through complementary base pairing between the miRNA seed region (nucleotides 2-8 from the 5' end) and target sequences, classically within 3' untranslated regions (3'UTRs) of mRNAs. However, accumulating evidence demonstrates that miRNAs can effectively target coding sequences (CDS), with some studies suggesting enhanced destabilization when targets exist in both CDS and 3'UTR regions (Fang & Rajewsky, 2011). The thermodynamic stability of miRNA-mRNA duplexes, quantified by free energy calculations, serves as a critical determinant of silencing efficiency, with more negative values indicating stronger binding and greater repression potential.

In viral contexts, miRNAs participate in complex host-pathogen interactions through multiple mechanisms. Viruses encode their own miRNAs to manipulate host cellular processes, evade immune surveillance, and optimize replication conditions. Conversely, host cellular miRNAs can restrict viral replication by targeting viral transcripts or modulating expression of host factors required for viral lifecycle completion. This bidirectional regulation creates an intricate molecular battleground wherein understanding specific interactions enables therapeutic exploitation (Foo et al., 2016; Fang & Rajewsky, 2011).

3. Viral-Encoded MiRNAs Targeting Host Genes

Gene ontology analysis of predicted human targets revealed enrichment in pathways critical to NiV pathogenesis. Malaysian variant miRNAs predominantly targeted neurological development genes including GRIA1 (glutamate receptor), DLG4 (synaptic scaffolding), and HDAC9 (transcriptional regulation), correlating with encephalitic manifestations (Dsouza et al., 2023). Notably, NM33 miRNA from NiV-M targeted 157 genes with 21.11% associated with neurocognitive disorders. The identification of protocadherin family members (PCDHA1-13) as targets of Bangladesh variant miRNA BD40 provides molecular explanation for respiratory complications, as these genes regulate bronchial epithelial integrity and have established roles in asthma pathogenesis.

Five critical host genes emerged as high-confidence targets with potential therapeutic implications. TLR3 (Toll-like receptor 3) mediates innate antiviral responses through recognition of viral dsRNA patterns; its suppression by viral miRNAs would facilitate

immune evasion. TJP1 (tight junction protein 1) maintains blood-brain barrier integrity, with degradation documented in Japanese encephalitis and HIV-1 neuroinvasion, suggesting a conserved mechanism exploited by NiV for CNS access. NOTCH2 participates in adult neurogenesis, with impaired signalling contributing to neurological disease manifestations. GRIA3 (glutamate receptor AMPA subunit 3) dysregulation associates with mental retardation and Rasmussen encephalitis, representing a convergence point between viral manipulation and clinical symptoms. The divergent miRNA landscapes between NiV strains provided molecular evidence explaining clinical differences. Bangladesh isolates uniquely generated 142 target genes related to respiratory distress pathways, including chronic obstructive pulmonary disease and vital capacity regulation genes, absent from Malaysian variant predictions. This strain-specific targeting suggests that miRNA-mediated host gene modulation contributes substantially to the enhanced respiratory pathology and increased mortality observed with Bangladesh isolates (Dsouza et al., 2023; Saini et al., 2018).

4. Synthetic Sirna Design Against Viral Genes

Complementary to understanding viral miRNA function, rational design of synthetic siRNAs targeting conserved viral sequences offers therapeutic potential. Mahfuz et al. (2022) employed a systematic computational pipeline analyzing 60 complete NiV genome sequences to identify conserved regions suitable for siRNA targeting. Using the siDirect algorithm with stringent filtering parameters (minimum hairpin size 70, minimum score 115), ten universal siRNAs were predicted against the nucleocapsid (N) gene, which encodes a protein essential for genome packaging and replication.

The designed siRNAs satisfied multiple criteria for effective gene silencing. All candidates maintain seed duplex stability (Tm) below 21.51500C, minimizing off-target effects while ensuring specific binding. Free energy calculations revealed favorable thermodynamic profiles ranging from -30.1 to -38.8 kcal/mol for guide strand-target interactions, indicating stable duplex formation. Melting temperature analysis demonstrated high thermal stability (>801500C) for siRNA-mRNA complexes, suggesting sustained binding under physiological conditions. Crucially, BLAST analysis confirmed absence of complementarity with human genomic sequences, eliminating potential off-target toxicity (Mahfuz et al., 2022).

Efficacy prediction using machine learning algorithms (siRNAPred and siPred) yielded scores indicating high silencing potential, with most siRNAs achieving efficacy scores >0.78 and inhibition percentages >82%. Molecular docking simulations with human AGO2 protein,

the catalytic component of RISC, revealed docking scores ranging from -191.11 to -294.98, with more negative values indicating stronger binding affinity. The 4E siRNA-AGO2 complex exhibited particularly stable dynamics during 100 ns molecular dynamics simulation, maintaining RMSD values below 2.5 1515 and demonstrating consistent hydrogen bonding patterns throughout the trajectory (Mahfuz et al., 2022).

The targeting of nucleocapsid protein holds strategic importance beyond simple replication inhibition. N protein facilitates viral genome encapsidation, protects RNA from degradation, and regulates RNA synthesis through interaction with the polymerase complex. Multiple siRNA binding sites across the N gene transcript (twelve sites total) enable cooperative repression, potentially compensating for any single siRNA's reduced efficacy due to secondary structure accessibility issues or sequence polymorphisms (Mahfuz et al., 2022; Harcourt et al., 2005).

5. Host Cellular MiRNAs Targeting Viral Genes

The inverse approach, identifying endogenous human miRNAs capable of restricting NiV replication through direct targeting of viral transcripts, revealed extensive potential for host-mediated antiviral defence. Kar and Chakraborty (2025) computationally screened 2,656 human mature miRNAs against nine NiV genes using 7mer-m8 seed matching algorithms, identifying 123 complementary binding sites across viral coding sequences. The L gene (RNA-dependent RNA polymerase) harboured the highest number of target sites (43), followed by P gene (17 sites), suggesting these essential replicative proteins face substantial host miRNA-mediated pressure.

Target site analysis revealed several favourable characteristics for effective silencing. Free energy calculations for all three regions (upstream, target, downstream) consistently yielded values <4 kcal/mol, indicating minimal secondary structure formation that might occlude miRNA access. GC content analysis demonstrated enrichment in target regions relative to flanking sequences, with higher GC percentage promoting stronger hydrogen bonding through Watson-Crick base pairing. Statistical correlations between free energy and GC content across eight viral genes (excluding M gene) confirmed thermodynamic principles governing RNA duplex stability.

Translational efficiency analyses provided additional evidence supporting miRNA-mediated repression potential. CompAI values (0.129-0.323) indicated low translational efficiency of viral transcripts, a condition favoring miRNA action according to established principles.

Similarly, COSM values (0.113-0.293) reflected minimal similarity between viral codon usage and host tRNA abundance, suggesting slow translation kinetics that enhance miRNA binding opportunity. mRNA stability index (MSI) calculations revealed predominantly negative values across target regions, indicating intrinsic transcript instability that synergizes with miRNA-induced degradation (Kar & Chakraborty, 2025).

Secondary structure predictions of miRNA-mRNA duplexes using RNAFold identified highly stable configurations with free energies ranging from -17.68 to -22.63 kcal/mol. Particularly noteworthy was hsa-miR-4687-5p targeting the N gene (position 52-73), exhibiting the most favorable binding energy (-22.63 kcal/mol). The F gene displayed susceptibility to multiple miRNAs (hsa-miR-517a-3p, hsa-miR-517b-3p, hsa-miR-517c-3p) at identical sites, suggesting functional redundancy that could prevent viral escape through mutation. These findings establish a comprehensive catalog of host miRNAs with predicted anti-NiV activity warranting experimental validation (Kar & Chakraborty, 2025).

RNA editing analysis revealed significant C-to-U transitions (126.22% frequency) in NiV genomes, substantially exceeding other modification types (A-to-G: 29.22%, A-to-C: 18.77%, T-to-G: 9.55%). These transitions, mediated by host APOBEC enzymes, may represent evolutionary pressure shaping viral genome composition and potentially influencing miRNA targeting through sequence alteration. The high frequency suggests active host-virus interaction at the RNA level, with implications for viral fitness and adaptability (Kar & Chakraborty, 2025).

6. Comparative Analysis And Therapeutic Implications

Integration of findings across studies reveals complementary insights into NiV biology and therapeutic opportunities. The divergent miRNA profiles between Malaysian and Bangladesh variants (no shared mature miRNAs despite 92% genomic similarity) provide molecular explanation for clinical differences, particularly the enhanced respiratory pathology and person-to-person transmission characteristic of Bangladesh isolates. Strain-specific targeting of host genes involved in vital capacity and pulmonary function by Bangladesh miRNAs establishes causative links between viral molecular mechanisms and epidemiological observations (Dsouza et al., 2023; Saini et al., 2018; Mire et al., 2016).

While some host miRNAs (e.g., miR-181 family) promote henipavirus infection through modulation of viral entry mechanisms (Foo et al., 2016), the majority identified in computational screens demonstrate anti-viral potential. This suggests that therapeutic

strategies might involve not only introduction of exogenous siRNAs but also modulation of endogenous miRNA expression to enhance antiviral responses.

Several host genes emerge as critical nodes in virus-pathogen interaction networks across multiple studies. The recurrent identification of GRIA1/GRIA3 (glutamate receptors) suggests excitotoxicity mechanisms contributing to encephalitic damage. TJP1 degradation compromising blood-brain barrier integrity represents a conserved neuro invasion strategy shared with other encephalitic viruses. The targeting of immune regulators (TLR3, STAT5B) indicates sophisticated viral immune evasion strategies that could be counteracted through miRNA-based therapeutic modulation (Dsouza et al., 2023; Foo et al., 2016).

Delivery challenges represent the primary obstacle to clinical translation of miRNA therapeutics. While nanoparticle-based delivery systems have demonstrated success in crossing the blood-brain barrier in animal models, achieving sufficient concentrations in neural tissues while avoiding off-target effects requires continued optimization. The identification of multiple miRNA candidates targeting different viral genes enables combination approaches that could reduce required doses while preventing viral escape through mutation.

7. Future Perspectives

The computational frameworks established in these studies provide templates for accelerated discovery against emerging viral threats. As next-generation sequencing makes viral genome data immediately available during outbreaks, these pipelines could generate therapeutic candidates within days rather than months required for traditional drug discovery.

Several research directions warrant priority attention. Experimental validation of predicted miRNA-target interactions through luciferase reporter assays, quantitative PCR, and western blotting remains essential for translating computational predictions to therapeutic candidates. Cell culture studies using BSL-4-approved systems should assess antiviral efficacy of designed siRNAs and identified host miRNAs, establishing dose-response relationships and identifying optimal candidates for in vivo studies. Animal model experiments in hamsters or ferrets, which recapitulate human NiV disease, would provide crucial pharmacokinetic and pharmacodynamic data.

The integration of CRISPR-based technologies offers additional opportunities. CRISPR activation (CRISPRa) systems could upregulate expression of protective host miRNAs identified in computational screens, while CRISPR interference (CRISPRi) might suppress

expression of proviral miRNAs. Combining these genetic approaches with exogenous siRNA delivery could create multi-layered antiviral strategies resistant to viral countermeasures.

Broader implications extend beyond NiV to other paramyxoviruses and emerging viral threats. The methodological approaches validated here apply to any pathogen with available genome sequences, enabling pre-emptive therapeutic development before outbreaks occur.

Conclusion

This chapter synthesizes computational advances in understanding miRNA-mediated gene regulation in Nipah virus infection, revealing complex bidirectional interactions between viral and host RNA molecules that determine disease outcomes. The identification of strain-specific miRNA profiles correlating with clinical manifestations provides molecular explanation for epidemiological observations and suggests diagnostic applications. Designed siRNAs against conserved viral sequences offer therapeutic potential with favorable thermodynamic and molecular properties. Host cellular miRNAs targeting viral genes represent endogenous antiviral mechanisms that could be therapeutically augmented. Collectively, these findings establish RNA interference as a promising therapeutic modality against NiV while providing frameworks applicable to emerging viral threats. Translation to clinical applications requires experimental validation and delivery system optimization but represents a rational, molecularly informed approach to combating one of the world's deadliest pathogens.

Conflict of Interest

The authors declare that they have no known competing financial interests in the work reported in this paper.

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Endophytic Bacteria and Their Applications in Agriculture: A Review

Bidisha Ghosh[1], Subhasis Sarkar[2*]

[1]*Dept. of Biotechnology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barrackpore, Barasat, Kolkata-700121*

[2]*Dept. of Microbiology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barrackpore, Barasat, Kolkata-700121*

**Corresponding author: subhasiss@svu.ac.in*

Abstract

Endophytic bacteria are microorganisms that inhabit internal plant tissues without causing disease symptoms and establish beneficial associations with their host plants. These bacteria contribute significantly to plant growth, nutrient acquisition, stress tolerance, and protection against pathogens. Increasing concerns over environmental degradation caused by excessive use of chemical fertilizers and pesticides have driven interest in sustainable agricultural alternatives, including microbial inoculants. Endophytic bacteria offer unique advantages over rhizospheric microorganisms due to their intimate association with plants and enhanced survival within host tissues. This review provides a comprehensive overview of endophytic bacteria, including their diversity, colonization mechanisms, plant–microbe interactions, and major plant growth-promoting traits. The role of endophytic bacteria in abiotic and biotic stress management, along with their applications as biofertilizers, biopesticides, and biostimulants, is discussed. Challenges related to field application, commercialization, and biosafety are also addressed, and future research directions are outlined. The review highlights the potential of endophytic bacteria as key components of sustainable and climate-resilient agriculture.

***Keywords*:** Endophytic bacteria, plant growth promotion, sustainable agriculture, biocontrol, biofertilizers

1. Introduction

Agriculture faces unprecedented challenges due to population growth, climate change, depletion of natural resources, and the negative environmental impacts of intensive farming practices. The indiscriminate use of chemical fertilizers and pesticides has resulted in soil degradation, water contamination, biodiversity loss, and increased resistance among plant pathogens (Lugtenberg & Kamilova, 2009). Consequently, there is a growing need for sustainable agricultural practices that maintain productivity while reducing environmental harm.

Beneficial plant-associated microorganisms have emerged as promising tools for sustainable agriculture. Among them, endophytic bacteria have gained considerable attention due to their ability to colonize internal plant tissues and exert direct effects on plant growth and health. Unlike rhizospheric bacteria, endophytes are protected from environmental fluctuations and competition, allowing them to establish stable and long-term associations with host plants (Hardoim et al., 2015).

Endophytic bacteria contribute to plant performance by improving nutrient acquisition, producing phytohormones, enhancing tolerance to abiotic stresses, and suppressing plant pathogens. Their multifunctional nature makes them attractive candidates for use as biofertilizers, biopesticides, and biostimulants. This review aims to summarize current knowledge on endophytic bacteria and their applications in agriculture, emphasizing their mechanisms of action, benefits, limitations, and future prospects.

2. Concept and Diversity of Endophytic Bacteria

The term “endophyte” refers to microorganisms that live inside plant tissues for at least part of their life cycle without causing apparent harm to the host (Strobel & Daisy, 2003). Endophytic bacteria have been isolated from virtually all plant species studied to date, including cereals, legumes, vegetables, fruit crops, and forest trees.

Taxonomically, endophytic bacteria are highly diverse and belong to several bacterial phyla, including **Proteobacteria**, **Firmicutes**, **Actinobacteria**, and **Bacteroidetes** (Hardoim et al.,

2015). Commonly reported genera include *Bacillus*, *Pseudomonas*, *Azospirillum*, *Rhizobium*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Paenibacillus*, and *Streptomyces* (Santoyo et al., 2016).

Endophytic bacteria can be classified based on their functional traits (e.g., nitrogen-fixing, phosphate-solubilizing, or biocontrol endophytes) or their mode of transmission. Some endophytes are vertically transmitted through seeds, while others are horizontally acquired from the soil or rhizosphere (Kandel et al., 2017).

3. Isolation, Identification, and Colonization of Endophytic Bacteria

3.1 Isolation and Identification

Isolation of endophytic bacteria typically involves surface sterilization of plant tissues to remove epiphytic microorganisms, followed by tissue maceration and culturing on appropriate growth media. The effectiveness of surface sterilization is confirmed by plating the final rinse water (Hallmann et al., 1997).

Traditional identification methods based on morphological and biochemical characteristics have largely been replaced by molecular techniques. Sequencing of the 16S rRNA gene is widely used for taxonomic identification, while advanced approaches such as metagenomics and high-throughput sequencing have revealed a vast diversity of unculturable endophytic bacteria (Hardoim et al., 2015).

3.2 Colonization Mechanisms

Endophytic bacteria gain entry into plant tissues through natural openings such as root hairs, lateral root emergence sites, stomata, or wounds. Once inside, they colonize intercellular spaces, xylem vessels, or even intracellular compartments (Kandel et al., 2017). Successful colonization depends on bacterial traits such as motility, biofilm formation, enzyme production, and the ability to evade or modulate plant defense responses.

4. Plant–Endophytic Bacteria Interactions

The interaction between plants and endophytic bacteria is generally mutualistic, with both partners benefiting from the association. Plants provide carbon sources and a protected niche, while endophytes enhance plant growth and health (Berg & Hallmann, 2006).

Plant–endophyte communication involves complex molecular signaling, including phytohormones, quorum-sensing molecules, and secondary metabolites. These interactions are influenced by plant genotype, bacterial strain, environmental conditions, and agricultural practices (Hardoim et al., 2015).

5. Mechanisms of Plant Growth Promotion

Endophytic bacteria promote plant growth through a variety of direct and indirect mechanisms.

5.1 Biological Nitrogen Fixation

Several endophytic bacteria, including *Azospirillum*, *Herbaspirillum*, and *Gluconacetobacter*, possess nitrogen-fixing能力 and convert atmospheric nitrogen into ammonia, which can be utilized by plants (James & Olivares, 1998). This reduces dependence on synthetic nitrogen fertilizers.

5.2 Phosphate Solubilization

Phosphorus is often present in insoluble forms in soil. Endophytic bacteria solubilize phosphate by producing organic acids and phosphatases, thereby enhancing phosphorus availability to plants (Vessey, 2003).

5.3 Phytohormone Production

Many endophytic bacteria synthesize phytohormones such as indole-3-acetic acid (IAA), cytokinins, and gibberellins. These hormones stimulate root development, increase nutrient uptake, and promote overall plant growth (Santoyo et al., 2016).

5.4 Siderophore Production

Iron is an essential micronutrient but is often poorly available in soil. Endophytic bacteria produce siderophores that chelate iron and facilitate its uptake by plants while limiting iron availability to pathogenic microorganisms (Lugtenberg & Kamilova, 2009).

5.5 ACC Deaminase Activity

Stress conditions often increase ethylene levels in plants, inhibiting growth. Endophytic bacteria producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase lower ethylene levels and enhance plant tolerance to stress (Glick, 2014).

6. Role of Endophytic Bacteria in Abiotic Stress Tolerance

Endophytic bacteria play a crucial role in enhancing plant tolerance to abiotic stresses such as drought, salinity, temperature extremes, and heavy metal toxicity. They improve water and nutrient uptake, regulate stress-related hormones, and enhance antioxidant enzyme activity (Yang et al., 2009).

For example, endophytic *Bacillus* and *Pseudomonas* species have been shown to improve drought and salt tolerance in crops by modulating osmolyte accumulation and reducing oxidative stress (Santoyo et al., 2016).

7. Biocontrol of Plant Pathogens

Endophytic bacteria protect plants against pathogens through multiple mechanisms, including:

- Production of antimicrobial compounds and lytic enzymes
- Competition for nutrients and ecological niches
- Induction of systemic resistance in host plants

These mechanisms make endophytic bacteria effective biocontrol agents against fungal, bacterial, and sometimes viral pathogens (Backman & Sikora, 2008).

8. Applications of Endophytic Bacteria in Agriculture

8.1 Biofertilizers

Endophytic bacteria are used as biofertilizers to improve nutrient availability and enhance crop yield. Their internal colonization ensures sustained interaction with the host plant, leading to improved nutrient use efficiency (Vessey, 2003).

8.2 Biopesticides

Due to their antagonistic activity against pathogens, endophytic bacteria are incorporated into biopesticide formulations, reducing reliance on chemical pesticides (Berg & Hallmann, 2006).

8.3 Biostimulants

Endophytic bacteria function as biostimulants by enhancing plant growth, stress tolerance, and crop quality, even under suboptimal conditions (Du Jardin, 2015).

8.4 Crop-Specific Applications

Positive effects of endophytic bacteria have been reported in major crops such as rice, wheat, maize, soybean, and horticultural crops, demonstrating their broad applicability in agriculture (Santoyo et al., 2016).

9. Challenges and Limitations

Despite their potential, the widespread adoption of endophytic bacteria in agriculture faces several challenges, including inconsistent field performance, host specificity, formulation and shelf-life issues, and regulatory constraints (Hardoim et al., 2015). Addressing these challenges requires extensive field trials and improved formulation technologies.

10. Future Prospects and Conclusion

Advances in genomics, transcriptomics, and metabolomics are expected to enhance our understanding of plant–endophyte interactions and facilitate the development of more effective microbial inoculants. The use of multi-strain consortia and integration with

precision agriculture systems may further improve the reliability of endophytic bacteria in the field.

In conclusion, endophytic bacteria represent a powerful and sustainable tool for modern agriculture. Their ability to promote plant growth, enhance stress tolerance, and protect against pathogens makes them valuable alternatives to chemical inputs. Continued research and supportive regulatory frameworks will be essential for their successful commercialization and large-scale application.

Conflict of Interest

The authors declare that they have no known competing financial interests in the work reported in this paper

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Evidence-Driven Clinical Documentation and Drug Surveillance

Suranjana Sarkar[1]*, Priyajit Banerjee[2], Rani Bhagat[2], Megha Bagh[2]

[1]Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata- 700121

[2]Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata- 700121

**Corresponding Author: suranjanas@svu.ac.in*

Abstract

Pharmacovigilance remains a cornerstone of modern healthcare systems, ensuring the detection, assessment, understanding, and prevention of adverse drug reactions (ADRs) throughout a product's life cycle. The increasing complexity of therapeutic regimens, biologics, and polypharmacy has intensified the need for accurate, real-time clinical documentation. In parallel, medical scribing has emerged as a critical digital health workforce that supports clinicians by capturing structured and unstructured clinical data within electronic health records (EHRs). This review critically examines the evolving intersection between pharmacovigilance and medical scribing, emphasizing how real-time clinical documentation contributes to drug safety surveillance, regulatory reporting, and clinical decision-making. Key domains reviewed include the role and responsibilities of medical scribes, foundational anatomy and physiology knowledge, medical terminology proficiency, EHR systems and workflows, and real-time patient data capture. Recent literature (≤5 years) demonstrates that optimized scribing workflows enhance documentation completeness, reduce clinician burnout, and improve the fidelity of safety-relevant data for post-marketing surveillance. However, challenges persist regarding data standardization, training variability, privacy, and regulatory harmonization. This review highlights knowledge gaps and outlines future directions, including artificial intelligence–assisted scribing, standardized pharmacovigilance training modules, and tighter integration between EHRs and national safety databases. Strengthening the role of medical scribes represents a translational opportunity to improve pharmacovigilance outcomes and patient safety in digitally driven healthcare ecosystems.

Keywords: Adverse drug reactions (ADRs), Biologics, Electronic health records (EHRs), Pharmacovigilance, Post-marketing surveillance

1. Introduction

Medicinal products are fundamental to disease prevention and treatment; however, their safety profiles frequently continue to evolve after regulatory approval. Clinical trials conducted prior to market authorization are inherently limited by controlled conditions, restricted sample sizes, short follow-up periods, and selective patient populations. As a result, rare, delayed, or population-specific adverse drug reactions (ADRs) often remain undetected until medicines are widely prescribed in routine clinical practice (Edwards & Aronson, 2000; World Health Organization [WHO], 2020). Landmark post-marketing safety failures, such as the withdrawal of rofecoxib following identification of increased cardiovascular risk, have demonstrated the potentially severe consequences of delayed adverse event detection and reinforced the necessity of continuous safety monitoring beyond approval.

Pharmacovigilance has therefore evolved into a critical public health function rather than a purely regulatory requirement. In real-world healthcare settings, patients frequently experience polypharmacy, comorbid conditions, age-related physiological changes, and genetic variability, factors that substantially influence drug response but are underrepresented in pre-approval trials (WHO, 2020). These complexities contribute to clinically significant ADRs including hepatotoxicity, cardiotoxicity, immune-mediated reactions, and drug–drug interactions, many of which are identified only during post-marketing use. Despite their clinical importance, global pharmacovigilance systems continue to face substantial underreporting; it is estimated that less than 10% of serious ADRs are formally captured through spontaneous reporting mechanisms (Uppsala Monitoring Centre [UMC], 2021).

The digitization of healthcare through electronic health records (EHRs) has created new opportunities for pharmacovigilance by enabling the large-scale capture of real-world clinical data. EHR-derived information has increasingly been leveraged for signal detection, pharmacoepidemiological studies, and regulatory decision-making (Bates et al., 2021). However, the quality of pharmacovigilance outputs is intrinsically dependent on the accuracy and completeness of frontline clinical documentation. Incomplete medication histories, inconsistent symptom descriptions, and non-standardized terminology remain major barriers to effective signal detection and causality assessment within digital systems (FDA, 2023).

In high-intensity clinical environments, such as emergency departments, oncology clinics, and intensive care units, documentation often competes with direct patient care for clinician time and cognitive resources. Evidence suggests that documentation burden contributes to clinician fatigue and increases the likelihood of omissions in medication and adverse event recording (Melnick et al., 2020). These gaps in documentation can delay the recognition of emerging safety signals, particularly for subtle or cumulative toxicities that rely on precise temporal relationships between drug exposure and symptom onset.

Medical scribes have emerged as an important workforce innovation within this evolving digital landscape. Operating under physician supervision, scribes document clinical encounters in real time, capturing patient histories, medication use, diagnostic assessments, and therapeutic decisions directly into EHR systems. While originally introduced to improve workflow efficiency, recent studies indicate that scribe-assisted documentation enhances the completeness and consistency of clinical records, including safety-relevant data elements essential for pharmacovigilance analyses (Melnick et al., 2020; Bates et al., 2021). Real-world observations suggest that early or mild ADRs, such as transient neurological symptoms, gastrointestinal intolerance, or laboratory abnormalities, are more likely to be documented when clinicians are supported by trained documentation personnel.

As pharmacovigilance increasingly relies on advanced analytics, machine learning, and real-world evidence frameworks, the integrity of source data has become a central determinant of system performance. Errors or omissions at the point of documentation propagate through downstream surveillance pipelines, potentially delaying regulatory action and patient risk mitigation (FDA, 2023). Consequently, the integration of accurate real-time clinical documentation into pharmacovigilance strategies represents a critical translational opportunity. Recognizing pharmacovigilance as an embedded clinical responsibility, supported by digital tools and specialized documentation professionals, will be essential for strengthening drug safety systems and protecting patient health in increasingly complex therapeutic landscapes.

2. Medical Scribing: Role and Responsibilities

Medicinal products form the backbone of modern healthcare; however, their safety profiles frequently evolve beyond the point of regulatory approval. Pre-marketing clinical trials, while essential for establishing efficacy and baseline safety, are conducted in controlled environments with limited patient diversity, short follow-up durations, and exclusion of complex clinical scenarios. Consequently, rare, delayed, or context-specific adverse drug reactions (ADRs) often remain undetected until medicines are introduced into routine clinical practice (Edwards & Aronson, 2000; World Health Organization [WHO], 2020). High-profile post-marketing safety failures, including drug withdrawals due to cardiovascular, hepatic, or neuropsychiatric toxicity, illustrate the critical need for continuous pharmacovigilance grounded in real-world clinical data.

Within this landscape, medical scribes have emerged as key contributors to clinical documentation. Their primary responsibility is to capture comprehensive, real-time clinical information under physician supervision, including medication histories, treatment decisions, and evolving patient symptoms. Although originally implemented to reduce clinician documentation burden, the scribe role has increasingly demonstrated relevance to pharmacovigilance by improving the completeness and temporal accuracy of safety-relevant data within electronic health records (EHRs).

2.1 Anatomy and Physiology Overview for Medical Scribes

Effective pharmacovigilance relies on the accurate clinical interpretation of adverse events, which in turn requires an understanding of basic anatomy and physiology. In real-world settings, ADRs often manifest as organ-specific toxicities such as hepatotoxicity, nephrotoxicity, cardiotoxicity, or immune-mediated reactions. These manifestations may be subtle, nonspecific, or delayed, making them particularly vulnerable to under-documentation. Medical scribes equipped with foundational biomedical knowledge are better positioned to document clinically meaningful symptom patterns and laboratory abnormalities that support downstream causality assessment (WHO, 2020). This competency strengthens the linkage between bedside observations and pharmacovigilance databases, where mechanistic interpretation of ADRs is essential.

2.2 Medical Terminology and Abbreviations

Standardized medical terminology plays a pivotal role in pharmacovigilance, particularly in the era of large-scale data mining and automated signal detection. Inconsistent abbreviations, ambiguous descriptors, and non-standard language reduce the interpretability of EHR-derived data and compromise the reliability of adverse event reporting systems. Underreporting and misclassification of ADRs remain global challenges, with estimates suggesting that fewer than 10% of serious reactions are formally reported (Uppsala Monitoring Centre [UMC], 2021). Medical scribes trained in standardized clinical vocabulary contribute to improved data harmonization, thereby enhancing the quality of safety signals extracted from routine clinical documentation.

2.3 Electronic Health Records (EHRs): Systems and Workflow

The widespread adoption of EHRs has transformed pharmacovigilance by enabling access to real-world evidence at an unprecedented scale. Regulatory agencies increasingly rely on EHR-derived data to complement spontaneous reporting systems and inform risk management decisions (U.S. Food and Drug Administration [FDA], 2023). However, EHR systems are only as effective as the workflows that populate them. High documentation burden, time pressure, and fragmented interfaces often result in incomplete or delayed recording of adverse events. Integration of medical scribes into EHR workflows has been shown to improve documentation completeness and consistency, thereby strengthening the foundational data upon which pharmacovigilance systems depend (Bates et al., 2021).

2.4 Real-Time Medical Documentation: Patient History, Symptoms, and Treatments

Temporal accuracy is a cornerstone of adverse drug reaction assessment, as causality frequently depends on the relationship between drug exposure and symptom onset. In busy clinical environments such as emergency departments and oncology units, real-time documentation is often deprioritized, increasing the risk of missed or poorly characterized ADRs. Documentation burden has been directly linked to clinician fatigue and increased omission of safety-relevant information (Melnick et al., 2020; Kopacheva et al., 2025). Medical scribes facilitate contemporaneous recording of patient histories, symptom evolution, and treatment modifications, enabling more reliable pharmacovigilance analyses and earlier detection of emerging safety signals.

Collectively, these domains highlight the evolving interface between pharmacovigilance and medical scribing. As drug safety surveillance increasingly incorporates real-world data analytics, machine learning, and proactive risk detection, the quality of frontline clinical documentation becomes a critical determinant of patient safety outcomes. Strengthening this interface represents a translational opportunity to embed pharmacovigilance within routine clinical practice rather than relegating it to post-hoc regulatory reporting.

3. Real-Time Medical Documentation

Real-time documentation is a high-yield leverage point for pharmacovigilance because most adverse drug reactions (ADRs) are adjudicated through time-dependent logic: exposure precedes symptom onset, dechallenge improves the event, and rechallenge (when it occurs) reproduces it. These causal features are routinely assessed in clinical practice and are embedded in computational approaches that mine EHR data for signals. In EHR-based pharmacovigilance, analytic performance depends heavily on the precision of timestamps (start/stop dates, dose changes, administration route), symptom onset chronology, and concurrent confounders such as intercurrent infection, organ dysfunction, or interacting co-medications. A major limitation highlighted across EHR signal-identification studies is heterogeneity and incompleteness of exposure and outcome documentation, which weakens confounding control and blurs temporal relationships, thereby reducing signal validity (Davis et al., 2023). Similarly, scoping evidence in AI-enabled adverse drug event (ADE) prediction/detection underscores that many models perform well technically but struggle to translate clinically when underlying data quality and temporal completeness are poor (Syrowatka et al., 2022).

Scribes can strengthen pharmacovigilance relevance by improving the clinical narrative structure and capturing “micro-timelines” that clinicians often omit under time pressure: when a new drug was initiated, when the first abnormal symptom was noticed, when the dose was escalated, when treatment was stopped, and what happened afterward. This is particularly important because a substantial fraction of ADE evidence resides in unstructured text (progress notes, triage narratives, discharge summaries), and extracting temporal relationships from such narratives is a recognized methodological priority in EHR-based safety surveillance (Ageno et al., 2023; Davis et al., 2023). By capturing details such as symptom progression, medication adherence, and over-the-counter or herbal exposures in

near real time, scribes can increase the amount of safety-relevant text that later becomes available to pharmacovigilance methods including natural language processing and hybrid structured–unstructured detection pipelines (Syrowatka et al., 2022).

Illustrative case vignette (real-world typical pattern): A 68-year-old patient with atrial fibrillation is stable on warfarin and presents to an emergency department with cough and fever. An antibiotic is started, and 3–5 days later the patient develops epistaxis and melena, with a markedly elevated INR. In many routine notes, the antibiotic start date, adherence, and timing of bleeding onset may be recorded imprecisely (for example “recent antibiotic” or “bleeding for a few days”), which can obscure causality and delay recognition of a clinically important interaction signal at the health-system level. In a scribe-supported encounter, the medication timeline is more likely to be captured explicitly (warfarin dose and schedule, antibiotic name and first dose date/time, onset and evolution of bleeding symptoms, relevant diet/alcohol changes, and follow-up INR checks), enabling clearer dechallenge documentation (warfarin held, INR improves, bleeding resolves) and better downstream case ascertainment for pharmacovigilance. While this vignette reflects a common ADR causality pattern rather than a single published patient, it illustrates the mechanism by which real-time documentation quality directly affects signal detectability and validity in EHR-driven surveillance (Davis et al., 2023; Syrowatka et al., 2022).

From a workflow science perspective, scribes do more than type notes: they change when and how clinical data are entered into the EHR, including edits to diagnoses, exam findings, and orders, which can influence the completeness of safety-relevant fields and the internal consistency between structured codes and narrative text. Audit-log–based analysis has shown that scribe contributions extend beyond the “progress note,” and that scribe impact on documentation workflows varies by physician and scribe training, emphasizing the need for best-practice implementation to maximize quality and minimize risk (Rule et al., 2022). Complementing this, qualitative safety-focused investigation has reported that organizations often perceive scribes as reducing EHR-related patient safety risks when best practices and appropriate oversight are in place, an important consideration for pharmacovigilance, where documentation errors can propagate into surveillance pipelines (Ash et al., 2021).

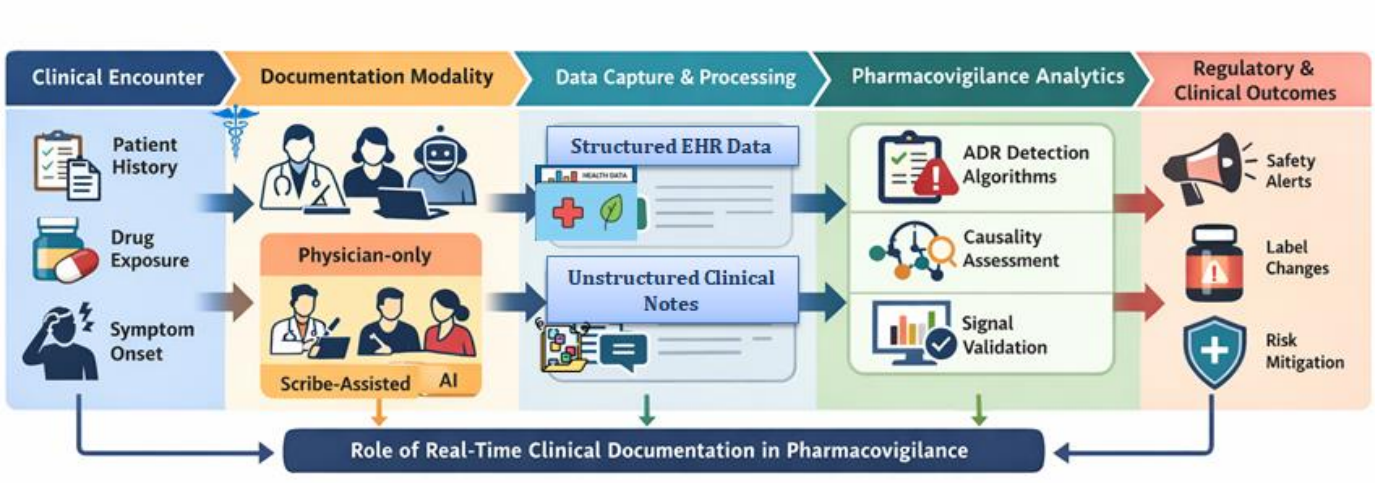

Figure 1: Overview of Pharmacovigilance Workflow

Virtual scribing models add another translational dimension: they can expand access to real-time documentation support and potentially standardize note structure across clinics, while raising distinct governance issues around latency, communication loops, and privacy. Quality-improvement evidence indicates that virtual scribes are associated with reductions in physicians' EHR time and changes in note and order composition, suggesting that documentation can be reshaped at scale; however, pharmacovigilance benefit still hinges on whether these workflow gains translate into more complete exposure–event timelines and improved capture of mild-to-moderate ADRs (Rotenstein et al., 2024). This matters because mild-to-moderate ADRs (for example early intolerance, mild rash, transient neurocognitive symptoms) are disproportionately under-recorded or recorded without temporal anchors, yet they can represent early warning signals, especially when clustered across patients or when they precede serious events.

Real-time documentation should be treated as a pharmacovigilance intervention: it strengthens causal interpretability at the individual patient level and improves case-finding fidelity at the population level. The strongest translational opportunity is not simply "more documentation," but higher-resolution medication timelines, standardized terminology, and narrative clarity that allows EHR-based pharmacovigilance to exploit both structured elements (medication orders, labs) and unstructured clinical text. Given that current EHR signal-identification efforts are limited by inconsistent temporal considerations and variable data quality, systematic reinforcement of real-time documentation, through trained scribes

and robust oversight, represents a practical route to improving signal validity and clinical actionability (Davis et al., 2023; Ash et al., 2021).

Table 1: Comparison of Clinical Documentation Approaches and Their Impact on Pharmacovigilance

Documentation approach	Key characteristics	Pharmacovigilance relevance	Strengths	Limitations	Translational potential
Physician-only documentation	Clinician documents encounter during or after visit	ADR detection depends on clinician recall and voluntary reporting	High clinical judgment; contextual insight	Underreporting; poor temporal resolution; high cognitive burden (Edwards & Aronson, 2000; Melnick et al., 2020)	Moderate
Scribe-assisted real-time documentation	Trained scribes document in real time under clinician supervision	Improved capture of exposure–event chronology and symptom evolution	More complete ADR timelines; reduced omission of mild/moderate ADRs (Ash et al., 2021; Bates et al., 2021)	Requires standardized training; governance and oversight needed	High
Structured EHR documentation tools	Checklists, coded fields, templates	Facilitates automated ADR detection and regulatory reporting	Standardization; interoperability; scalable analytics (Davis et al., 2023)	Limited narrative nuance; may miss early or atypical ADRs	High
AI-assisted documentation (NLP, speech recognition)	Automated extraction from clinical text or speech	Enables large-scale ADE signal detection from unstructured data	Scalable; supports real-world evidence generation (Syrowatka et al., 2022)	Susceptible to bias; dependent on data quality	Emerging
Spontaneous ADR reporting systems	Voluntary reporting to national/international databases	Regulatory signal validation and risk communication	Global reach; regulatory acceptance (WHO, 2020)	Severe underreporting; delayed signal detection (UMC, 2021)	Moderate

5. **Thematic Critical Review**

Thematic critical review provides a structured framework to synthesize heterogeneous evidence across disciplines while moving beyond descriptive summarization toward analytical integration. In the context of pharmacovigilance and clinical documentation, this approach is particularly valuable because relevant evidence spans regulatory science, clinical

pharmacology, health informatics, and healthcare workflow research. Rather than evaluating individual interventions in isolation, a thematic lens enables comparison of mechanisms, strengths, and limitations across emerging strategies that influence drug safety surveillance in real-world settings. The overview of the pharmacovigilence workflow is schematically illustrated in Figure 1.

5.1 Efficacy of Enhanced Documentation for Pharmacovigilance

High-quality documentation from scribes improves the signal quality available for EHR-based surveillance by ensuring that key data elements, including medication exposure and clinical outcomes, re accurately recorded**.** This enhances temporal causal inference in pharmacovigilance analyses that depend on precise timing of events and interventions.

5.2 Limitations and Risk Factors

Limitations include variability in scribe proficiency, incomplete clinical context, and inconsistencies in terminology usage. Digital brace technologies may introduce errors if models misinterpret clinical language or if transcription biases exist. These documentation inaccuracies can degrade the signal-to-noise ratio in pharmacovigilance data and potentially generate spurious associations.

6. Challenges & Knowledge Gaps

Major challenges remain in standardized integration of documentation workflows with pharmacovigilance analytics. Specific gaps include:

- Training standards for scribes that incorporate pharmacovigilance-relevant data needs.
- EHR interoperability and data harmonization across institutions.
- Methodologic consensus on handling confounding and bias in EHR-based signal detection.

7. **Future Directions & Clinical Translation**

Future research should:

- Establish curricula for scribes that emphasize clinical coding and drug safety documentation.
- Develop validated informatics pipelines linking EHR narratives to pharmacovigilance systems using advanced NLP.
- Conduct multicenter clinical outcome studies assessing documentation quality impacts on drug safety signal detection.

6. Conclusion

Integrating medical scribing practices with pharmacovigilance frameworks offers a potent strategy to enrich drug safety surveillance. Optimized documentation, whether human or AI-augmented, enhances the utility of EHR data for real-world pharmacovigilance and ultimately improves patient safety. Strategic investments in training, technology, and methodological rigor are essential to realize this potential.

Conflict of Interest

The authors declare that they have no known competing financial interests in the work reported in this paper

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Nipah Virus Infection: Mechanisms of Disease, Clinical Care, and Neonatal Management

Priyanka Chakraborty*

Dept. of Microbiology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barrackpore, Barasat, Kolkata-700121

**Corresponding author: priyankachakraborty44@gmail.com*

Abstract

Nipah virus (NiV) is a highly pathogenic zoonotic virus associated with severe encephalitis and respiratory disease in humans, with case fatality rates among the highest reported for emerging viral infections. Since its discovery in the late 1990s, recurrent outbreaks in South and Southeast Asia have highlighted its epidemic and pandemic potential. Beyond understanding viral biology, effective management of Nipah virus disease requires comprehensive knowledge of infection mechanisms, clinical care across all age groups, and special considerations for vulnerable populations such as infants and neonates. This book chapter provides an in-depth discussion of Nipah virus infection mechanisms at the molecular and cellular levels, outlines evidence-based clinical care and supportive management strategies, and presents detailed guidance on neonatal and infant care in the context of Nipah virus exposure or infection. The chapter is intended for clinicians, researchers, nurses, public health professionals, and postgraduate students, offering an integrated perspective on pathogenesis and patient-centred care.

Keywords: Nipah virus, infection mechanisms, clinical management, neonatal care, encephalitis, emerging viruses

1. Introduction

Nipah virus (NiV) is an emerging zoonotic pathogen that poses a significant threat to global health due to its high mortality rate, lack of approved antiviral therapies, and ability to transmit from animals to humans and between humans. Nipah virus belongs to the genus *Henipavirus* in the family *Paramyxoviridae*. Human infection is characterized by acute febrile illness that rapidly progresses to severe neurological and respiratory complications. Wang, L.et al. (2018)

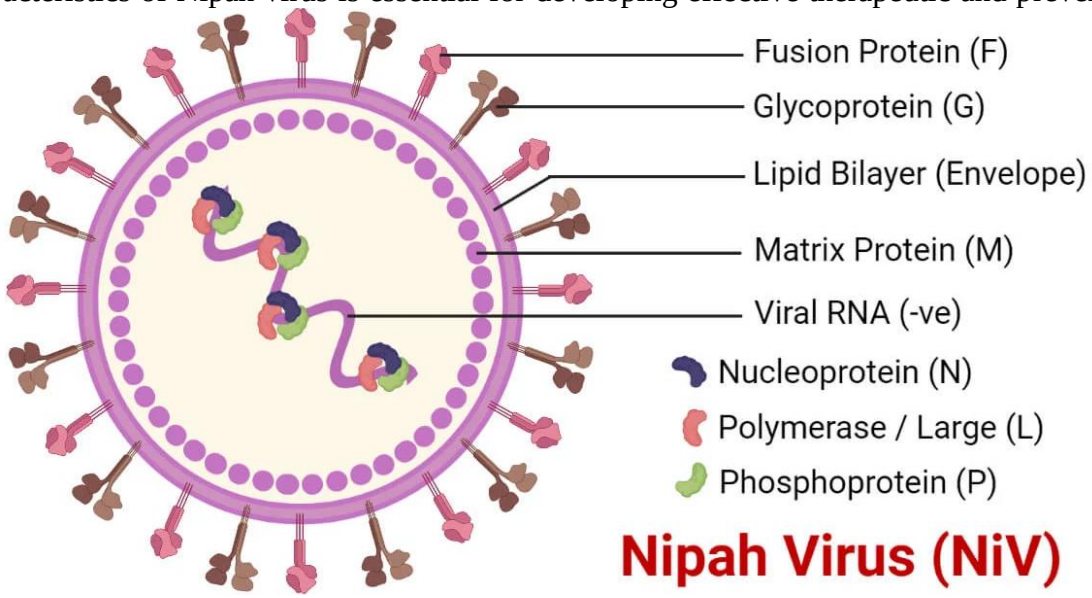
Outbreaks of Nipah virus infection have been reported primarily in Malaysia, Bangladesh, and India, with sporadic cases elsewhere. D. T., et al. (2019). The repeated emergence of NiV reflects increasing human–animal–environment interactions driven by deforestation, agricultural intensification, climate change, and urban expansion**.**Mackenzie, J.et al. (2001). While much attention has been given to epidemiology and virology, there remains a critical need for comprehensive resources addressing infection mechanisms alongside practical clinical care, including neonatal and infant management. This chapter aims to fill that gap by integrating mechanistic insights with clinical and caregiving perspectives.

2. Overview of Nipah Virus Biology

Nipah virus (NiV) is an envelope, negative-sense, single-stranded RNA virus with a genome of approximately 18.2 kilobases. The viral genome encodes six major structural proteins: nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), attachment glycoprotein (G), and large polymerase (L). Khetawat, D., et al. (2008). Each of these proteins plays a distinct role in viral replication, structural stability, and host interaction. The nucleocapsid (N) protein encapsidates the viral RNA genome, forming a ribonucleoprotein complex that protects the RNA from degradation and serves as the template for transcription and replication. The phosphoprotein (P) functions as a cofactor for the viral polymerase and also participates in antagonizing host immune responses. The matrix (M) protein is responsible for viral assembly and budding. It connects the ribonucleoprotein complex with the viral envelope and coordinates the release of newly formed virions from infected cells. The large polymerase (L) protein acts as the RNA-dependent RNA polymerase that catalyzes transcription of viral mRNA and replication of the viral genome. Together, these proteins enable efficient viral propagation within host cells. The G and F glycoproteins are critical determinants of viral entry and host range. Aguilar, H. C.et al. (2014). The attachment glycoprotein (G) binds to specific receptors on the host cell surface, initiating the infection process. Following receptor

binding, the fusion glycoprotein (F) mediates the merging of the viral envelope with the host cell membrane, allowing the viral genome to enter the cytoplasm. This coordinated interaction between G and F proteins is essential for viral infectivity and cell-to-cell spread.

NiV exhibits a remarkably broad host tropism, infecting a wide range of mammalian species. The primary cellular receptors for Nipah virus are ephrin-B2 and ephrin-B3, Rahman, M. et al. (2011). These receptors are highly conserved across mammalian species and are widely expressed in endothelial cells, neurons, and respiratory epithelial cells. The presence of these receptors in vital organs explains the systemic nature of Nipah virus infection.The wide distribution of ephrin receptors also accounts for the virus's strong affinity for the central nervous system and vascular endothelium. As a result, infection frequently leads to neurological disease, vasculitis, and respiratory complications. In addition, the high conservation of these receptors among mammals contributes to the virus's ability to cross species barriers, facilitating zoonotic transmission from animal reservoirs, particularly fruit bats of the genus Pteropus, to humans and other animals. Understanding the biological characteristics of Nipah virus is essential for developing effective therapeutic and preventive



strategies. Knowledge of viral proteins, receptor interactions, and host cell tropism provides important insights into viral pathogenesis and informs the design of vaccines, antiviral drugs, and diagnostic tools.

Figure 1: Structure of Nipah Virus: Nipah virus is an enveloped, negative-sense single-stranded RNA virus of the *Henipavirus* genus. The virion contains surface glycoproteins G (attachment) and F (fusion) embedded in a lipid envelope. Internally, the RNA genome is associated with nucleocapsid (N), phosphoprotein (P), matrix (M), and polymerase (L) proteins essential for replication and assembly Salleh.et al. (2025).

3. Mechanisms of Nipah Virus Infection

3.1 Viral Entry and Cell Tropism

Infection begins when the viral G glycoprotein binds to ephrin-B2 or ephrin-B3 receptors on host cells. This interaction triggers conformational changes in the F glycoprotein, leading to fusion of the viral envelope with the host cell membrane. Unlike many viruses that rely on endocytosis, Nipah virus can fuse directly at the plasma membrane, allowing efficient viral entry**.**Satterfield, B. A., Cross, R. W., & Geisbert, T. W. (2016).

The widespread expression of ephrin receptors enables Nipah virus to infect multiple cell types, including endothelial cells, neurons, smooth muscle cells, and respiratory epithelial cells. This broad tropism underlies the multisystem nature of Nipah virus disease**.**Ksiazek, T. G., et al. (2001).

3.2 Viral Replication and Spread

Following entry, the viral ribonucleoprotein complex is released into the cytoplasm, where transcription and replication occur. The viral RNA-dependent RNA polymerase synthesizes viral mRNAs and genomic RNA, leading to the production of new viral proteins. Assembly occurs at the host cell membrane, and progeny virions are released by budding.Bergfeld, J., et al. (2016),Luby, S. P., Gurley, E. S., & Hossain, M. J. (2009).

Cell-to-cell fusion mediated by the F and G proteins results in the formation of multinucleated syncytia, facilitating viral spread while partially evading host immune surveillance**.**Clayton, B. A. (2017).

3.3 Endothelial Dysfunction and Vasculitis

Syrian hamsters as a model of Nipah virus disease. Feldmann, H.et al. (2016). One of the hallmark features of Nipah virus infection is widespread vasculitis. Infection of endothelial cells leads to inflammation, increased vascular permeability, thrombosis, and microhemorrhages. These changes contribute to cerebral edema, hemorrhagic lesions, and multi-organ dysfunction.

3.4 Neuroinvasion and Encephalitis

Nipah virus gains access to the central nervous system through both hematogenous spread and retrograde axonal transport. Infection of neurons and supporting glial cells results in

severe encephalitis characterized by neuronal necrosis, inflammation, and disruption of the blood–brain barrier. Clinically, this manifests as altered consciousness, seizures, and coma.

3.5 Immune Evasion and Dysregulation

NiV has evolved mechanisms to suppress host innate immune responses. Viral proteins interfere with interferon signaling pathways, impairing antiviral defenses. The resulting dysregulated immune response contributes to uncontrolled viral replication and tissue damage.Lo, M. K., Lowe, L., Hummel, K. B., et al. (2012).

4. Clinical Manifestations Across Age Groups

The incubation period for Nipah virus infection typically ranges from 4 to 14 days, although longer incubation periods of up to 45 days have occasionally been reported. During this period, infected individuals may remain asymptomatic while viral replication occurs within the body. The variability in incubation duration can complicate early detection and outbreak control, particularly in areas with limited surveillance systems. The initial symptoms of Nipah virus infection are generally nonspecific and resemble those of many other viral illnesses. Early manifestations often include fever, headache, myalgia, fatigue, sore throat, dizziness, and vomiting. These symptoms may persist for several days before progressing to more severe disease. In some patients, respiratory symptoms such as cough, shortness of breath, and chest discomfort may develop during the early phase of infection. As the disease progresses, severe complications may arise involving the respiratory and nervous systems. Respiratory manifestations may include acute respiratory distress syndrome (ARDS), pneumonia, and severe hypoxia requiring ventilatory support. In many outbreaks, respiratory involvement has been associated with increased person-to-person transmission due to the presence of virus in respiratory secretions.

Neurological involvement is one of the most serious manifestations of Nipah virus infection. Adults and older children frequently develop acute encephalitis characterized by inflammation of the brain. Clinical signs of encephalitis may include altered consciousness, confusion, drowsiness, seizures, disorientation, and coma. Rapid deterioration can occur within 24 to 48 hours after the onset of neurological symptoms, making early clinical recognition critically important. In infants and neonates, the clinical presentation may differ significantly from that observed in adults. Young infants may display subtle or atypical symptoms such as poor feeding, lethargy, irritability, apnea, temperature instability, or

seizures. Because these signs are common to many neonatal infections and metabolic disorders, diagnosis may be challenging without epidemiological context or laboratory confirmation.

Another important feature of Nipah virus infection is the possibility of relapsing or late-onset encephalitis. Some patients who initially recover from acute infection may later develop neurological symptoms months or even years after the initial illness. These relapses are believed to result from persistent viral infection within the central nervous system. Symptoms during relapse may include seizures, behavioural changes, personality alterations, and cognitive decline. The severity of clinical manifestations can vary depending on factors such as viral strain, host immune response, age, and underlying medical conditions. Overall case fatality rates remain high, often ranging from 40% to 75% in documented outbreaks, highlighting the need for rapid clinical intervention and strong public health response.

5. Clinical Care and Management of Nipah Virus Infection

5.1 Principles of Clinical Care

There is currently no licensed antiviral treatment for Nipah virus infection. Rota, P. A., et al. (2000). Therefore, clinical care is primarily supportive and aimed at managing complications, maintaining vital functions, and preventing secondary infections**.**Tan, K. S., Tan, C. T., & Goh, K. J. (1999). Early recognition and prompt supportive care are critical for improving outcomes**.**Prescott, J., de Wit, E., Feldmann, F., et al. (2015).

5.2 Supportive Medical Management

Supportive care includes aggressive management of fever, hydration, electrolyte balance, and nutritional support. Patients with respiratory involvement may require supplemental oxygen, noninvasive ventilation, or mechanical ventilation. Neurological complications require close monitoring, seizure control, and measures to reduce intracranial pressure**.**Hossain, M. J.,Gurley, E. S., Montgomery, J. M., et al. (2008),Schountz, T., Prescott, J., & Feldmann, H. (2014).

5.3 Infection Prevention and Control in Healthcare Settings

Strict infection control measures are essential to prevent nosocomial transmission. These include patient isolation, use of personal protective equipment, hand hygiene, and safe

handling of bodily fluids. Healthcare workers should receive training in high-risk pathogen management**.**Hossain, M. J., et al. (2016).

5.4 Experimental Therapies

Several experimental therapies have been explored, including ribavirin and monoclonal antibodies targeting the viral G glycoprotein. While promising results have been observed in animal models, clinical evidence remains limited, and such interventions should be considered investigational.

6. Neonatal and Infant Care in Nipah Virus Infection

6.1 Risk of Infection in Neonates and Infants

Neonates and infants represent a particularly vulnerable population due to immature immune systems and dependence on caregivers**.** Senthil Kumar, D.et al. (2013). Potential routes of exposure include vertical transmission, close contact with infected caregivers**,**J. M., Hossain, M. J., et al. (2007)., Wong, K. T., Shieh, W. J., Kumar, S., et al. (2002). and nosocomial exposure.Daszak, P.et al. (2006).

6.2 Clinical Presentation in Infants

Infected infants may present with nonspecific symptoms such as poor feeding, irritability, hypothermia or fever, respiratory distress, apnea, or seizures.Feldmann, H., & Geisbert, T. W. (2011)**.**Because these signs overlap with other neonatal conditions, a high index of suspicion is required in outbreak settings. Chua, K. B. (2010).

6.3 Principles of Neonatal Care

Care of neonates potentially exposed to Nipah virus should prioritize infection prevention, supportive management, and close monitoring. Isolation of the infant, use of appropriate personal protective equipment by caregivers, and minimization of unnecessary handling are essential.DeBuysscher, & Prescott, J.et al. (2014).

6.4 Feeding and Nutrition

Breastfeeding decisions should be individualized based on maternal infection status and local public health guidance. If the mother is infected or suspected of infection, expressed breast milk may need to be avoided unless safety can be assured. Alternative feeding strategies

should ensure adequate nutrition while minimizing infection risk**.**Eaton, B. T., Broder, C. C., Middleton, D., & Wang, L. F. (2006).

6.5 Family-Centered and Psychological Care

Families of infected infants experience significant psychological stress. Clearcommunication, counseling, and psychosocial support are essential components ofcomprehensive care. Ethical considerations, including parental presence and bonding, mustbe balanced against infection control requirements**.**Broder, C. C., Xu, K., Nikolov, D. B., et al. (2013).

7. Public Health Measures and Community Care

Public health measures play a critical role in preventing and controlling outbreaks of Nipah virus infection. Because the virus is zoonotic and capable of human-to-human transmission, effective prevention strategies must involve coordinated actions at the community, healthcare, and governmental levels. Community-based interventions are particularly important for protecting vulnerable populations, including infants, pregnant women, elderly individuals, and immuno-compromised persons. Public health education programs should focus on increasing awareness about the sources of Nipah virus infection, modes of transmission, and preventive practices that can reduce the risk of exposure.

One of the major sources of Nipah virus infection in several outbreaks has been the consumption of raw date palm sap contaminated by fruit bats. Public health authorities should therefore promote safe food practices such as avoiding raw palm sap, thoroughly washing fruits, and preventing bats from accessing food collection containers. These preventive strategies have been shown to significantly reduce the risk of zoonotic transmission.

Reducing direct contact with bats and other potentially infected animals is another important preventive measure. Communities living near bat habitats should be informed about the risks associated with handling sick animals, consuming partially eaten fruits, or entering areas heavily populated by bats. Early detection and reporting of suspected cases are also essential components of outbreak control. Community health workers and local healthcare providers should be trained to recognize early symptoms of Nipah virus infection and promptly notify public health authorities. Rapid case identification allows for timely isolation of infected individuals, contact tracing, and implementation of quarantine measures. Community engagement and risk communication are equally important during outbreaks. Transparent communication helps build trust between health authorities and the public, reducing

misinformation and panic. Public awareness campaigns through media, schools, and local organizations can encourage individuals to seek medical care promptly when symptoms develop. In addition, community care initiatives should include psychosocial support for affected families. Outbreaks often lead to fear, stigma, and social disruption. Providing counseling services, mental health support, and accurate information can help communities cope with the psychological impact of infectious disease outbreaks.

Overall, effective public health interventions require collaboration between healthcare systems, veterinary services, environmental agencies, and local communities. This integrated strategy aligns with the One Health approach, which recognizes the interconnectedness of human, animal, and environmental health. Guillaume, V., Contamin, H., Loth, P., et al. (2004).

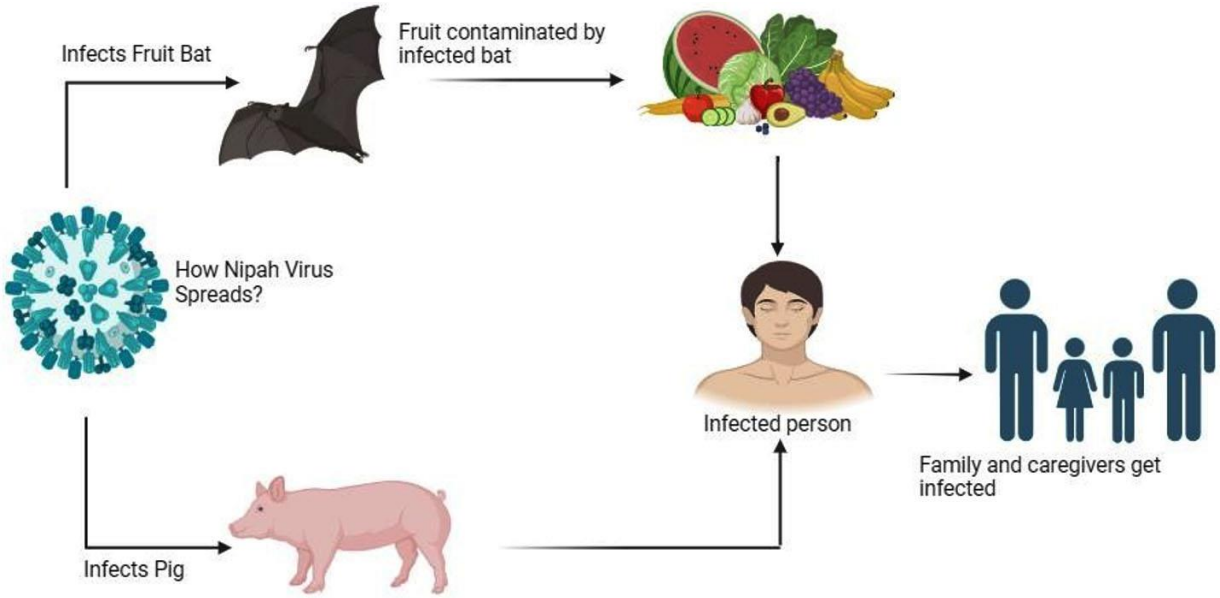


Figure 2: Route of transmission of Nipah virus: The diagram illustrates the transmission cycle of Nipah virus. Fruit bats act as the natural reservoir, contaminating fruits or food sources. Infection may spread to pigs or directly to humans through contaminated food. Infected individuals can further transmit the virus to family members and caregivers through close contact Pandey.et al. (2024).

8. Future Directions and Research Needs

Despite significant advances in understanding Nipah virus biology and pathogenesis, many aspects of the disease remain incompletely understood. Continued research is essential to develop effective preventive and therapeutic strategies that can reduce morbidity and mortality associated with Nipah virus infection.

One of the most urgent research priorities is the development of safe and effective vaccines. Several vaccine candidates, including recombinant viral vector vaccines, subunit vaccines, and mRNA-based vaccines, are currently under investigation. Experimental studies in animal models have demonstrated promising results, but further clinical trials are needed to evaluate safety, immunogenicity, and long-term protection in humans. Another critical area of research involves the development of antiviral therapies specifically targeting Nipah virus replication. Currently, treatment options remain largely supportive, with limited evidence for the effectiveness of existing antiviral drugs. Investigational treatments such as monoclonal antibodies targeting the viral glycoproteins and small-molecule inhibitors of viral replication are being explored. These therapeutic approaches have shown encouraging results in laboratory and animal studies. Understanding the mechanisms of vertical transmission is also an important research priority, particularly for protecting pregnant women and neonates. Studies investigating how the virus crosses the placental barrier, as well as the potential consequences for fetal development, could provide valuable insights for maternal and neonatal care guidelines.

Improved diagnostic tools are also needed to enable rapid and accurate detection of Nipah virus infection. Development of point-of-care diagnostic tests that can be used in resource-limited settings would significantly enhance early detection and outbreak response. Advances in molecular diagnostics, including real-time PCR and next-generation sequencing technologies, may further improve surveillance capabilities. Strengthening global surveillance systems is another critical aspect of future preparedness. Enhanced monitoring of wildlife reservoirs, particularly fruit bats, can help identify early warning signs of viral spillover events. Integrating animal health surveillance with human disease monitoring can provide a more comprehensive understanding of outbreak dynamics.

Finally, adopting a One Health approach is essential for preventing future Nipah virus outbreaks. This approach emphasizes collaboration among medical professionals, veterinarians, ecologists, and public health experts to address the complex interactions between humans, animals, and the environment. Sato, H., et al. (2013). Continued investment in multidisciplinary research, international collaboration, and capacity building will be crucial for improving preparedness against Nipah virus and other emerging zoonotic pathogens.

9. Conclusion

Nipah virus infection represents one of the most serious emerging zoonotic diseases of the modern era. Characterized by high mortality rates, severe neurological complications, and the absence of licensed antiviral therapies or vaccines, the virus poses a substantial threat to global public health. Its ability to infect multiple species, combined with increasing environmental and ecological changes, increases the likelihood of future outbreaks. The pathogenesis of Nipah virus infection is driven by complex interactions between viral proteins and host cellular mechanisms. The virus enters host cells through ephrin receptors, replicates efficiently within the cytoplasm, and spreads through direct cell-to-cell fusion. These processes result in widespread endothelial damage, vasculitis, and severe involvement of the central nervous system, ultimately leading to encephalitis and multi-organ dysfunction.

Effective management of Nipah virus disease requires a comprehensive approach that includes early recognition of symptoms, prompt supportive clinical care, and strict infection control practices. Healthcare systems must be prepared to manage severe respiratory and neurological complications while preventing nosocomial transmission among healthcare workers and other patients. Special attention must also be given to vulnerable populations such as infants, neonates, and pregnant women. These groups may experience atypical clinical presentations and require tailored care strategies to ensure optimal outcomes. Neonatal and infant care during Nipah virus outbreaks must balance infection prevention measures with the essential needs of early-life development and parental bonding.

Public health interventions remain the cornerstone of outbreak prevention and control. Community education, safe food practices, avoidance of wildlife exposure, and rapid surveillance systems are critical for reducing transmission. Collaboration across sectors through a One Health framework is essential for addressing the environmental and ecological drivers of disease emergence. Looking ahead, continued research efforts aimed at vaccine development, antiviral therapies, improved diagnostics, and understanding viral transmission dynamics will be vital. Strengthening global preparedness and response systems will help mitigate the impact of future outbreaks. In conclusion, combating Nipah virus infection requires an integrated strategy that combines scientific research, clinical excellence, public health preparedness, and community engagement. Through sustained global cooperation and multidisciplinary efforts, it may be possible to reduce the burden of this deadly disease and prevent future epidemics.

Conflict of Interest

The authors declare that they have no known competing financial interests in the work reported in this paper.

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