Published by
AkiNik Publications®
169, C-11, Sector - 3, Rohini,
Delhi - 110085, India
Toll Free (India): 18001234070
Email: akinikbooks@gmail.com

ISBN 9789361352362
9 789361 352362
₹ 687 US$ 14

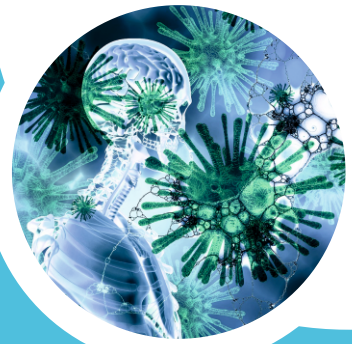
Interdisciplinary Review Articles: Assisting As a Bridge between Research & Development

AkiNik Publications

Interdisciplinary Review Articles

Assisting as a Bridge between Research & Development

Dr. Sibashish Bakshi



AKINIK PUBLICATIONS

Interdisciplinary Review Articles

Assisting as a Bridge between Research & Development

Editor

Dr. Sibashish Bakshi
Assistant Professor, Swami Vivekananda University, Kolkata, West Bengal, India

AkiNik Publications ®
New Delhi

Published By: *AkiNik Publications*

AkiNik Publications
169, C-11, Sector - 3,
Rohini, Delhi-110085, India
Toll Free (India) – 18001234070
Phone No.: 9711224068, 9911215212
Website: www.akinik.com
Email: akinikbooks@gmail.com

Editor: *Dr. Sibashish Bakshi*

Publication Year: *2024*

Edition: *1st*

Pages: *131*

ISBN: *978-93-6135-236-2*

Book DOI: *https://doi.org/10.22271/ed.book.2879*

Price: *₹687/-*

Registration Details

- *Printing Press License No.: F.1 (A-4) press 2016*
- *Trade Mark Registered Under*
  - *Class 16 (Regd. No.: 5070429)*
  - *Class 35 (Regd. No.: 5070426)*
  - *Class 41 (Regd. No.: 5070427)*
  - *Class 42 (Regd. No.: 5070428)*

Preface

In today's rapidly evolving scientific landscape, the boundaries between disciplines are increasingly blurred, and the importance of interdisciplinary research has never been more apparent. This book, “Interdisciplinary Review Articles: Assisting as a Bridge between Research & Development”, is born out of the recognition of this crucial need for integration. It aims to serve as a conduit, connecting the dots between diverse areas of expertise and fostering a deeper understanding of how interdisciplinary efforts can propel both research and development forward. By bringing together knowledge from multiple disciplines, these articles not only provide comprehensive insights but also serve as a resource for researchers, developers, and practitioners looking to bridge the gap between theory and practice. As we delve into the content, may we be reminded of the power of collaboration and the boundless possibilities that emerge when we transcend traditional boundaries in pursuit of a common goal.

The book begins with the first chapter titled "The Impact of Climate Change on Microbial Diversity: A Comprehensive Review." This chapter sets the stage by exploring how climate change is reshaping the microbial world, highlighting the critical role that microorganisms play in ecosystems and how shifts in their diversity could have far-reaching consequences.

The book transitions into the second chapter "The Utility of Biologically Competent Polymers and Tuning Natural Bioactive Compounds in Modern Dermatological Formulations: A Concise Review”, which focuses on the innovative use of biologically compatible polymers and the optimization of natural bioactive compounds in skincare and dermatological products.

Continuing the journey, the book explores "Microbiome Study of COVID-19 Variants Using Metagenomics Approaches: A Systematic Review of Current Evidence and Future Directions”, reviews how metagenomics has been used to study the microbiomes of COVID-19 variants, providing insights into current findings and potential future research avenues.

Another crucial component of the book includes "The Regulatory Genes of Lung Cancer - A Brief Review”, which highlights the key regulatory genes involved in lung cancer.

Next, the book addresses the "Relationship between Gut Microbial Incidence and Metabolic Disorders in Humans", investigating the intricate connections between gut microbiota and the development of metabolic disorders, highlighting how changes in microbial communities can influence human health and disease.

To further enhance the interdisciplinary aspect, the book features a crucial chapter "Diagnosis of Alagille Syndrome: A Genetic Disorder', plunging into the genetic basis of Alagille Syndrome.

Moreover, the book also features "A Short Review of Microbiota and Their Ecological Significances in the Stress of Fly Ash Contaminated Soil." This chapter reviews the impact of fly ash contamination on soil microbiota, highlighting the ecological roles of these microorganisms and their responses to environmental stressors.

As the book nears its conclusion, it centers on "Paving Progress, Breathing Challenges: Navigating the Complex Intersection of Aggressive Urban Development and Air Pollution", discussing how rapid urban development contributes to air pollution.

To further enhance its versatility, the book also includes "Pioneering the Next Frontier: Innovative Approaches to Tackle Antibiotic Resistance", which talks about cutting-edge strategies and novel solutions in the fight against antibiotic resistance.

The book concludes with the final chapter, "Nanoparticle and Antibiotic: Combinatorial Therapy for Biofilm Prevention', summing up the synergistic potential of combining nanoparticles with antibiotics to prevent and disrupt biofilm formation, offering insights into innovative approaches to enhance treatment efficacy and address persistent infections.

Each chapter in this volume is crafted by leading experts, offering in-depth analyses, the latest developments, and critical perspectives in the field of microbiology. We hope this anthology becomes a valuable resource for scholars, researchers, and enthusiasts, enhancing their understanding of the complexities and marvels of microbial life.

Thank you for joining us on this enlightening journey through the fascinating world of Life Sciences.

List of Authors

1. Dr. Aritri Laha, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
2. Dr. Bidisha Ghosh, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
3. Dr. Debjit De, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
4. Dr. Priyankar Pal, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
5. Mr. Rupesh Dutta Banik, Swami Vivekananda University, Kolkata, 700121, India
6. Dr. Semanti Ghosh, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
7. Dr. Sabyasachi Ghosh, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
8. Dr. Srijan Haldar, Associate Professor, Swami Vivekananda University, Kolkata, 700121, India
9. Ms. Suranjana Sarkar, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
10. Dr. Subhasis Sarkar, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India

Contents

Chapter - 1

Climate Change's Impact on Microbial Diversity: A Short Review

Authors

Esha Dutta
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Sarmistha Saha
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Sabarni Sarkar
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Keshab Ghosh
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Aritri Laha
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Chapter - 1

Climate Change's Impact on Microbial Diversity: A Short Review

Esha Dutta, Sarmistha Saha, Sabarni Sarkar, Keshab Ghosh and Aritri Laha

Abstract

Microbes being omnipresent in nature, exhibits numerous diversities in different habitats. As these microorganisms, execute a number of essential functions, like carbon storage regulation, nutrient recycling, crop fertility, pollution detoxification, crop fertility, and the generation and utilisation of greenhouse gases, they are necessary for all life on Earth. Change in Earth's climate is occurring more intensely, glaciers are thawing due to extreme weather changes, leading to the rise in water-levels. The alterations in climate conditions jeopardise the lives of all living things. Humans and Microorganisms both, are not immune to such climatic changes. In reality, microorganisms are already evolving to a shifting environment, which may lead to major consequences for the mankind. The effect of climatic changes on microbiota has been discussed for many years, but it has generally been neglected. Subsequent alterations in climate can have a substantial influence on the diversity, quantity, and functioning of microorganisms, which is frequently related to global warming, depletion in the soil carbon levels, and changes in the concentration of atmospheric greenhouse gases in the environment. Pathogens are redistributing globally due to these modifications. In this review paper, we will be discussing the various climate change's effect on microbial diversity, and the way they affect our environment.

Keywords: Climate changes, microbial community, environment, global warming.

Introduction

Changes in Climate is an increasing concern in the 21st century, impacting not only the weather but also the water, air, food, and living conditions of all living beings. Extreme weather has been increasingly common in recent decades, with records being broken on a regular basis

(Ibáñez *et al*., 2023). Climate change is influencing distributions of species and affecting interactions between organisms. Microorganisms have been present since at least 3.5 billion years ago (Yang *et al*., 2021). Microorganisms being ubiquitous in nature are the major contributors of biological cycles and survivability of the Earth. They have a immense impact on the Earth's environment and atmosphere. The frequent climatic change is also affecting the microbial community directly or indirectly (Ibrahim *et al*., 2021). Due to these frequent shifts in climate, soil fertility and soil organic carbon (SOC) are getting adversely affected, which in turn is affecting the soil microbiome and its influence on soil carbon sequestration. But, not only the soil microbiome is getting influenced, the marine food webs and carbon exchanges are also being affected (Naylor *et al*., 2020). The sudden changes in the climate over the last few decades, have triggered the microbial communities adapt to various survival strategies, which in turn have increased their chances to survive in extreme environments. Microorganisms that have faced dryness and rewetting throughout the rainy season are more likely to adapt to unfavourable conditions than those unfamiliar with climate change issues (Ibrahim *et al*., 2021).

Temperature fluctuations can impact microbial biodiversity through multiple methods. Temperature increases metabolism, leading to faster population doubling times. This additionally influences evolutionary and ecological processes like as mutation, interactions, and speciation. High temperatures help in the production of large amount of plant species, and these large number of plant diversities distributes the nutrient levels in the underground ecosystems. These shift in nutrients distribution can result in the alterations in plant-microbe interactions, affecting PGPR (plant-growth promoting rhizobacteria) which depends on rhizodeposition. The changes in the pattern of precipitation and drought pressure linked with climatic changes, also unfavorably affects the mycorrhizal mycelium formation in plant roots. Climate change induces environmental stress, leading to increased activity of pathogens and heterotrophic bacteria. This can also result in a distribution shift of beneficial microbes across ecological niches (Ibáñez *et al*., 2023).

Ecosystem variation generates considerable alterations in climate, including droughts, heat waves, greenhouse gas emissions, floods, and ozone depletion. These factors considerably impact the resilience and resistance of various microorganisms and ability to adapt to detrimental environmental changes. Limited research exists on how climate change impacts microbial communities, particularly the transition of diversity and population. Climate

change requires a comprehensive understanding of its impact on microbial communities, including their unpredictability, variety, and contributions, as well as their effects on ecosystems (Ibrahim *et al*., 2021).

In this review our aim is to be aware of the impact of climatic changes on microbial community, and how it alters the composition and function of microorganisms. We will also discuss the impact of these changes on our environment, and what are the various diseases caused by it.

Impact of changes in climate on microbial community's function and composition: Extreme climatic scenarios such as flood, drought, high temperature, and emission of greenhouse gases can change the structural distribution of microorganisms and their growth in a particular niche. The microorganisms include bacteria, archea, virus, protista, and fungi which are found in different parts of our ecosystem. There is little information on how microbial populations change throughout transitions. Evidence suggests that climate change may contribute to these shifts. Furthermore, the responsiveness to developmental changes in microbial communities are immeasurable (Naylor *et al*., 2020).

- **Soil microbial community:** Climate alteration-related disruptions can drastically alter soil microbial communities and functional characteristics. Microbial community of soil consists of various forms of microorganisms. These may include symbionts, mutualists, decomposers, producers, soil pathogens, etc. It has been predicted that by 2100 there will be a rough increase of 3.7°C. As microorganisms responds to the increase in temperature, this warming will also affect them. First, organic carbon breakdown rates will increase, leading to increased microbial biomass. And it is predicted that the soil microbial community will increase by 40-150%. As the temperature will increase the rate of eCO_2 utilization, which in short-term will stimulate plant growth, which in turn will enhances rhizodeposition, stimulates microorganisms to mineralize soil organic carbon (SOC), and emits CO_2 into the atmosphere via respiration. Plant growth will absorb more CO_2 from the atmosphere, reducing greenhouse gas emissions overall. But in long-term, increased microbial activity depletes soil stocks of easily digested carbon, leading to increased catabolism of SOC reservoirs and higher ambient CO_2 concentrations that plants can absorb over time. Thus, the CO_2 in the atmosphere will keep on increasing, leading to more severe global warming (Naylor *et al*., 2020).

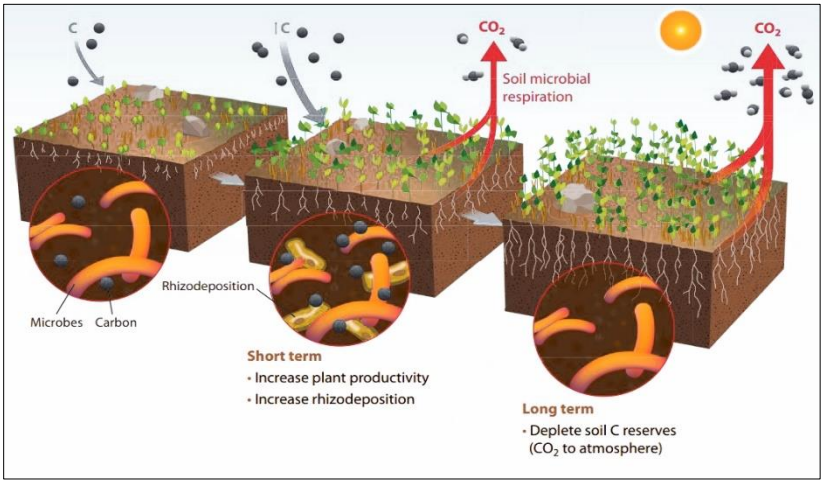


Fig 1: Impact of increased eCO_2 in Soil (Naylor *et al*., 2020)

Marine microbial community: Microorganisms present in the ocean contribute most of the secondary production and are responsible for all primary production, which affects important biogeochemical cycles and environmental processes. Microorganisms helps in regulating the ocean biogeochemistry, by possessing adaptable metabolism that control the nutrient irregularities such as phosphorus, nitrogen, sulphur, hydrogen, oxygen, and carbon. Marine microbes influence global climate through photosynthetic carbon fixation from atmospheric CO_2. Global warming alters the rate of biological and chemical reactions, which at disproportionate amounts, causes deleterious biochemical changes such as enzyme denaturation. It also influences climate changes such as increased precipitation and melting of glaciers. Certain microorganisms may find the changes in the environment favourable due to minimum energy use for respiration, lessened competition, calcification, increased availability of nutrients, and acid-base balance. Alterations in geographical conditions outside the normal range may trigger strain on organisms, leading to suboptimal physiological Effectiveness and limitations of environmental shifts. High levels of pressure may lead to decreased size, growth, mortality, and reproduction (Abirami *et al*., 2021).

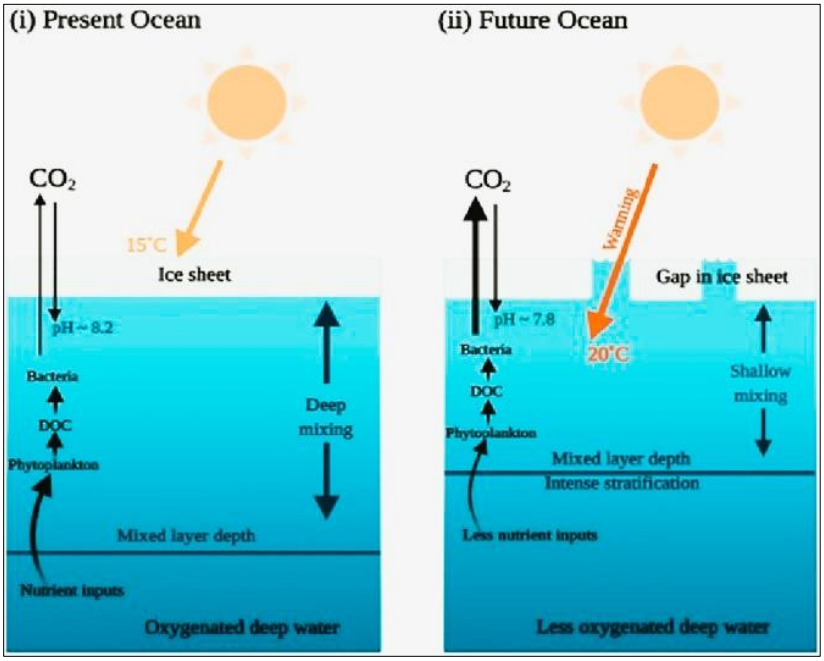


Fig 2: Impact of Climate Change on Marine Microbial Community (Abirami *et al*., 2021)

Changes in climate: Contributors and effect on microbial communities

- **Temperature:** The top contributor which affects the rate of metabolism is temperature. Temperature is crucial to the performance of metabolic processes. Alterations in temperature can affect microbial metabolisms which in turn can cause transitional alterations in the configuration of the microbial community. Sustainability of many microorganisms present in different environments gets disturbed by the discharge of greenhouse gases, caused by rise in temperature. High temperature also increases the amount of nitrogen in soil, which then affects the fungi present in the soil. As fungi can easily degrade organic components in the unavaibility of nitrogen in the soil, but the rise in temperature prevents that process (Ibrahim *et al*., 2021).

Change in temperature also affects the microbial community in waters, as it affects the population, metabolisms, and also the resistivity of the microorganisms. Algae present in the marine water bodies like cyanobacteria is also influenced by the temperature change (Ibrahim *et al*., 2021).

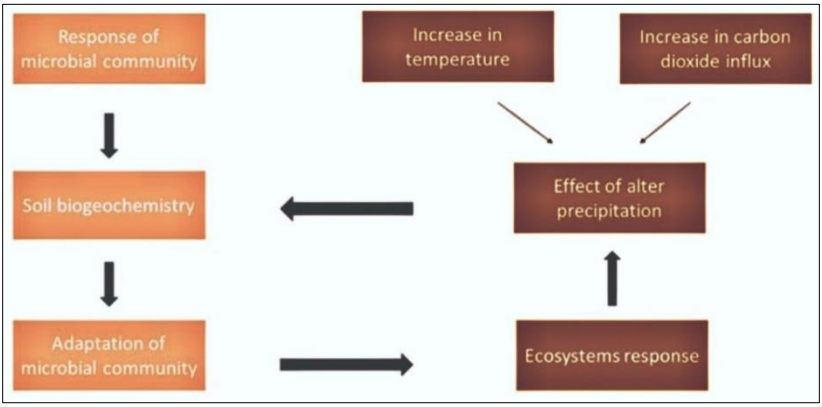


Fig 3: Climate change and other environmental factors' impact on microbial communities (Ibrahim *et al*., 2021)

- **Water content:** Water is essential for the microbial population to function properly. The presence or lack of water impacts bacteria in ecological systems. The presence of water affects microbial activity and composition. Furthermore, it encourages microorganisms to respond to soil respiration, including temperature and moisture. Moisture levels in terrestrial and soil habitats impacts microbial community composition and organic material breakdown. Changes in climate has a significant effect over fungus, bacteria, and other microbial communities, particularly when water precipitation changes. Thus, Increased or decreased water precipitation impacts the ecosystem's microbial community, including its roles, structures, and metabolism.

In low-water settings like dirt and saltwater, microbial activity may be repressed due to reduced enzymatic activity and hydration. Soil moisture influences respiration and may impact CO_2 emissions and production in the atmosphere and ecosystem (Ibrahim *et al*., 2021).

Climate change effects on various infections: Lastly, human health is also affected by climate changes. Infectious disease epidemiology is also continuously changing due to environmental factors, and interconnection between infections, reservoirs, hosts, and vectors (Ibáñez *et al*., 2023).

Disease	Microorganisms	Climate change effects on infections
1. Chikungunya	1. Chikungunya	Elevated temperatures, variations

2. Zika 3. Dengue	virus 2. Zika virus 3. Dengue virus	in rainfall, and the rise in the number and extent of severe weather conditions elevated the risk of disease and infection spread.
4. Diarrheal disease 5. Salmonellosis	4. *Campylobacter spp.* 5. *Salmonella spp.*	Rise in temperature favors the growth of microorganisms on the food, thus contaminating the food that cause diseases
6. Cholera 7. Leptospirosis	6. *Vibrio cholerae* 7. *Leptospira spp.*	Increasing temperatures and intense weather conditions can generate ideal circumstances for Leptospira bacteria to thrive and stay in the environment.

Conclusion

Microbes perform an essential function in regulating biotic and abiotic elements in the ecosystem. Microbial communities have a significant role in directing life processes and influencing global changes in the climate. Human activities and industrial revolutions are also two factors which also contribute to the climatic changes. These Regulations may impact the diversity and community of microbes. Microbiome changes can have an impact on the environment and ecology, either positively or negatively. The feedback may have direct or indirect effects on the microbial population and macro-organisms present in the environment. Further research is needed to be done to understand the interconnection between climate change and microbial communities, as well as the effects of transitions after and before these phenomena occur.

References

1. Naylor, D., Sadler, N., Bhattacharjee, A., Graham, E. B., Anderton, C. R., McClure, R., ... & Jansson, J. K. (2020). Soil microbiomes under climate change and implications for carbon cycling. *Annual Review of Environment and Resources*, *45*(1), 29-59.
2. Ibáñez, A., Garrido-Chamorro, S., & Barreiro, C. (2023). Microorganisms and Climate Change: A Not so Invisible Effect. *Microbiology Research*, *14*(3), 918-947.
3. Yang, Y., Li, T., Wang, Y., Cheng, H., Chang, S. X., Liang, C., & An, S. (2021). Negative effects of multiple global change factors on soil microbial diversity. *Soil Biology and Biochemistry*, *156*, 108229.

4. Ibrahim, A. D., Uhuami, A. O., Abdulkadir, N. U., & Uzoh, I. M. (2021). Climate change alters microbial communities. *Climate Change and the Microbiome: Sustenance of the Ecosphere*, 13-29.

5. Abirami, B., Radhakrishnan, M., Kumaran, S., & Wilson, A. (2021). Impacts of global warming on marine microbial communities. *Science of The Total Environment*, *791*, 147905.

6. Zhou, Z., Wang, C., & Luo, Y. (2020). Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. *Nature communications*, *11*(1), 3072.

7. Jansson, J. K., & Hofmockel, K. S. (2020). Soil microbiomes and climate change. *Nature Reviews Microbiology*, *18*(1), 35-46.

Chapter - 2

The Utility of Biologically Competent Polymers and Tuning Natural Bioactive Compounds in Modern Dermatological Formulations: A Concise Review

Authors

Anushka Singh
Dept of Biotechnology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barasat- Barrackpore Rd, Bara Kanthalia, West Bengal, India

Madiha Perween
Dept of Biotechnology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barasat- Barrackpore Rd, Bara Kanthalia, West Bengal, India

Palak Bhardwaj
Dept of Biotechnology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barasat- Barrackpore Rd, Bara Kanthalia, West Bengal, India

Priyanka Mandal
Dept of Biotechnology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barasat- Barrackpore Rd, Bara Kanthalia, West Bengal, India

Riya Lama
Dept of Biotechnology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barasat- Barrackpore Rd, Bara Kanthalia, West Bengal, India

Authors

Semanti Ghosh
Dept of Biotechnology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barasat- Barrackpore Rd, Bara Kanthalia, West Bengal, India

Subhasis Sarkar
Dept of Microbiology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barasat- Barrackpore Rd, Bara Kanthalia, West Bengal, India

Suranjana Sarkar
Dept of Microbiology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barasat- Barrackpore Rd, Bara Kanthalia, West Bengal, India

Bidisha Ghosh
Dept of Biotechnology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barasat- Barrackpore Rd, Bara Kanthalia, West Bengal, India

Chapter - 2

The Utility of Biologically Competent Polymers and Tuning Natural Bioactive Compounds in Modern Dermatological Formulations: A Concise Review

Anushka Singh, Madiha Perween, Palak Bhardwaj, Priyanka Mandal, Riya Lama, Semanti Ghosh, Subhasis Sarkar, Suranjana Sarkar and Bidisha Ghosh

Abstract

Nowadays, irrespective of all age groups and regardless of social and financial status people are more attentive and conscious towards personalised skin care and overall cosmetic routine. Undoubtedly cosmetic products of vogue play a significant role in alterations and improvement over natural appearance which lead to partial enhancement of self-confidence and esteem. Popular ingredients in skin care products contain a number of chemical compounds which can lead to deleterious side effects upon constant use and in the long run. This review paper exhibits the involvement of biopolymers and bioactive compounds extracted from some basic natural sources in the formulation of cosmetics products. We have focused on different types of biopolymers obtained from both natural and synthetic origin such as collagen, cellulose, alginate, PVA, PLA, pullulan, etc., and their applications. We have also discussed about the plant-based ingredients like aloe vera, neem, sandalwood oil, fruit peel and their effective tuning in skin care formulation which tend to be dermatologically favourable.

Keywords: Alginate, biopolymers, collagen, cosmetics, skin care.

1. Introduction

Materials that are produced using biotechnological methods or that are created by chemically changing naturally occurring biological molecules are referred to as biopolymers (Muñoz *et al*., 2023). These substances are non-toxic, biodegradable, and biocompatible. Biopolymers are key chemicals that serve as emulsifiers, moisturizers, hydrators, antimicrobials, and more recently, materials with skin metabolic activity. These benefits have led to their widespread use in both traditional and innovative cosmetics (Muñoz *et al*., 2023). The skin, which makes up the largest organ in the human body

and weighs 8% of the body, is crucial for fluid homeostasis and sensory detection. The epidermis, dermis, and subdermal layers are the three primary structural constituents of skin (Sahana *et al*., 2018). The largest organ in the body, the skin is typically treated with cosmetic products. When it comes to appearance, skin is charge of outward appearance, crafting a distinct and recognizable persona that is recognizable to others. The sebaceous gland distributed in the face, scalp, mid-back, and mid-chest contain the majority of sebaceous glands generating secretions that are sent into the follicular canal through the sebaceous duct where the secretion acts as a natural moisturizer that improves the skin's barrier function, and may have antifungal and antibacterial qualities (Mitura *et al*., 2020). Cosmetics are a highly developed application of polymers, and new developments in polymer science and nanoscience are propelling the development of extremely sophisticated scientific products (Lochhead *et al*., 2007). The chemicals generally present in cosmetics and skin care products exhibit problems like skin irritation, transient skin discolouration (Nigam *et al*., 2009), acute toxicity, skin sensitization, ocular irritation and many more (JN *et al*., 2015). There are two primary categories of biopolymers that are generally utilized in cosmetic applications: those based on proteins and polysaccharides. The hydrophilic property of polysaccharides enables the creation of gels with high viscosity, as well as high adhesion and reservoir-type systems that have significant effects on ageing and inflammatory skin conditions. Several anti-aging cosmetic products have proteins as key ingredients. Retaining water in the stratum corneum, minimizing wrinkles, and promoting the production of new extracellular matrix proteins are the main mechanisms that promote skin moisture. Hydrolysed soluble protein can therefore be added to formulations to favour cosmetic results (Muñoz *et al*., 2023). Biopolymers that are frequently employed in biological processes include hydrocolloids, hydrogels, alginates, polyurethane, collagen, poly (lactic-co-glycolic acid), chitosan, proteins, peptides, pectin, and hyaluronic acid (Basavaraj *et al*., 2010). Due of these qualities, they can be sold in a variety of sectors, including the food, pharmaceutical, medical, and environmental industries (Elgarahy *et al*., 2023). There are various raw ingredients utilized in cosmetic preparation. There are a wide variety of cosmetic ingredients accessible in the market. Among them, biopolymers and synthetic polymers are widely utilized. Living organisms are rich source of natural polymers. There are variety of natural polymers found in nature, including cellulose, which is the primary component of leaves and wood. These biopolymers and other polysaccharides are widely used in formulations for cosmetics. A large number of natural polymers are used as thickeners and moisturizers in

cosmetic formulations. For possible use in biomedical and cosmetic applications, a variety of hydrogels can be created using biopolymers and synthetic polymers (Mitura *et al*., 2020).

The aim of this review is to highlight the ill effects of synthetic polymers which are generally used in cosmetic and skin care products and introducing biopolymers as their innovative substitute. By employing the dermatological friendly properties of biopolymers we are promoting the production of skin care and cosmetic products which would tend to be economically sustainable and cause no harm to the environment.

1.1 Existing Polymers and their shortcomings

Synthetic polymers are a desirable excipient for cosmetic formulations. Silicon, polyacrylamides, acrylic acid-based polymers, and alkylene oxide-based homopolymers and copolymers are the synthetic polymers most frequently encountered in cosmetics. The cosmetics sector has begun using silicon materials in recent years. In cosmetics like deodorants, shampoos, antiperspirants, and lotions, silicon and its composites (such biomethane and cyclomethycaine) are employed as suspending agents and in processes like emulsification and associative thickening (Alves *et al*., 2020). Siloxanes are synthetic compounds derived from silicone that are used to smooth and soften a range of cosmetic items. They are frequently utilized in moisturizers and face treatments. It is categorized as an endocrine disruptor that interacts with human hormone function and may harm fertility (Nayak *et al*., 2021). Polyethylene glycols (PEGs) and their anionic or non-ionic derivatives are widely used in cosmetics as emulsifiers, emollients (helping to soften and lubricate the skin), and penetration enhancers. They are found in shampoo, hair conditioners, deodorant, bath and shaving products, skin care products, makeup, and skin cleansing products. Poly(lactic acid) (PLA), poly(ε caprolactone) (PCL), and poly(3-hydroxybutyrate-co-3 hydroxy valerate) are examples of aliphatic polyesters (Nam *et al*., 2020). BHA -Artificial ingredients like butylated hydroxyl anisole (BHA) used in moisturizers and lipsticks can irritate skin. BHA is recognized as a human carcinogen by the International Agency for Research on Cancer. Parabens are also recognized as the most widely used preservative in makeup that is again an endocrine disruptor affecting male reproductive function and mimic hormones like oestrogen (Boberg *et al.,* 2010).

1.2 Types of biopolymers and their potential useful derivatives in cosmetic products

In recent times a vast majority of natural polymers are employed in cosmetics. These are considered safe, biocompatible, environmentally

friendly and appropriate for a wide range of uses, such as skin and hair care, makeup, and as stabilisers and modifiers. Naturally occurring polysaccharides, starch (Thanyapanich *et al.,* 2021), xanthan gum (Jamshidan *et al.,* 2014), guar gum (Kim *et al.,* 2017), carrageenan (Valenta *et al.,* 2004), alginate (Song *et al.,* 2018), pectin (Shalaka *et al.,* 2009), gelatin (Al-Nimry *et al.,* 2021), collagen (Al-Atif, 2022). and the most recently used hyaluronic acid (Bukhari, S *et al.,* 2018) are among the most widely utilised natural polymers. Natural polymers can be sub-divided into plant based and animal based polymer, a short discussion of which is provided as follows:

Plant based natural biopolymer

- **Chitosan**

In tissue engineering, chitosan is one of the most researched polysaccharide polymers because of its biocompatible properties, biodegradability, and negligible or non-existent cytotoxicity. It can also bind to DNA, proteins, and polyanions synthetic polymers or components of the extracellular matrix by electrostatic contact. It can also be changed into specific morphologies and shapes after it has been solubilized. In fact, employing neutralising bases like sodium hydroxide, chitosan can be extruded, gelled, and crosslinked in the form of hydrogels. Multiple studies have conclusively demonstrated the antimicrobial properties of chitosan (Elango *et al*., 2023). Thus, it makes the chitosan as an important tool in various fields, like drug carrier for controlled release, bone tissue building, anti-bacterial and anti-acid (Aggarwal *et al*., 2020).

- **Alginate**

Alginate has been widely utilized as a food and cosmetic additive because it is non-toxic. Hydrogels of alginate have being investigated as hyaluronic acid substitute injectable materials (Mitura *et al*., 2020). Alginate is widely employed in biomedical applications such as tissue building, because of its biodegradability, biocompatibility, non-antigenicity, and chelating limit (Aggarwal *et al*., 2020). Numerous materials, including hydrogels, films, wafers, foams, nanofibres, and topical preparations, have been prepared for use as wound dressings using alginate (Aderibigbe *et al*., 2018).

Animal based natural biopolymers

- **Collagen**

One of the most popular polymers in tissue engineering is collagen, and

because of its special qualities, it is more appealing for skin regeneration. In skin regeneration, collagen is applied in a variety of forms, including films, gels, scaffolds, mats, composites. Collagen has many benefits for skin regeneration, including self-regeneration, biocompatibility, biomimetics, proliferation, breakdown, mineralization, flexibility, and skin restoration (Elango *et al*., 2023).

- **Keratin**

An assembly of filament proteins rich in cysteines is represented by the chemical substance known as keratin. By interacting with cosmetics, the keratin that is present in the skin's outer layer and the skin under the nails and hair helps retain moisture in the skin (Aggarwal *et al*., 2020). Hydrolyzed keratin that is soluble in water is a constituent of hair conditioners, body lotions, and sprays.Hydrogels based on keratin produced from hair are presumed to have potential use in cosmetics (Mitura *et al*., 2020).

Microbial based natural biopolymer

- **Levan**

Film-forming ability, biodegradability, non-toxicity, water retention, as well as antibacterial and anticancer capabilities, are just a few of the many attributes that Levan demonstrates. Levan is a desirable option for nature-based products in food production, contemporary cosmetics, medicine, and pharmacy because of these remarkable qualities. Levan may function as a product regulating skin pigmentation by reducing tyrosinase activity and lowering melanin formation (Domżał-Kędzia *et al*., 2023).

Fungal based natural biopolymer

- **Pullulan**

Pullulan and its derivatives play a crucial role in many biomedical applications because of their special chemical and physical features. The pullulan composite scaffolds can be utilized to promote cell differentiation and proliferation for tissue regeneration (Singh *et al*., 2016). Furthermore, because of its ability to retain water and build films, it might make an intriguing moisturizing and protective element for face packs, lotions, and powders as well as a protective ingredient for hair shampoos, hair dressings, and teeth powders (Coltelli *et al*., 2020).

- **Synthetic biopolymers**

Synthetic biopolymers that react to stimuli are being developed for a

variety of uses, such as wrinkle-masked makeup, easy emulsion processing, and thermally responsive systems that react to the skin's surface (Lochhead *et al*., 2007). They have a long shelf life, can be produced uniformly on a big scale, and are frequently less expensive than natural polymers. Alkylene oxide- and acrylic acid-based homopolymers and copolymers, silicon, polyacrylamides, and acrylic acid-based polymers are the synthetic polymers most frequently encountered in cosmetics (Alves *et al*., 2020).

- **PGA (Polyglycolic Acid)**

Synthetic polymer PGA is frequently employed in biomedical applications and skin regeneration due to its strong biodegradability and biocompatibility (Elango *et al*., 2023). Recently, glycolic acid has been used into many dermatological and cosmetic compositions.

- **PVA (Polyvinyl alcohol)**

PVA has been widely employed as a polymeric matrix for the creation of polymers for skin and tissue repair, including hydrogels, membranes, xerogels. Its low cytotoxicity, excellent water absorption, advantageous mechanical qualities, and biocompatibility all contribute to its enormous potential in regenerative medicine (Elango *et al*., 2023).

Name of polymer	Source of origin	Application	Reference
1. Chitosan	Crustacean waste materials and shellfish.	Personal hygiene products and cosmetic industries.	(Aggarwal *et al*., 2020).
2. Collagen	Invertebrates in the cuticles and body walls.	Skin regeneration templates and cosmetics.	(Aggarwal *et al*., 2020).
3. Cellulose	Plant tissues (Cotton trees etc.)	Thickener, controlled drug delivery devices.	(Aggarwal *et al*., 2020).
4. Alginate	Brown algae of the genera *Nacrocystis, Alario*	Emulsifier, beauty products, antioxidants.	(Aggarwal *et al*., 2020).

Application of biopolymers in dermatological treatments (cosmetics and skin care product). Chitosan is frequently used in cosmetic application, such as hair and skin care and lotions. It is a very effective moisturizing ingredient that is also applied to lips and skin. Additionally, it can be utilized to give sunscreens water-resistant qualities (Alves *et al*., 2020). Collagen showed an excellent ability to hold onto water, making it appropriate for use as a moisturizer on the skin. It is used in anti-aging and anti-wrinkling products (Alves *et al*.,2020).Cellulose fibrils are used to make skin care products that are non-irritating, have good skin adhesion and spreadability, and are perfect

for usage as face masks(Alves *et al*.,2020).Silk fibroin improves elasticity and hydrates, making it a useful ingredient in lotions and shampoos (Alves *et al*.,2020).Curcumin as a cosmetic has been used to reduce wrinkles, calm inflamed skin, and enhance general skin health (Selvasudha *et al*.,2023). Hydrolyzed keratin that is soluble in water is a constituent of hair conditioners, body lotions, and sprays (Mitura *et al*., 2020). Levan may function as a substance regulating skin pigmentation since it inhibits tyrosinase activity, which lowers the formation of melanin and it can be used in De-Tan products such as scrubs, masks, etc. (Domżał-Kędzia *et al*., 2023).

2. Plants accommodating majestic effects in dermatology

Aloe Vera is a component in skin lotions, face creams, and sun protection product. It has special antiaging compounds that are said to keep skin appearing young and vibrant (Javed *et al*., 2014). Neem is an excellent natural source of raw materials for cosmetics. It can be used as an option for making multipurpose sunscreens because its SPF values were higher than those of the commercial cream. Different skin conditions like acne, psoriasis, eczema, mycosis, and warts have all been treated using organic neem oil(Baby *et al*., 2022).With several beneficial effects on the skin and body as a whole, sandalwood oil is one of the most important raw resources and is widely used in natural medicine and cosmetics. It has been utilized for its calming, antibacterial, and antidepressant properties. (Hartman-Petrycka *et al*., 2021). Numerous biological benefits of bamboo, including antioxidant, anti-free radical, anti-aging, and antibacterial properties, are known. A multitude of compounds make up the majority of sunscreen formulas, each of which absorbs UV radiation at a particular wavelength (Wróblewska *et al*., 2019). The value of fruit peels in the creation of cosmetics and skin care products has been extensively studied. Focusing on the antibacterial, anti-UVB, and antioxidant qualities of fruit peel extracts, such as those from apples, pomegranates, oranges, and bananas (Khang *et al*., 2021). Pomegranate extracts are known for their strong antioxidant (Boggia *et al*., 2016). The antioxidants in extract from banana peels have a vital role for eliminating toxins from the skin (Bhavani *et al*., 2023).

3. Hydrogels: An alternative strategy for dermatological treatments

Because of their great capacity to swell, hydrogels are used in a wide range of biomedical applications, including drug delivery, tissue engineering, and regenerative medicine. There are several benefits of using bio-adhesive hydrogels for skin care. Numerous biopolymers, such as collagen, gelatine, hyaluronic acid, alginate, chitosan, starch, and cellulose derivatives, can be

the basis for hydrogels used in cosmetic preparations. Hydrogels based on biopolymers are utilized to create novel cosmetics, like "beauty masks."

Conclusion

Numerous biopolymers have been applied in cosmetics in the recent years, which serves as the innovative alternative for the chemicals based skin care and cosmetic products. The biopolymers have proved to be biodegradable, biocompatible and environmentally sustainable which enable them to be the ideal substitute of the currently used chemical compounds. Here we tried to elucidate the remarkable characteristics of the above mentioned biopolymers which constitute several benefits for skin.

References

1. Mendoza-Muñoz, N., Leyva-Gómez, G., Piñón-Segundo, E., Zambrano-Zaragoza, M. L., Quintanar-Guerrero, D., Del Prado Audelo, M. L., & Urbán-Morlán, Z. (2023). Trends in biopolymer science applied to cosmetics. International Journal of Cosmetic Science, 45(6), 699-724.
2. Sahana, T. G., & Rekha, P. D. (2018). Biopolymers: Applications in wound healing and skin tissue engineering. Molecular biology reports, 45, 2857-2867.
3. Nigam, P. K. (2009). Adverse reactions to cosmetics and methods of testing. Indian Journal of Dermatology, Venereology and Leprology, 75, 10.
4. JN, O., Udebuani, A. C., Ezeji, E. U., Obasi, K. O., & Nnoli, M. C. (2015). Possible Health Implications Associated with Cosmetics: A. Science, 3(5-1), 58-63.
5. Basavaraj, K. H., Johnsy, G., Navya, M. A., & Rashmi, R. (2010). Biopolymers as transdermal drug delivery systems in dermatology therapy. Critical Reviews™ in Therapeutic Drug Carrier Systems, 27(2).
6. Elgarahy, A. M., Eloffy, M. G., Guibal, E., Alghamdi, H. M., & Elwakeel, K. Z. (2023). Use of biopolymers in wastewater treatment: A brief review of current trends and prospects. Chinese Journal of Chemical Engineering.
7. Alves, T. F., Morsink, M., Batain, F., Chaud, M. V., Almeida, T., Fernandes, D. A., ... & Severino, P. (2020). Applications of natural, semi-synthetic, and synthetic polymers in cosmetic formulations. Cosmetics, 7(4), 75.
8. Elango, J., Zamora-Ledezma, C., & Maté-Sánchez de Val, J. E. (2023). Natural vs Synthetic Polymers: How Do They Communicate with Cells

for Skin Regeneration - A Review. Journal of Composites Science, 7(9), 385.

9. Aggarwal, J., Sharma, S., Kamyab, H., & Kumar, A. (2020). The realm of biopolymers and their usage: an overview. J Environ Treat Tech, 8(2), 1005-1016.
10. Coltelli, M. B., Danti, S., De Clerck, K., Lazzeri, A., & Morganti, P. (2020). Pullulan for advanced sustainable body-and skin-contact applications. Journal of Functional Biomaterials, 11(1), 20.
11. Singh, R. S., Kaur, N., Rana, V., & Kennedy, J. F. (2016). Recent insights on applications of pullulan in tissue engineering. Carbohydrate Polymers, 153, 455-462.
12. Lochhead, R. Y. (2007). The role of polymers in cosmetics: Recent trends.
13. Aderibigbe, B. A., & Buyana, B. (2018). Alginate in wound dressings. Pharmaceutics, 10(2), 42.
14. Selvasudha, N., Goswami, R., Subi, M. T. M., Rajesh, S., Kishore, K., & Vasanthi, H. R. (2023). Seaweeds derived ulvan and alginate polysaccharides encapsulated microbeads–Alternate for plastic microbeads in exfoliating cosmetic products. Carbohydrate Polymer Technologies and Applications, 6, 100342.
15. Mitura, S., Sionkowska, A., & Jaiswal, A. (2020). Biopolymers for hydrogels in cosmetics. Journal of Materials Science: Materials in Medicine, 31, 1-14.
16. Domżał-Kędzia, M., Ostrowska, M., Lewińska, A., & Łukaszewicz, M. (2023). Recent developments and applications of microbial levan, a versatile polysaccharide-based biopolymer. Molecules, 28(14), 5407.
17. Eshun, K., & He, Q. (2004). Aloe vera: a valuable ingredient for the food, pharmaceutical and cosmetic industries—a review. Critical reviews in food science and nutrition, 44(2), 91-96.
18. Javed, S. (2014). Aloe vera gel in food, health products, and cosmetics industry. Studies in Natural Products Chemistry, 41, 261-285.
19. Baby, A. R., Freire, T. B., Marques, G. D. A., Rijo, P., Lima, F. V., Carvalho, J. C. M. D., ... & Morocho-Jácome, A. L. (2022). Azadirachta indica (Neem) as a potential natural active for dermocosmetic and topical products: a narrative review. Cosmetics, 9(3), 58.

20. Hartman-Petrycka, M., & Lebiedowska, A. (2021). The assessment of quality of products called sandalwood oil based on the information provided by manufacturer of the oil on Polish, German, and English websites. Evidence-Based Complementary and Alternative Medicine, 2021.

21. Wróblewska, K. B., Baby, A. R., Guaratini, M. T. G., & Moreno, P. R. H. (2019). *In vitro* antioxidant and photoprotective activity of five native Brazilian bamboo species. Industrial Crops and Products, 130, 208-215.

22. Prommaban, A., Kuanchoom, R., Seepuan, N., & Chaiyana, W. (2021). Evaluation of fatty acid compositions, antioxidant, and pharmacological activities of pumpkin (Cucurbita moschata) seed oil from aqueous enzymatic extraction. Plants, 10(8), 1582.

23. Khang, D. T., Tien, L. T. T., Men, T. T., & Thuy, N. P. (2021). Potential of Fermented Fruit Peel Liquid in Cosmetics as a Skin Care Agent. Cosmetics, 8(2), 33.

24. Boggia, R., Turrini, F., Villa, C., Lacapra, C., Zunin, P., & Parodi, B. (2016). Green extraction from pomegranate marcs for the production of functional foods and cosmetics. Pharmaceuticals, 9(4), 63.

25. Bhavani, M., Morya, S., Saxena, D., & Awuchi, C. G. (2023). Bioactive, antioxidant, industrial, and nutraceutical applications of banana peel. International Journal of Food Properties, 26(1), 1277-1289.

26. Alves, T. F., Morsink, M., Batain, F., Chaud, M. V., Almeida, T., Fernandes, D. A., ... & Severino, P. (2020). Applications of natural, semi-synthetic, and synthetic polymers in cosmetic formulations. Cosmetics, 7(4), 75.

27. Bilal, M., & Iqbal, H. M. (2019). An insight into toxicity and human-health-related adverse consequences of cosmeceuticals—a review. Science of the total environment, 670, 555-568.

28. Nayak, M., Sreedhar, D., Prabhu, S. S., & Ligade, V. S. (2021). Global trends in cosmetics use-related adverse effects: A bibliometric analysis of literature published during 1957–2021. Cosmetics, 8(3), 75.

29. Nam, H. C., & Park, W. H. (2020). Aliphatic polyester-based biodegradable microbeads for sustainable cosmetics. *ACS Biomaterials Science & Engineering*, *6*(4), 2440-2449.

30. Boberg, J., Taxvig, C., Christiansen, S., & Hass, U. (2010). Possible endocrine disrupting effects of parabens and their metabolites. *Reproductive Toxicology*, *30*(2), 301-312.

31. Thanyapanich, N., Jimtaisong, A., & Rawdkuen, S. (2021). Functional properties of banana starch (Musa spp.) and its utilization in cosmetics. *Molecules*, *26*(12), 3637.

32. Jamshidian, M., Savary, G., Grisel, M., & Picard, C. (2014). Stretching properties of xanthan and hydroxypropyl guar in aqueous solutions and in cosmetic emulsions. *Carbohydrate polymers*, *112*, 334-341.

33. Kim, J. H., Min, H. J., Park, K., & Kim, J. (2017). Preparation and evaluation of a cosmetic adhesive containing guar gum. *Korean Journal of Chemical Engineering*, *34*, 2236-2240.

34. Valenta, C., & Schultz, K. (2004). Influence of carrageenan on the rheology and skin permeation of microemulsion formulations. *Journal of Controlled Release*, *95*(2), 257-265.

35. Song, J., & Chen, H. (2018). Preparation of aroma microcapsules with sodium alginate and tetradecylallyldimethylammonium bromide (TADAB) and its potential applications in cosmetics. *Flavour and fragrance journal*, *33*(2), 160-165.

36. Shalaka, D., Naik, S. R., Amruta, A., & Parimal, K. (2009). Vitamin E loaded pectin alginate microspheres for cosmetic application. *J. Pharm. Res*, *2*(6).

37. Al-Nimry, S., Dayah, A. A., Hasan, I., & Daghmash, R. (2021). Cosmetic, biomedical and pharmaceutical applications of fish gelatin/hydrolysates. *Marine drugs*, *19*(3), 145.

38. Al-Atif, H. (2022). Collagen supplements for aging and wrinkles: a paradigm shift in the fields of dermatology and cosmetics. *Dermatology practical & conceptual*, *12*(1).

39. Bukhari, S. N. A., Roswandi, N. L., Waqas, M., Habib, H., Hussain, F., Khan, S., ... & Hussain, Z. (2018). Hyaluronic acid, a promising skin rejuvenating biomedicine: A review of recent updates and pre-clinical and clinical investigations on cosmetic and nutricosmetic effects. *International journal of biological macromolecules*, *120*, 1682-1695.

Chapter - 3

Microbiome Study of COVID-19 Variants Using Metagenomics Approaches: A Systematic Review of Current Evidence and Future Directions

Authors

Sudipta Chakraborty
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Debjit De
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Chapter - 3

Microbiome Study of COVID-19 Variants Using Metagenomics Approaches: A Systematic Review of Current Evidence and Future Directions

Sudipta Chakraborty and Debjit De

Abstract

The severe acute respiratory syndrome coronavirus, or SARS-CoV-2, is the source of the extremely infectious illness COVID-19, which mostly affects the respiratory system. But besides affecting the respiratory system, it also a multiorgan disease that damages the neurological and gastrointestinal systems. The significance of Human microbiota in health and illness has become more evident in the past ten years. The microbiome of the upper respiratory tract (URT) can mimic the lung microbiota and affect the host's immune response to the infection. There are some bacteria which predominates the URT of Covid patients are - *Pseudomonas aeruginosa*, *Pelagibacterium halotolerans, Halomonas piezotolerans* etc. Our gut has a diverse range of bacteria. Imbalances in the makeup of digestive tract microorganisms can cause dysbiosis. In COVID-19 alteration of the gut microbiome is also observed. There are some bacteria which predominate in gut during COVID-19 are- *Bacteroides nordii*, *Actinomyces viscosus*, they are Opportunistic pathogens associated in causing bacteremia. The metagenomics is the study of genetic material extracted directly from clinical or environmental samples using a sequencing technique is known as metagenomics. In metagenomics besides the culturable bacteria we can able to know about unculturable bacteria. So, it gives us a overall scenario of total microorganisms present in a particular environment. Different COVID-19 variants like- omicron, delta have different microbiome profiles. Using metagenomics microbiome profiles of covid19 variants are studied. Understanding SARS-CoV-2's effects on gut flora are crucial this review give clear resolve picture on metagenomic approaches used to study the modification of microbial members in COVID-19 patients and their impact on disease compared to healthy individuals.

Keywords: Contagious, microbiome, Sars-Cov2, metagenomics, gut flora, dysbiosis, URT, sequencing.

Introduction

Before starting microbiome study of COVID-19 variants we need to know about microbiome. Microbiome is referred to as the population of microorganisms that live in a certain habitat, including bacteria, viruses, and fungus, is known as the microbiome. The phrase is frequently used to refer to microorganisms that reside in or on certain sections of the human body, including the gastrointestinal system or skin. These dynamic groupings of microorganisms adapt to a wide range of environmental stimuli, including food, exercise, medications, and other exposures. The severe acute respiratory syndrome coronavirus (SARS-CoV-2) is the source of the highly contagious coronavirus illness 2019 (COVID-19). With over 6 million fatalities globally, the effects of COVID-19 have been catastrophic for the earth. After the first cases of this mostly respiratory viral illness were discovered in Wuhan, Hubei Province, China, at the end of December 2019, SARS-CoV-2 rapidly spread around the world. On March 11, 2020, the World Health Organization (WHO) was forced to declare it a global pandemic as a result. (Cascella *et al*., 2023). It is an enclosed, forward single stranded RNA virus. SARS-CoV-2 is closely linked to both Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV). (Jiang *et al*., 2020).

We need to know about metagenomics also, metagenomics is the study of genetic material extracted directly from clinical or environmental samples using a technique called sequencing is known as metagenomics. In this review we want to review the microbiome composition in the patients infected with different COVID-19 variants using metagenomics.

COVID-19 discussion

In the twenty-first century, SARS-CoV-2 infection is now a major risk to the public's wellbeing. Since Wuhan, China, first reported the new coronavirus illness known as COVID-19, it has swiftly spread to practically every country in the world (Zhu *et al*., 2020). The nasal cavity is the main entry point for COVID-19 infections. The virus first proliferates in the oropharynx and/or nasopharynx before aspirating into the lungs and invading the lungs more severely (Hou *et al*., 2020) Therefore, in order to avoid serious complications during a COVID-19 infection, it is essential to maintain dental and nasal health (Atukorallaya *et al*., 2021). The upper respiratory tract (URT) microbiome, which is made up of healthy oral and/or

nasal microbiomes, is thought to protect respiratory health and provide resistance against invasive infections (Kumpitsch *et al*., 2019). The host immune response to viral and secondary bacterial infections may be modified by changes in the URT microbiota (Man *et al.,* 2017 & Hanada *et al.,* 2018). COVID-19 is not just a pulmonary illness that affects the lungs, but also a multiorgan disease that damages the neurological and gastrointestinal systems. (Mao *et al*., 2020; Patel *et al*., 2020; Tian J. *et al*., 2020; Wong *et al*., 2020). The beta coronavirus known asSARS-CoV-2 belongs to the family Coronaviridae. Single-stranded positive ribonucleic acid (RNA) viruses constitute the family (Klein *et al*., 2020). There are four genera of coronaviruses, and it is known that people may contract viruses from the alpha and beta genera. These viruses are zoonotic, meaning they may spread from animals to people. When this happens for the first time, it's called a spill over event. It has been discovered that the SARS-CoV-2 and coronaviruses that are prevalent in bat populations are closely related (Emma *et al*., 2021).

Epidemiology of COVID-19

With over 375,000 fatalities, COVID-19 ranked as the third most common cause of death in the United States (USA) in 2020, behind cancer and heart disease. Individuals across all age groups are vulnerable to this infection. Patients 60 years of age or older, as well as those with underlying medical conditions (for example, diabetes, obesity, cancer, patients receiving solid organ or hematopoietic stem cell transplants, obesity, cardiovascular illness, and chronic renal disease) are at an increased risk of contracting a severe COVID-19 infection. The CDC states that among COVID-19 patients, age is still the best indicator of poor prognosis and serious disease. According to data from the CDC's National Vital Statistics System (NVSS), individuals with COVID-19 between the ages of 50 and 64 had a 25-fold increased chance of dying than adults (Cascella *et al*., 2023).

Table 1: Representing different covid strains and their comparisons (Ref: Begum *et al.,* 2022)

S. No.	Viral strain	Variety	Genome	Similarity ratio to the SARS-CoV-2 Genome (%)
1	Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV)	Beta (β)	SARS-CoV	82.45
2	Middle East Respiratory	β	MERS-	69.58

	Syndrome Coronavirus (MERS-CoV)		CoV	
3	Human Coronavirus-NL63 (hCoV-NL63)	Alpha (α)	hCoV NL63	65.11
4	Human Coronavirus-229E (hCoV-229E)	α	hCoV 229E	65.04
5	Human Coronavirus-HKU1 (hCoV-HKU1)	β	hCoV HKU1	67.59
6	Human Coronavirus-OC43 (hCoV-OC43)	β	hCoV OC43	68.93

Besides these variants there are other variants like -delta (2022) & omicron (2023) variants are also identified.

Metagenomic approach used in microbiome study

Before studying metagenomic approach we need to know about metagenomics. So, metagenomics is basically the study of genetic material extracted directly from clinical or environmental samples using a technique called sequencing. It is exactly the opposite of microbiological techniques. We can directly annotate our target sequence without any isolation or purification procedure.

Sepsis, pneumonia, and other lung infections are among the most common long-term consequences and unfavourable results of SARS-CoV-2 infection. Since the clinical signs of these conditions are frequently identical to those of a bacterial infection and are frequently seen. This clearly shows serious bacterial imbalance, even after the patient has eliminated the virus. In addition to increasing the likelihood of a future lung infection, coughing, lung hypoxia, and SARS-CoV-2-induced immune modulation may also promote the proliferation of facultative and anaerobic anaerobes. Based on the available data and the tight relationship between the oropharynx and lung airways, we proposed the hypothesis that human oropharyngeal microbiome (HOPM) will change in response to SARS-CoV-2 infection and may serve as a marker for co-infections in the lungs (Paine *et al*., 2022).

Though by molecular and microbial and biochemical tests we can be able to know its pathogenicity and other details like molecular level, biochemical nature, but we can't get to know about its structural details. So, for this reason we are using this metagenomic approach to completely know its structural details without prior isolation and purification. It is not enough to know only about its molecular or biochemical nature, to know more about this virus which has severe pathogenicity, and which was responsible for the

severe epidemic we must know it from depth that's why the need of metagenomic study.

Outcomes of the study

This study shows that microbial diversity decreased in the people infected with COVID-19 rather than people without COVID history (healthy individuals). Also, it is observed that people infected with any of the covid variants has dysbiosis in the gut flora. Also, it is shown that people with COVID history also faced the opportunistic pathogens in their body.

Table 2: Significant difference between the two COVID-19 variants (Nath *et al*., 2023)

Characteristics	Omicron	Delta
Transmissibility	higher	Lesser
Symptoms	milder	Higher
Immunological reactions	milder	Higher

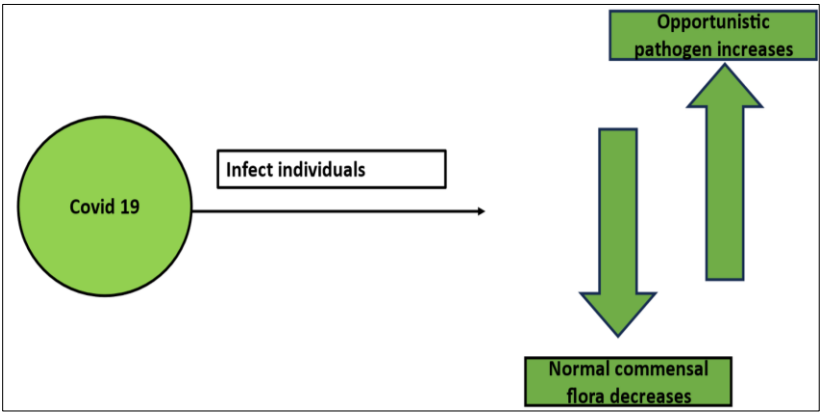


Fig 1: Schematic diagram to understand the URT microbiome composition in COVID-19 infected patients compared to healthy individuals

Table 3: List of bacteria present in patients infected with covid 19 variants (Nath *et al*., 2023)

Species	% of occurrence in covid patients
Pseudomonas aeruginosa	12.36%
Pelagibacterium halotolerans	2.18%
Halomonas piezotolerans	0.79%
Vibrio tritonious	1.85%

Microbiome association

Using metagenomics microbiome association was observed. It is seen that in different COVID variants like, Omicron, delta cluster formation of the microbiome was observed. In healthy individuals there was association of commensal flora, but in case of COVID infected individuals' commensal flora decreased significantly and increased occurrence of opportunistic pathogen. In several studies it is seen that the certain proteobacteria, such as *Stenotrophomonas*, *Acenetobactor*, *Enterobactor*, *Bifidobacterium*, and *Chryseobacterium*, were discovered to be more common in COVID instances. In earlier studies with model organisms and human corpses, these proteobacteria were found to be the cause of lung pneumonia. Furthermore, we demonstrate that not every person reacts the same way to the medicines (doxycycline and azithromycin). While we did see a decrease in the number of dangerous bacteria in some patients when antibiotics were administered, this trend frequently reversed in other patients, particularly those with extremely low baseline concentrations of harmful bacteria (Paine *et al*., 2022). It was also observed that delta infected patients has lesser diversity of URT (Upper Respiratory Tract) microbiome than omicron and healthy individuals.

Table 4: Phylum predominant in COVID 19 and healthy individuals (Nath *et al*., 2023)

Covid 19/Healthy controls	Phylum predominant	% of occurance
COVID +VE	Proteobacteria	73.57%
Healthy controls	Firmicutes	42.67%
Healthy controls	Bacteriodota	19.72%
Healthy controls	Fusobacteroidota	6.30%

Table 5: Genera segregating healthy and COVID affected individuals (Nath *et al*., 2023)

Genera (Healthy)	% Occurance	Genera (Affected)	% of occurance
Streptococcus	23.94%	*Pseudomonas*	18.98%
Neisseria	6.86%	*Acinetobacter*	6.00%
Prevotella	12.13%	*Pelagibacterium*	2.32%
Actinobacillus	6.33%	*Halomonas*	1.27%
Veillonella	11.43%	*Stenotrophomonas*	2.09%

Table 6: Genera segregating omicron and delta affected individuals (Nath *et al*., 2023)

Genera	(Omicron) % occurance	(Delta) % occurance
Pseudomonas aeruginosa	21.45%	5.17%
Rothia mucilaginosa	3.74%	0.03%
Veilonella nakazawae	2.68%	0.07%
Prevotella melaninogenica	2.20%	0.68%
Neisseria perflava	1.56%	0.12%

Gut microbiome

In COVID 19 alteration of gut microbiome is also observed. It is observed that individuals with COVID-19 have a severely compromised gut microbial ecological network that is sparse and less diverse in their gut microbiome. Predominance of opportunistic bacteria, fungi, eukaryotic viruses is identified with a significant decrease in normal beneficial gut flora. Even after disease resolution some microorganisms persist which is also termed as long COVID. Additionally, an unfavourable gut milieu may result from an active SARS-CoV-2 virus in the stomach and changed gut microbiome ecology. This could hinder the community assembly and Function of the gut microbiota and impair the host immune system by facilitating the opportunistic bloom of certain fungi and pathogenic bacteria. The excrement of COVID-19 patients was screened for opportunistic microorganisms such as *Actinomyces viscosus, Clostridium hathewayi, and Bacteroides normandii,* which are known to induce bacteremia (Zuo *et al*., 2021). As a disrupted gut microbiome, chances of secondary infections are also increased.

Antibiotic-treated hospital patients showed a further reduced the amount of bacterial species, especially beneficial symbionts such as *Faecalibacterium prausnitzii, Lachnospiraceae bacteria 5_1_63FAA, Eubacterium rectale, Rubinococcus obeum*, and *Dorea formicigenerans.* These alterations in the ecology of the bacterial microbiome persisted throughout the COVID-19 disease, even after SARS-CoV-2 was removed from the respiratory system. Comparing COVID-19 patients to controls, the number of butyrate-producing bacteria, including *Eubacterium rectale, Clostridium leptum, Clostridium butyricum, and Faecalibacterium prausnitzii*, was considerably lower (Tang *et al*., 2020 & Yousef *et al*., 2024). The prevalence of Enterobacteriaceae and Enterococcus, two frequent opportunistic infections, was, however, much higher in in COVID-19

patients versus controls. A situation called "long COVID." Remarkably, the GI tract is also long-term impacted by COVID-19, as evidenced by the extended 42-day viral RNA shedding in stool specimens and the persistence of SARS-CoV-2 virus in the gut epithelium in certain patients up to 90 days after the sickness resolves (Wang *et al*., 2020 & Gaebler *et al*., 2021).

A more severe COVID-19 disease course was linked to a high baseline abundance of opportunistic bacteria *Coprobacillus, Clostridium ramosum, and Clostridium hathewayi* in hospitalised patients' faeces (Zuo *et al*., 2021). In contrast, an inverse association was found with the anti-inflammatory bacterium *Faecalibacterium prausnitzii*, suggesting that host immunity is calibrated by the gut microbiome at baseline, which in turn affects disease response upon SARS-CoV-2 infection. Furthermore, the gut bacterial diversity was much lower in COVID-19 patients (Gu *et al.,* 2020 & Ren *et al.,* 2021). The stability of the microbial ecosystem is significantly influenced by microbial diversity (Lahti *et al*., 2014). Opportunistic infections are resistant to colonisation in stable environments (Buffie *et al.,* 2013). Thus, in patients with COVID-19, the decrease in gut microbiota variety and richness may have a long-term effect and may contribute to the growth of opportunistic microorganisms. *Streptococcus infantis, Morganella morganii, Collinsella aerofaciens,* and *Collinsella tanaka*ei were among the bacterial species that were most prevalent in the intestines of individuals with a high SARS-CoV-2 infection rate. Since COVID-19 is basically a lung illness, it is known that the gut-lung axis can have an impact on the lung (Enaud *et al*., 2020 & Dang *et al.,* 2019). So, the impact of gut microbiome is very much essential in COVID 19 patients.

Using microbiome in beneficial way

- Nasal probiotic spray has been found in studies to effectively treat sinusitis and respiratory syncytial virus infection (Tran *et al*., 2022).
- On another hand, oral Bifidobacterium longum supplementation is recommended for treating dental inflammation and influenza infection (Wong *et al*., 2019 & Ribeiro *et al.*, 2021).
- A functional investigation also demonstrated that oral Bifidobacterium longum supplementation can prevent *P. aeruginosa*-induced infection in mice (Matsumoto *et al*., 2008).
- We found a modest abundance (<0.5%) of Bifidobacterium longum in patients infected with Delta and Omicron. As a result, our findings can aid in the ongoing development of nasal probiotic spray for COVID-19 patients, taking into consideration both

existing variations in the community and newer emergent variants (Nath *et al*., 2023).

Recently, there has been increased interest in combination therapy. Research suggests that combining multiple therapies, including microbial therapy, can effectively treat specific diseases (Gutiérrez-Gutiérrez *et al*., 2017). Mager *et al.* (2020) found that *Bifidobacterium pseudolongum* in mouse faeces can be used as a marker for immunotherapy. Inosine, a metabolite, can boost Th1 cell differentiation improves the efficiency of immunological checkpoint blockade (ICB) therapy in certain settings. Combining immunotherapy and microbial therapy has been shown to improve anti-tumor immunity. Our study found a significant reduction in Bifidobacterium sp. in COVID-19 patients' faecal samples, potentially contribute to a deterioration in immune response. Increasing the amount of this bacteria and thus increasing the level of this bacteria and its metabolite, inosine, may improve the patient's immune response (Sijia *et al*., 2021).

Conclusion

In conclusion it can be said that metagenomics is an emerging field in bioinformatics. It helps us in every way possible. Using bioinformatics, it is easier to fully understand the structural details of our target pathogen or interest. By using conventional approaches, it takes so much time to get in depth about any target but using metagenomics the process become easier.

We can get to understand about the microbiota makeup in various COVID-19 variations

infected individuals and we get to know about how we can use the microorganisms in beneficial way, what are the approaches. Also, we observed that the effect of antibiotic is not same in all the individuals, it depends upon several factors.They are some of things we can get to know.

Acknowledgement

I would like to offer my heartfelt gratitude to our honourable Chairman Sir, V.C. Sir, Dean Sir, COO Sir, Director Sir of the School of Life Sciences, and other Swami Vivekananda University officials. I am also grateful to our esteemed HOD, my project guide, Dr. Debjit De, and other faculty members of Swami Vivekananda University's School of Life Sciences for providing me with this excellent opportunity.

References

1. Cascella M, Rajnik M, Aleem A, *et al*. Features, Evaluation, and

Treatment of Coronavirus (COVID-19) [Updated 2023 Aug 18]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554776/

2. Zhu H, Wei L, Niu P. 2020. The novel coronavirus outbreak in Wuhan, China. Glob Health Res Policy 5:6. https://doi.org/10.1186/s41256-020-00135-6

3. Hou YJ, Okuda K, Edwards CE, Martinez DR, Asakura T, Dinnon KH, Kato T, Lee RE, Yount BL, Mascenik TM, *et al*. 2020. SARS-CoV-2 reversegenetics reveals a variable infection gradient in the respiratory tract. Cell. 182:429https://doi.org/10.1016/j.cell.2020.05.042

4. Atukorallaya DS, Ratnayake RK. 2021. Oral mucosa, saliva, and COVID-19 infection in oral health care. Front Med (Lausanne) 8:656926. https://doi.org/10.3389/fmed.2021.656926

5. Kumpitsch C, Koskinen K, Schöpf V, Moissl-Eichinger C. 2019. The microbiome of the upper respiratory tract in health and disease. BMC Biol 17:87. https://doi.org/10.1186/s12915-019-0703-z

6. Man WH, de Steenhuijsen Piters WAA, Bogaert D. 2017. The microbiota of the respiratory tract: gatekeeper to respiratory health. Nat Rev Microbiol 15:259–270. https://doi.org/10.1038/nrmicro.2017.14

7. Hanada S, Pirzadeh M, Carver KY, Deng JC. 2018. Respiratory viral infection-induced microbiome alterations and secondary bacterial pneumonia. Front Immunol 9:2640. https://doi.org/10.3389/fimmu.2018.02640

8. Mao, R., Qiu, Y., He, J. S., Tan, J. Y., Li, X. H., Liang, J., *et al*. (2020). Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. Lancet Gastroenterol. Hepatol. 5, 667–678. doi: 10.1016/S2468-1253(20)30126-6

9. Patel, K. P., Patel, P. A., Vunnam, R. R., Hewlett, A. T., Jain, R., Jing, R., *et al*. (2020). Gastrointestinal, hepatobiliary, and pancreatic manifestations of COVID-19. J. Clin. Virol. 128:104386. doi: 10.1016/j.jcv.2020.104386

10. Tian, J., Yuan, X., Xiao, J., Zhong, Q., Yang, C., Liu, B., *et al*. (2020). Clinical characteristics and risk factors associated with COVID-19 disease severity in patients with cancer in Wuhan, China: a multicentre, retrospective, cohortstudy. Lancet Oncol. 21, 893–903. doi: 10.1016/S1470-2045(20)30309-0

11. Wong, M. C. S., Huang, J., Lai, C., Ng, R., Chan, F. K. L., and Chan, P. K. S. (2020). Detection of SARS-CoV-2 RNA in fecal specimens of patients with confirmed COVID-19: a meta-analysis. J. Infect. 81, e31–e38.

12. Emma B. Hodcroft. 2021. “CoVariants: SARS-CoV-2 Mutations and Variants of Interest.” https://covariants.org/.

13. Klein S, Cortese M, Winter SL, Wachsmuth-Melm M, Neufeldt CJ, Cerikan B, Stanifer ML, *et al*. SARS-CoV-2structure and replication characterized by in situ cryoelectron tomography. Nat Commun. 2020;11(1):5885.

14. Cosar B, Karagulleoglu ZY, Unal S, Ince AT, Uncuoglu DB, Tuncer G, Kilinc BR, Ozkan YE, Ozkoc HC, Demir IN, Eker A, Karagoz F, Simsek SY, Yasar B, Pala M, Demir A, Atak IN, Mendi AH, Bengi VU, Cengiz Seval G, Gunes Altuntas E, Kilic P, Demir-Dora D. SARS-CoV-2 Mutations and their Viral Variants. Cytokine Growth Factor Rev. 2022 Feb;63:10-22. doi: 10.1016/j.cytogfr.2021.06.001. Epub 2021 Jul 2. PMID: 34580015; PMCID: PMC8252702.

15. Paine SK, Rout UK, Bhattacharyya C, Parai D, Alam M, Nanda RR, Tripathi D, Choudhury P, Kundu CN, Pati S, Bhattacharya D, Basu A. Temporal dynamics of oropharyngeal microbiome among SARS-CoV-2 patients reveals continued dysbiosis even after Viral Clearance. NPJ Biofilms Microbiomes. 2022 Aug 24;8(1):67. doi: 10.1038/s41522-022-00330-y. PMID: 36002454; PMCID: PMC9400563.

16. Wang Y, Zhang L, Sang L, Ye F, Ruan S, Zhong B, *et al*. Kinetics of viral load and antibody response in relation to COVID-19 severity. J Clin Invest 2020;130:5235–44.

17. Gaebler C, Wang Z, Lorenzi JCC, Muecksch F, Finkin S,Tokuyama M, *et al*. Evolution of antibody immunity to SARSCoV-2. Nature 2021;591:639–44.

18. Ren Z, Wang H, Cui G, Lu H, Wang L, Luo H, *et al*. Alterations in the human oral and gut microbiomes and lipidomics in COVID-19. Gut 2021;70:1253–65.

19. Lahti L, Saloja¨ rvi J, Salonen A, Scheffer M, de Vos WM. Tipping elements in the human intestinal ecosystem. Nat Commun 2014;5:4344.

20. Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol 2013;13:790–801

21. Enaud R, Prevel R, Ciarlo E, Beaufils F, Wiee¨ rs G, Guery B, *et al*.The

gut–lung axis in health and respiratory diseases: a place for inter-organ and inter-kingdom cross talks. Front Cell Infect Microbiol 2020;10:9.

22. Dang AT, Marsland BJ. Microbes, metabolites, and the gut–lung axis. Mucosal Immunol 2019;12:843–50.

23. Tran DM, Tran TT, Phung TTB, Bui HT, Nguyen PTT, Vu TT, Ngo NTP, Nguyen MT, Nguyen AH, Nguyen ATV. 2022. Nasal-spraying *Bacillus* spores as an effectivesymptomatic treatment for children with acute respiratory syncytial virus infection. Sci Rep 12:12402. https://doi.org/10.1038/s41598-022-16136-z

24. Ribeiro WR, Queiroz AG, Mendes E, Casaro MB, Nascimento CM, Coelho LSSF, Martins FS, Leite-Silva VR, Ferreira CM. 2021. Preventive oral supplementation with *Bifidobacterium*longum 5(1A) alleviates oxazolone-induced allergic contact dermatitis-like skin inflammation*in mice*. Benef Microbes 12:199–209. https://doi.org/10.3920/BM2020.0134

25. Matsumoto T, Ishikawa H, Tateda K, Yaeshima T, Ishibashi N, Yamaguchi K. 2008. Oral administration of *Bifidobacterium*longum prevents gut-derived *Pseudomonas aeruginosa* sepsis *in mice*. J Appl Microbiol 104:672–680. https://doi.org/10.1111/j.1365-2672.2007.03593.x

26. Mager, L. F., Burkhard, R., Pett, N., Cooke, N. C. A., Brown, K., Ramay, H.,et al. (2020). Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. Science 369:1481. doi: 10.1126/science.abc3421

27. Yousef, M., Rob, M., Varghese, S., Rao, S., Zamir, F., Paul, P., & Chaari, A. (2024). The effect of microbiome therapy on COVID-19-induced gut dysbiosis: A narrative and systematic review. Life Sciences, 122535. https://doi.org/10.1016/j.lfs.2024.122535

Chapter - 4

The Regulatory Genes of Lung Cancer - A Brief Review

Authors

Priti Goswami
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Priyankar Pal
Department of Biotechnology and Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Malir Math, Kolkata, Bara Kanthalia, West Bengal, India

Chapter - 4

The Regulatory Genes of Lung Cancer - A Brief Review

Priti Goswami and Priyankar Pal

Abstract

Although lung cancer usually starts in the cells that line the airways, it can also start in the bronchi, bronchioles, or alveoli, among other areas of the lung. Lung cancer in India is associated with various factors like changing lifestyles, air pollution, and increased tobacco use. The regulatory genes, including EGFR, ALK, ROS1, TP53, PTEN, and STK11 are involved in the genetic aspects of lung cancer. The function, mutations, and consequences of each gene in lung cancer offer information on possible treatment targets and prognostic markers. There have been reports regarding the role of nAChR receptors and RNA genes in relation to bronchial epithelial cells and their possible contribution to the development of lung cancer. The section covers the signs and forms of lung cancer, specifically small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Lung cancer is highly prevalent worldwide, accounting for 1.6 million deaths annually from 1.8 million cases that are diagnosed. The main objective is to ascertain risk factors, such as the established correlations with smoking, exposure to secondhand smoke, air pollution, radiation gas (radon), and Cannabis sativa. Lung cancer patients have a much better prognosis when the disease is discovered and treated early. For this reason, it's critical to understand risk factors and symptoms and to get help as soon as you notice anything suspicious. Furthermore, quitting smoking is essential for preventing lung cancer and enhancing general health.

Keywords: Lung cancer, small cell lung cancer, non-small cell lung cancer, regulatory genes.

Introduction

The uncontrolled growth of abnormal lung cells is an early precursor to lung cancer. It's a serious health issue that could be lethal. Lung cancer symptoms include shortness of breath, chest pain, and a chronic cough. Seeking medical attention as soon as possible is essential to avoiding serious

health consequences. The stage of the disease and the patient's medical history dictate the treatment plan.

The two most common types of lung cancer are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC is more common and often progresses slowly, while SCLC is less common and often grows quickly. Lung cancer is a major public health issue because of its high global death rate.

Global pervasiveness of lung cancer

According to recent research, diseases that were more common in the past have been surpassed by lung cancer, also known as lung cancer or carcinoma of the lungs. Currently, 1.6 million people die from the disease each year from over 1.8 million cases that are diagnosed globally. Less than one million cases were reported in the US in 2018, according to records, and between 650 and 700 new cases per month, involving both sexes, were regularly found. Lung cancer risk is significantly increased later in life in places like Asia, where about 65% of men begin smoking at age 20.

Distinguishing itself from Utah is Kentucky, where the diagnosis rate is three times higher at 120 per 100,000 cases. The states of Alabama, Louisiana, West Virginia, Arkansas, and Tennessee are among those with higher incidence and death rates. Recent years have seen a steady or sharp rise in cases in the southern and western states, which has been linked to declining air quality and rising smoking rates.

Of all the carcinogenic diseases, lung carcinoma is the most common malignant neoplasm in the modern world and has the highest death rate. Its prevalence is attributed to several factors, such as smoking, genetic predisposition, air pollution, and unhealthy eating patterns. The use of tobacco increases mortality rates because it puts nonsmokers at risk in addition to endangering smokers.

Diverse factors affecting lung cancer

Tobacco smoking

Tobacco is one of the most common causes of lung cancer around the world, whether it is from cigarettes, cigars, or pipes (Bade & Dela Cruz, 2020). In cigarette smoke, many cancer-causing substances damage the cells lining the lungs, causing tumours to form. According to the Centers for Disease Control and Prevention (CDC), smoking causes about 85 percent of the 4,444 2 lung cancers in the United States (Bade & Dela Cruz, 2020).

Second-hand Smoke

Recognized by the term passive smoking, second-hand smoke exposure raises the risk of lung cancer in nonsmokers (Bade & Dela Cruz, 2020). It is not always safe to inhale smoke from other people's pipes, cigars, or cigarettes, as this might cause lung cancer. Complete details about the dangers of second hand smoke exposure may be found at the American Cancer Society (Bade & Dela Cruz, 2020).

Radiation gas

Radon (222Rn) is known to be the second most common cause of lung cancer, accounting for around 10% of all lung cancer-related deaths (Bade & Dela Cruz, 2020). It is a naturally occurring radioactive gas that can leak into houses and other buildings through foundational fractures and gaps. Lung cancer risk increases with prolonged exposure to high radon levels. Radon and its impact on health are covered in materials provided by the Environmental Protection Agency (EPA) (Bade & Dela Cruz, 2020).

Air pollution

The World Health Organization (WHO) emphasises how lung health is impacted by air pollution (Bade & Dela Cruz, 2020). There is a link between being exposed to exterior air pollution and an increased probability of lung cancer. These pollutants include sulphur dioxide, nitrogen dioxide, and particle matter. Lung cancer can arise from prolonged exposure to contaminated air, particularly in metropolitan areas and close to industrial operations (Bade & Dela Cruz, 2020).

Cannabis sativa

We know that, as opposed to other narcotics, *Cannabis sativa*, commonly recognized as marijuana, is perhaps the most often used illicit substance (de Groot *et al*., 2018). It is estimated that 17% of teenagers who start using cannabis will eventually develop a dependence, and between 30 and 50% of frequent users have already developed a cannabis addiction (de Groot *et al*., 2018). Approximately 15% of adults and teenagers worldwide have acknowledged smoking cannabis (de Groot *et al*., 2018). THC, or delta-9-tetrahydrocannabinol, is a psychotropic compound found in cannabis that is equally addictive to nicotine and not known to cause (de Groot *et al*., 2018). Studies suggests that THC has a negative impact on the neurological growth of teenage brains (de Groot *et al*., 2018).

According to studies, smoking cannabis has been scientifically demonstrated to create organic material that causes cancer, and cannabis

produces tar at a rate that is greater than that of (de Groot *et al*., 2018). In comparison to tobacco, there is not only more tar, but also more polyaromatic hydrocarbons (de Groot *et al*., 2018). Finally, research indicates that marijuana usage is known to cause malignant molecular histological alterations in the bronchial epithelium (de Groot *et al*., 2018).

Pervasiveness of lung cancer in India

Starting around my last update in January 2022, cellular breakdown in the lungs has been progressively perceived as a critical medical problem in India. Numerous factors, including increased tobacco use, indoor and outdoor air pollution, occupational exposures, and shifting lifestyles, have contributed to the rising incidence of lung cancer in India (Kumar *et al*., 2022). Tobacco smoking, both as cigarettes and customary structures, for example, bidi and hookah, remains a significant gambling factor for cellular breakdown in the lungs in India. Also, openness to indoor air contamination from biomass fills utilised for cooking and warming in rustic regions contributes altogether to the weight of cellular breakdown in the lungs, particularly among ladies (Kumar *et al*., 2022). Another significant factor in the rising incidence of lung cancer in urban areas is outdoor air pollution, primarily caused by vehicle emissions, industrial activities, and construction.

The pervasiveness of cellular breakdown in the lungs fluctuates across various locales of India, with higher rates detailed in metropolitan regions and among specific segment bunches with higher smoking commonness and openness to natural poisons (Kumar *et al*., 2022). To address the growing burden of lung cancer in India, it is essential to promote early detection and treatment, reduce air pollution, improve access to smoking cessation programs, and raise awareness of the risks associated with tobacco use. In addition, effective strategies for prevention and control require research into the genetic, environmental, and lifestyle factors that influence lung cancer incidence (Kumar *et al*., 2022).

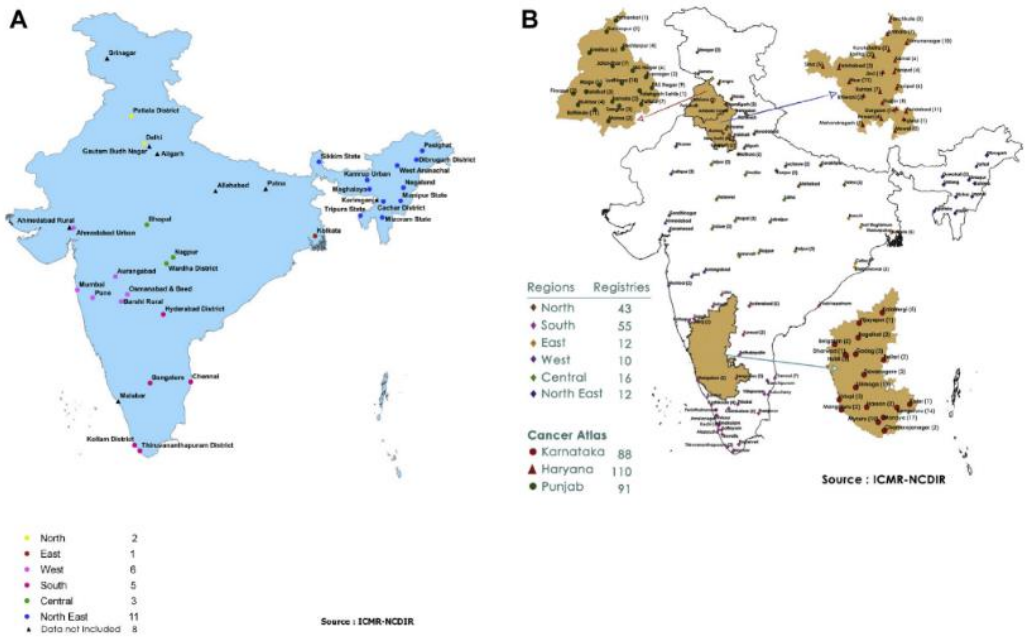


Fig 1: Network of (A) PBCR an (B) HBCR of the NCRP coordinated and steered by the ICMR-NCDIR. Source:https://www.ncdirindia.org/All_Reports/Report_2020/default.aspx & Mathur *et al*. Reproduced with permission. HBCR, hospital-based cancer registry; ICMR-NCDIR, Indian Council for Medical research-National Centre for Desease Informatics and research; NCRP, National Cancer Registry Progremme; PBCR, population-based cancer registry

Demographic variable	Numerical value
Total population	1.38 billion[a]
Urban population	34.5%[a]
Life expectancy	70.42 y[a]
Languages	216
Sex ratio	924 females per 1000 males[b]
Nominal GDP	$3.202 trillion[c]
PPP	$11.33 trillions
Total health care expenditure	3.6% of GDP
Public health expenditure	1.29% of GDP
Health insurance coverage	20% of women and 23% of men[d]
Doctor-population ratio	1:1456 (WHO recommendation 1:1000)
Noncommunicable diseases	60% of all deaths
Tobacco use	28.6% of adults

GLOBOCAN India statistics 2018

Number of new cancer cases	1.16 million
Cancer deaths	784,821
Number of prevalent cancer cases (5-y)	2.26 million
Lung cancer	5.9% of all cancer cases (fourth most common)
Lung cancer incidence	67,795
Lung cancer mortality	63,475 (8.1% of all cancer deaths)

Projected incidence, 2020

All sites	1,392,179
Males	679,421
Females	712,758
Lung cancer	98,278
Males	71,788
Females	26,490

Regulatory genes involved in lung cancer

There are various genes involved in the regulation of lung carcinoma. Some of those genes are EGFR, KRAS, RAS, TP53, STK11, ROS1 etc.

EGFR

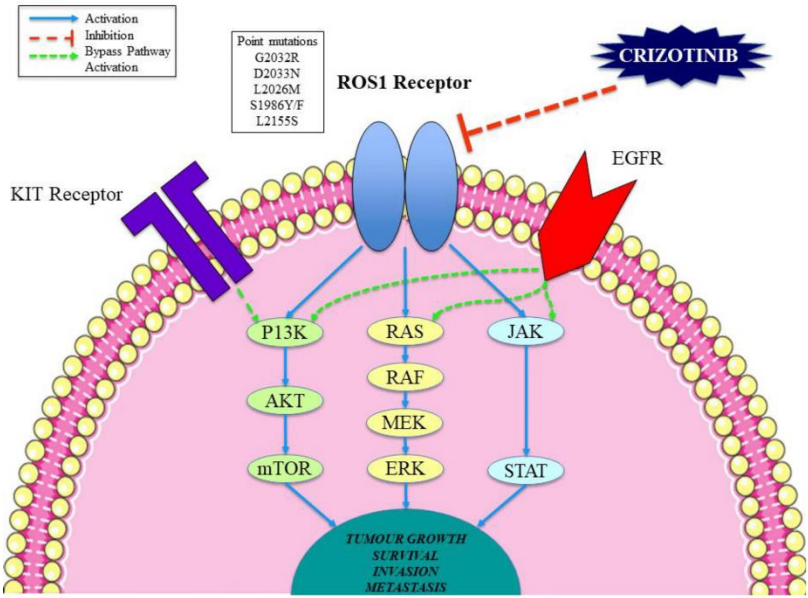
The term "EGFR" stands for Epidermal Growth factor Receptor, which is a protein that is encoded by the gene. It is involved in the regulation of the growth and division of cells (El-Telbany & Ma, 2012).Certain regions of the "EGFR" gene, including exon (19) of the gene, have been linked to various cancers, especially Non-Small Cell Lung Cancer (Huang *et al*., 2022). Exon (19) refers to a part of the DNA within the "EGFR gene" that codes for the

"EGFR protein". Exon 19 mutations are particularly sensitive to specific targeted therapies used to treat lung cancer (Huang *et al*., 2022). Mutation of the gene "EGFR" is most commonly found in "Non-Small Cell Lung Cancers" (NSCLCs), particularly in the "Adenocarcinoma Subtype" of the disease (Huang *et al*., 2022). The "EGFR" mutations can stimulate the growth of the cancer cells and affect the response to the targeted therapies.

ALK

Although ALK has long been known to have a role in human cancer, EML4-ALK fusion in non-Hodgkin lymphoma was just recently identified in the literature as a novel possible oncogenic driver mutant kinase in non-small cell lung cancer (NSCLC) by Soda *et al*. in 2007. ALK fusions are seen in 3–7% of lung cancers. There are several distinct EML4-ALK fusion polymorphisms that have been reported in NSCLC; these variations usually have different fusion sites at EML4 but the same fusion site inside ALK. The majority of light (<10 pack years) or never smokers with EML4-ALK fusions are often younger in age. It was also discovered that various ethnic groups have distinct EML4 ALK oncogenic rearrangements. Numerous studies examining the Asian cohort found that the prevalence of oncogenic mutation ranged from 2.3% to 6.7%, with no discernible variation when compared to Asian individuals who had never smoked. However, it was shown that white people had a significantly lower rate of EML4-ALK rearrangement; most research supported a range of 1% to 3%. Remarkably, an investigation carried out on a group of NSCLC specimens gathered from Italy and Spain revealed that the incidence rate, at 7.5%, was more akin to the Asian group. EML4-ALK fusions did not often coincide with other oncogenic mutations of KRAS or EGFR. Furthermore, linked to EGFR TKI resistance is the existence of EML4-ALK fusions. ALK-positive NSCLC patients receiving the first-generation A treatment had a better rate of survival than crizotinib-naive controls, according to recent research by Shaw *et al* (El-Telbany & Ma, 2012). However, individuals ultimately become resistant to crizotinib, just like they do to other focused cancer medications. New mutations that confer resistance are always being discovered. In the near future, more effective and reasonably priced NGS efforts applied to various human populations should yield more fusion variants of human oncogenic translocations, possibly in lung cancer (El-Telbany & Ma, 2012).

ROS1

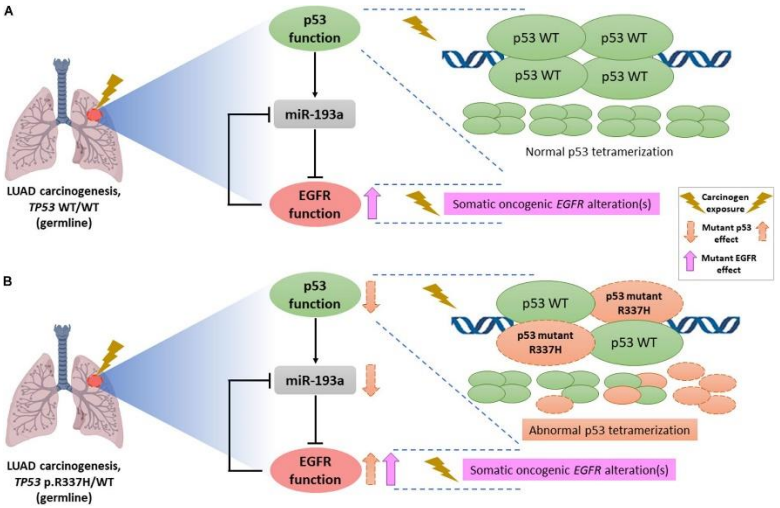


ROS1 is one of the insulin receptor family RTKs. Similar to ALK, ROS1 rearrangements occur in a subset of lung adenocarcinomas and can be targeted with specific drugs. Glioblastomas are the first type of cancer in which the gene is fused to the adjacent FIG gene. ROS1 fusions were found to be present in approximately 2% of non-small cell lung cancer (NSCLC) and may be a contributing mutation (El-Telbany & Ma, 2012). While the specific downstream signalling that the transduced ROS1 fusion elicited is still unknown, these fusions resulted in RTK activation. The study also demonstrated a patient's rapid and lasting full response. A ROS-positive status is associated with sensitivity to TKIs, particularly crizotinib (El-Telbany & Ma, 2012).

TP53

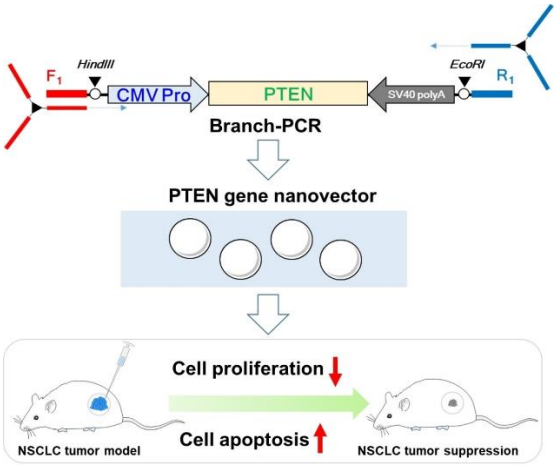
The "guardian of the genome," or TP53 gene, is an essential component in controlling cell division and minimising the risk of cancer. One of the many cancers that is commonly associated with TP53 gene mutations is lung cancer. Given that non-small cell lung cancer (NSCLC) is the most prevalent form of the disease, it is thought that TP53 gene mutations are rather common in lung cancer. These mutations may render TP53's tumour-suppressive characteristics inactive, allowing cancerous cells to proliferate and evade cell death.TP53 mutations may affect how well a patient responds

to treatment and are frequently linked to more aggressive forms of lung cancer. Treatment choices and prognosis can be influenced by knowledge about the presence or absence of TP53 mutations in lung cancer patients. Further investigation is being conducted into targeted therapies that specifically target TP53 mutations in the context of lung cancer treatment approaches (Dong *et al*., 2017).



PTEN

Tumour development and progression: Lung cancer is influenced by the loss of PTEN function in its early stages. PTEN mutations are more likely to be seen in squamous cell carcinoma and adenocarcinoma, two subtypes of lung cancer.

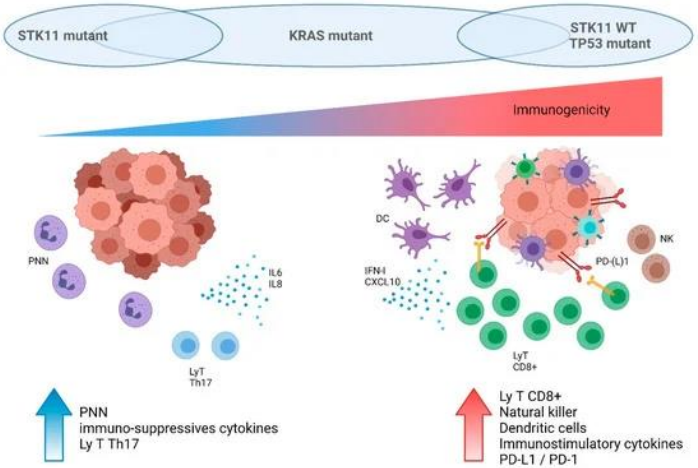


Prognostic marker: PTEN mutations can indicate aggressive tumour behaviour and a poor prognosis in lung cancer patients. Compared to patients whose PTEN function is intact, patients with lung cancers that lack PTEN may experience a worse clinical outcome.

Therapeutic objective: PTEN loss causes the PI3K/AKT/mTOR pathway to become dysregulated, which makes it a desirable target for treatment. Lung cancer patients with PTEN alterations are especially being studied for treatment with targeted agents and inhibitors that attempt to block this pathway.

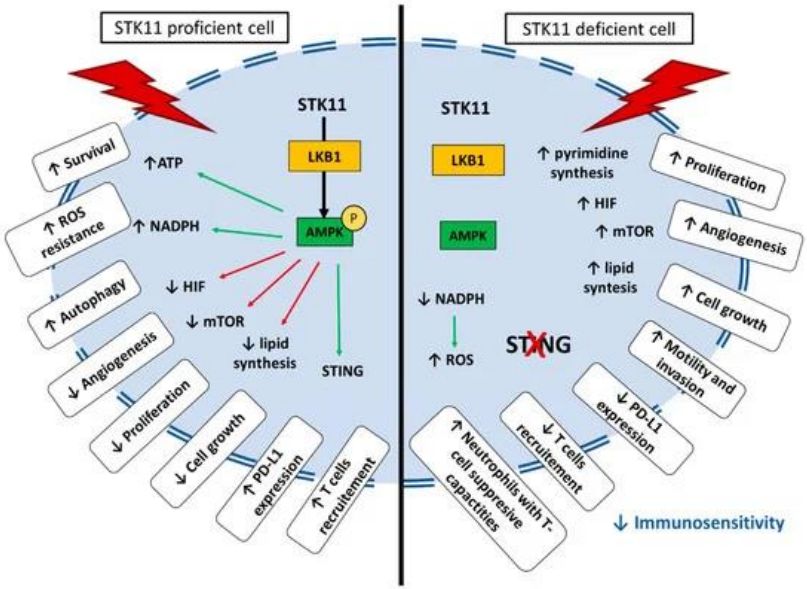
Resistance to therapy: Patients with lung cancer who have PTEN loss may develop resistance to some targeted therapies and chemotherapy treatments. Determining the PTEN status may be useful in determining the best course of action and forecasting therapy response (Lu *et al*., 2022).

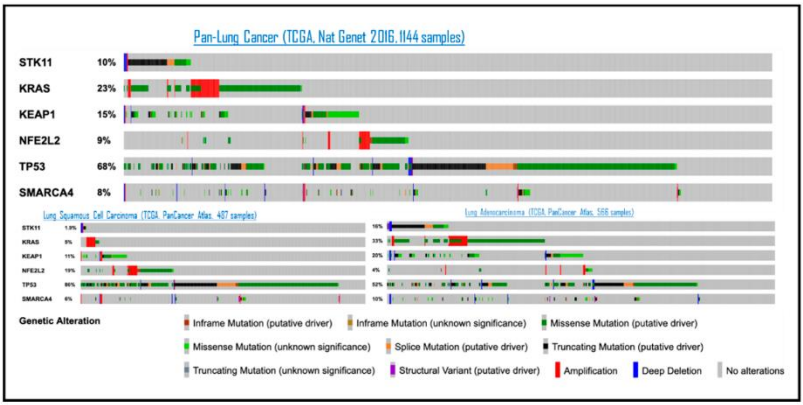
STK11



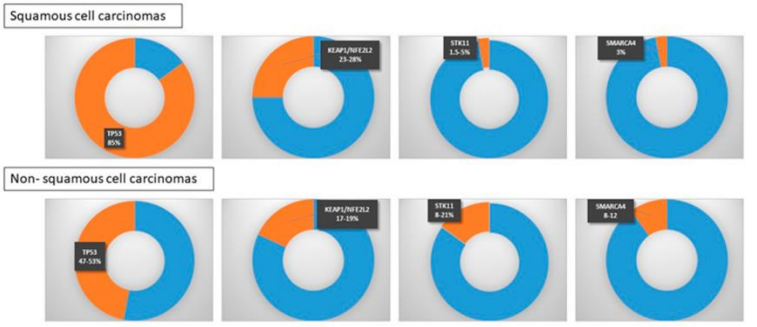
Biological basis of susceptibility with CHRNA genes nAChR are ubiquitous cell-surface receptors composed of five subunits, each a transmembrane protein with four membrane-spanning domains (Figure 3). By binding to a subunit and causing a conformational change on the internal side of the membrane that permits cations (Ca^{2+}, Na^{+}, and K^{+}) to flow into the cytoplasm, nicotine mimics the action of acetylcholine. The voltage-activated calcium channels are opened by this Ca^{2+} influx, which has a variety of effects on the activation of calcium-dependent signalling pathways. The two primary nAChR forms found in bronchial epithelial cells are α7 homopentamers and α3α5β2 heteropentamers. Within the epithelium, these two receptor types exhibit disparate distributions. While α3α5β2-

nAChr is highly expressed in migrating cells at the wound edge, α7-nAChR is mainly expressed at the lateral edges of differentiated, non-migrating cells in wounded epithelium. Furthermore, the surface of basal cells the compartment that produces progenitors for normal differentiation and for flat cells that migrate during wound repair also exhibits low levels of the latter receptor. Based on these findings, α3α5β2-nAChR may be involved in the healing process of injured bronchial epithelium. Changes in nAChR signalling may be a factor in lung cancer. According to studies on gene expression, non-smokers with NSCLC have higher levels of α3-nAChR and α6-nAChR than smokers do. These studies have also found a 65-gene expression signature linked to the α3/α6-nAChR expression pattern. In a recent study, we discovered that CHRNA3 is systematically down-regulated and hypermethylated in lung cancers, but not CHRNA5. Increased apoptosis was observed in lung cancer cells that were forced to express CHRNA3, indicating that hypermethylation may give cancer cells an advantage over healthy cells in terms of survival. Additionally, it is possible that these receptors could improve the way tobacco carcinogens target bronchial cells because the tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosonornicotine (NNN) bind to different nAChRs with affinities higher than for nicotine itself (Pons-Tostivint *et al*., 2021).





(**A**)



(**B**)

Genes upregulated	Genes downregulated	Pathways involved
	RAS	PI3K Pathway
KRAS, BRAF		MAPK Pathway
	MIR-145, HER2	EGFR Pathway
TP53		P53 Pathway
	mTOR	mTOR pathway
	ROS1	ROS1 pathway
	TRIM52	Wnt/β-catenin pathway

Transcription factors affected by SNP

Genes upregulated	Genes downregulated
EGFR	RAS
KRAS, BRAF	TP53
ALK	MIR-145, HER2
ROS1	mTOR

	STK11
	TRIM52
	PTEN

Gene	SNP	Effected TF	Function
APC	rs1012191	STAT2:STAT1	STAT1 and STAT2 proteins are key mediators of type I and type III interferon (IFN) signalling and are essential components of the cellular antiviral response and adaptive immunity. They associate with IFN regulatory factor 9 (IRF9) to form a heterotrimeric transcription factor complex known as ISGF3.

Conclusion

Lung cancer has an influence on families, communities, and healthcare systems in addition to individuals. Lung cancer has a significant financial impact due to the high expense of diagnosis, treatment, and supportive care. Furthermore, it is impossible to ignore the emotional toll that patients and their loved ones endure. The main targets of lung cancer prevention and early detection efforts have been these two areas. Tobacco usage has been decreased and public health regulations, educational initiatives, and anti-smoking campaigns have been put into place to increase awareness of the risks associated with smoking. These programs, however, encounter difficulties in nations where smoking is still socially and culturally acceptable and tobacco control laws are not adequately implemented.

In terms of treatment, Lung cancer treatment has changed dramatically as a result of developments in immunotherapies and targeted medicines. Patients with particular genetic alterations, such EGFR or ALK, have demonstrated extraordinary success when treated with targeted therapy, such as tyrosine kinase inhibitors (TKIs). These medications work by selectively targeting the aberrant proteins that these mutations create, improving results and extending survival. Conversely, immunotherapies use the immune system's strength to combat cancerous cells. By preventing immune cells from attacking cancer cells, checkpoint inhibitors such as PD-1 and PD-L1 inhibitors have shown to be highly beneficial in a subset of patients with lung cancer. Results from these treatments have been encouraging, especially in individuals with metastatic or advanced illness.

Nonetheless, obstacles persist in guaranteeing fair access to these inventive treatments, given their potential cost and potential lack of

accessibility in some medical environments. Furthermore, resistance to immunotherapies and targeted therapies might emerge over time, requiring continued study and the creation of novel therapeutic approaches.

To sum up, lung cancer is a complicated and diverse global health issue that necessitates an all-encompassing strategy. The incidence of lung cancer can be significantly decreased by implementing preventive measures, such as quitting smoking and limiting exposure to environmental risk factors.

Additionally, advancements in understanding the genetic and molecular mechanisms of lung cancer have paved the way for targeted therapies and immunotherapies, offering new hope for patients. However, addressing the economic, social, and healthcare challenges associated with lung cancer remains a priority to improve outcomes and reduce the global impact of this devastating disease.

References

1. Bade, B. C., & Dela Cruz, C. S. (2020). Lung Cancer 2020: Epidemiology, Etiology, and Prevention. In *Clinics in Chest Medicine* (Vol. 41, Issue 1). https://doi.org/10.1016/j.ccm.2019.10.001
2. de Groot, P. M., Wu, C. C., Carter, B. W., & Munden, R. F. (2018). The epidemiology of lung cancer. *Translational Lung Cancer Research*, *7*(3), 220–233. https://doi.org/10.21037/TLCR.2018.05.06
3. Dong, Z. Y., Zhong, W. Z., Zhang, X. C., Su, J., Xie, Z., Liu, S. Y., Tu, H. Y., Chen, H. J., Sun, Y. L., Zhou, Q., Yang, J. J., Yang, X. N., Lin, J. X., Yan, H. H., Zhai, H. R., Yan, L. X., Liao, R. Q., Wu, S. P., & Wu, Y. L. (2017). Potential predictive value of TP53 and KRAS mutation status for response to PD-1 blockade immunotherapy in lung adenocarcinoma. *Clinical Cancer Research*, *23*(12). https://doi.org/10.1158/1078-0432.CCR-16-2554
4. El-Telbany, A., & Ma, P. C. (2012). Cancer Genes in Lung Cancer: Racial Disparities: Are There Any? In *Genes and Cancer* (Vol. 3, Issues 7–8). https://doi.org/10.1177/1947601912465177
5. Huang, L. T., Zhang, S. L., Han, C. B., & Ma, J. T. (2022). Impact of EGFR exon 19 deletion subtypes on clinical outcomes in EGFR-TKI-Treated advanced non-small-cell lung cancer. In *Lung Cancer* (Vol. 166). https://doi.org/10.1016/j.lungcan.2022.01.014
6. Kumar, H., Akash, K., Bhardwaj, K., Taneja, A., Singh, S., Manickam, S., Valko, M., & Kuča, K. (2022). *Applications of Metallic*

Nanoparticles in Lung Cancer Treatment. https://doi.org/10.1007/16833_2022_51

7. Lu, L., Fang, T., Pang, T., Chen, Z., Cheng, L., Ma, D., & Xi, Z. (2022). The Potential Application of Branch-PCR Assembled PTEN Gene Nanovector in Lung Cancer Gene Therapy. *ChemBioChem*, *23*(21). https://doi.org/10.1002/cbic.202200387
8. Pons-Tostivint, E., Lugat, A., Fontenau, J. F., Denis, M. G., & Bennouna, J. (2021). Stk11/lkb1 modulation of the immune response in lung cancer: From biology to therapeutic impact. In *Cells* (Vol. 10, Issue 11). https://doi.org/10.3390/cells10113129

Chapter - 5

Relationship between Gut Microbial Incidence and Metabolic Disorders in Human

Authors

Diptika Dey
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Rupesh Dutta Banik
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Chapter - 5

Relationship between Gut Microbial Incidence and Metabolic Disorders in Human

Diptika Dey and Rupesh Dutta Banik

Abstract

Over 100 trillion microbial cells live in the human gut, which have a symbiotic connection with the host and play a crucial role in regulating human metabolism. The gut microbiota has a significant impact on controlling the immunological responses of the host as it gets older, and it is characterized by varying levels of bacterial presence in different age categories. Furthermore, it has been confirmed that there are molecular connections between the gut microbiota and the immune system of the host, as well as the storage of fat and the metabolism of energy. The precise processes that link specific alterations in the makeup of the gut microbiota to the development of metabolic disorders and obesity in humans remain unknown due to the intricate causes of these ailments. This study examines the current knowledge of the molecular connections between the immune system of the host, the gut microbiota, and the host's energy metabolism in relation to obesity and metabolic diseases. This study highlights the significance of the connection between metabolic inflammation in the host and gut microbes. The primary objective of this study was to investigate the age-related variations in the gut microbiota, the composition of which changes throughout the course of an individual's lifespan, and explore potential methods to modify it.

Keywords: Gut microbiota, host immunity, energy metabolism, obesity, metabolic inflammation.

Introduction

The human body harbors a multitude of cells that are not exclusively human, a fact that has just recently come to our attention. According to (Whitman 1998), there are at least 100 trillion microbial cells and a quadrillion virus both within and on humans (Haynes *et al*. 2011). The human microbiota consists of the various microorganisms that reside within

and on the human body, while the microbiome refers to the complete set of genes encoded by these microorganisms. This complex ecosystem comprises creatures from several branches of the biological tree, such as bacteria, eukaryotes, viruses, and at least one archaeon. These organisms interact with the host and with each other, leading to major effects on human physiology and health. Just a tiny percentage of them are cultivable, and recently, high-throughput sequencing that is not dependent on culture has significantly increased the number of known microorganisms found in human bodies and the surrounding environment (Shendure *et al*. 2008) (Whitman 1998). Our microbiota's geographical, temporal, and disease-associated patterns may now be found because to highly multiplexed studies' ability to quickly describe and analyze large numbers of samples (Caporaso 2010) (Hamady 2008).

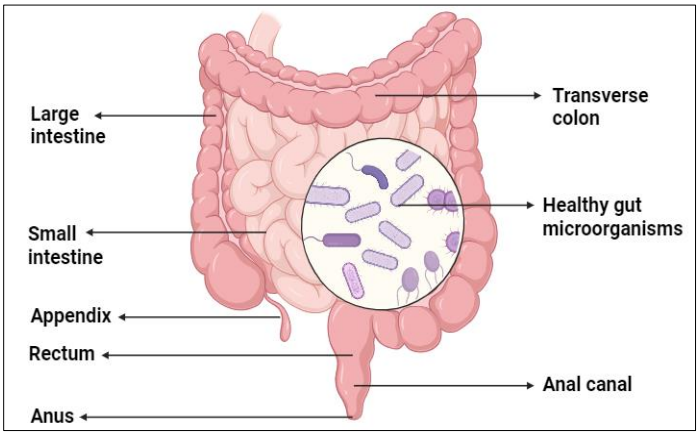


Fig 1: Human gut microbiota

Human health and illness are significantly influenced by the microbiota, which is why it's frequently called our "forgotten organ" (O'Hara *et al*. 2006). Furthermore, the microbiota has a role in other metabolic processes, such as the breakdown and assimilation of unprocessed carbohydrates, in addition to its contribution to energy generation and storage (Gill 2006). This characteristic has likely played a significant evolutionary role in the establishment of bacteria as human symbionts. The gut microbiota plays a significant role in immune system interactions by promoting immune cell maturation and appropriate immune function development through the provision of signals (Chow 2010).

Crucial significance of gut microbiota in human health

Our digestive tracts are home to trillions of microorganisms known as

the "gut microbiota."(Bruzzese *et al*. 2006). Following birth, the human gut continues to colonize and is influenced by a number of variables, such as gestational age, delivery method (natural or caesarean), nutrition (breastfeeding versus baby formula), cleanliness and exposure to antibiotics. The development of an adult-like microbiota and the bacterial-host symbiosis, which impacts the neurological and immune systems, are largely dependent on the environment and diet during the first three years of life. The human gut microbiota develops the characteristics of an adult microbiome between the ages of two and five. The process of colonization with these microbes is started by prenatal microbial transfer from the mother to the fetus (Rodríguez *et al*. 2015)

The gut microbiota also produces pharmacologically active signalling molecules that interact with the metabolism of the host. As an illustration, gut bacteria degrade food fibers to create short-chain fatty acids (SCFAs). Their interactions with G protein couple receptor (GPCRs) influence insulin sensitivity in peripheral organs and adipocytes, which in turn controls energy consumption. Although there is a wide variety of bacterial species in healthy people, gene sequencing data have revealed that the gut metagenome all the genes in the community of gut microorganisms is involved in essential processes like the breakdown and digestion of nutrients that would otherwise be indigestible and the growth and stimulation of the host's immune system and digestive tract (Dis 2006).

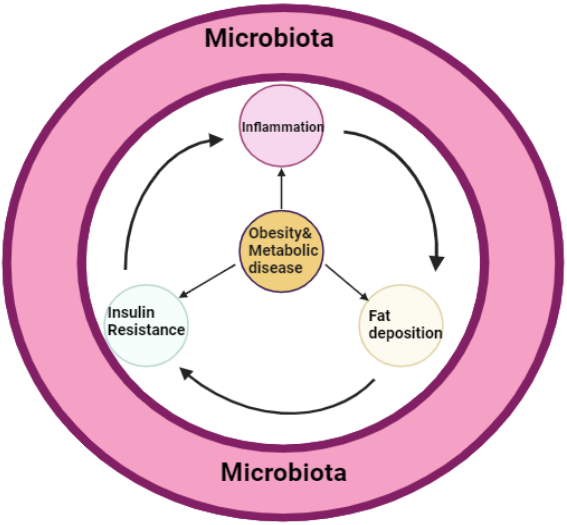


Fig 2: Interactions between the host mammalian gut microbiota and metabolism and inflammation

It is currently unknown, nevertheless, whether the primary cause of the host's metabolic and inflammatory diseases is the existence of keystone bacterial species or the overall loss of microbial core activities (Marchesi *et al*. 2016). Interactions between the mammalian host's gut flora and metabolism and inflammation through many molecular interactions, the gut microbiota can indirectly contribute to the genesis of obesity and metabolic disorders by promoting insulin resistance, low-grade inflammation, and fat deposition in the host.

Metabolic syndrome in relation to obesity

An imbalance between energy intake and energy expenditure results in obesity, which is defined by an excess of adipose tissue (Costello *et al*. 2012). People with the metabolic syndrome are officially diagnosed when they satisfy three or more of these requirements (Nicholson *et al*. 2012).

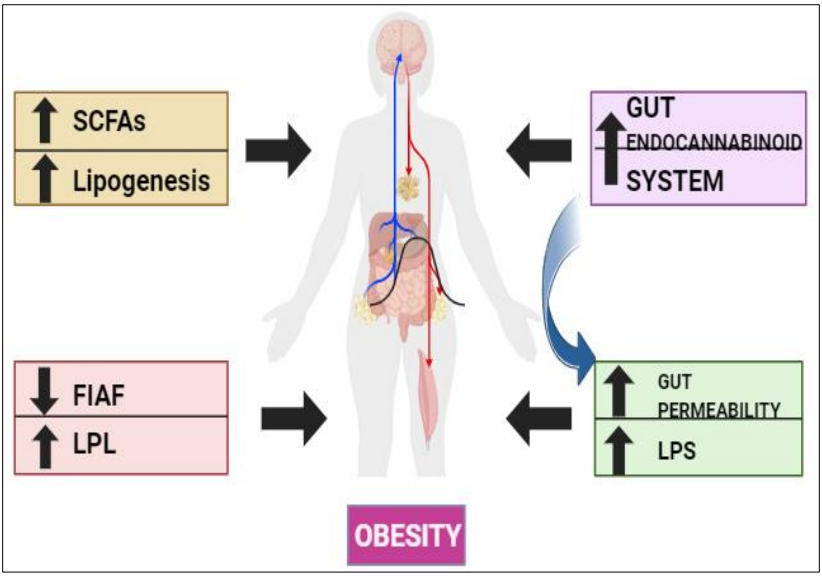


Fig 3: Mechanisms underlying the association with obesity

This raises the possibility of acquiring cardiovascular disorders and metabolic illnesses like type 2 diabetes. Since excessive fat build-up characterizes the majority of those with metabolic syndrome, it is likely that an excess of adipose tissue plays a causal role in the condition (Hooper *et al*. 2012). The clinical diagnosis of metabolic syndrome should be viewed as a result of a complicated interplay between abnormal fat storage, insulin action, and immunity (Kahn *et al*. 2006).

Energy balance and calorie harvesting are influenced by the gut bacteria

Many studies demonstrate that the gut bacteria aids in energy harvesting and increases the amount of fat stored by the host (Gill *et al*. 2006). Furthermore, germ-free mice are shielded against diet-induced glucose intolerance and the emergence of insulin resistance, and they weigh less than mice grown normally (Marchesi *et al*. 2016).

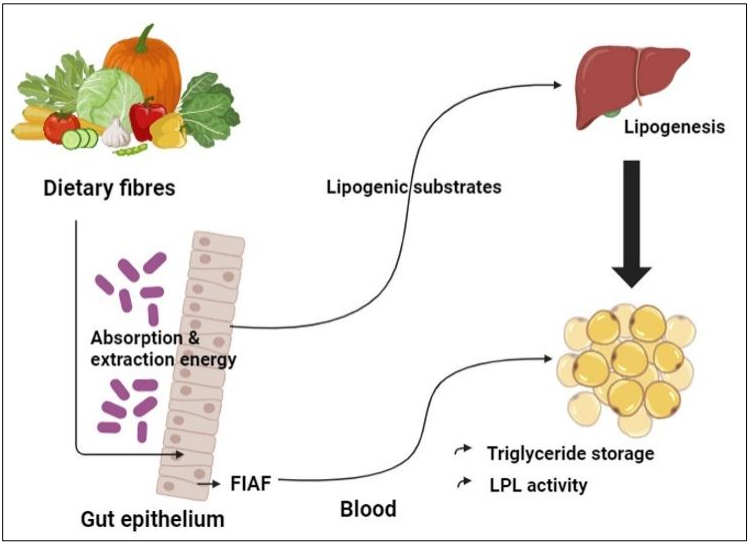


Fig 4: The microbiota in the gut aids in obtaining energy from food and promotes lipogenesis

There are noticeable differences between germ-free mice and conventionally grown mice in the expression of host genes related to energy homeostasis, lipid metabolism, and mitochondrial metabolism in various gastrointestinal segments, as well as in the liver and adipose tissues (Larsson *et al*. 2012). Compared to their traditionally grown littermates, germ-free mice consume 29% more calories, yet they have 40% less total body fat (Gill *et al*. 2006). Moreover, insulin resistance, hepatic triglyceride levels, and body fat percentage all increased by 57% when the fecal microbiota of mice grown normally was transferred to animals lacking germs. This occurred without changing the mice's food intake. The host's gut microbiota interacts with the immune system and metabolism in obesity and metabolic syndrome. Through molecular interactions, the gut microbiota and host regulate the host's physiology, metabolism, and inflammatory state. The gut microbiota specifically influences the physiology and motility of the digestive tract,

which in turn affects the digestion of polysaccharides and directly affects the host's energy availability (Bäckhed *et al*. 2004).

Links among inflammatory diseases, inflammation, and the gut microbiome

The composition of the gut microbiota is impacted by various factors and is negatively associated with body mass index (BMI) and dietary choices. Additionally, it has a bidirectional interaction with inflammation, and its production method determines whether it promotes or inhibits inflammatory pathways. These, in turn, can promote the onset of specific inflammatory illnesses.

Once the inflammatory process is under way, it will follow a certain path until the source of the inflammation is eliminated, at which point the healing process may begin. On the other hand, the inflammation will persist and frequently change in intensity over time if the underlying cause of the inflammation cannot be addressed. While neutrophil granulocytes, also known as neutrophils, will accumulate in the inflammatory tissue during acute inflammation, lymphocytes, macrophages, and plasma cells will accumulate in the tissue as well as infiltrate the connective tissue during chronic inflammation. On the other hand, eosinophil granulocytes (eosinophils) and T-lymphocytes and occasionally neutrophils will quickly accumulate during an allergic response.

Acute inflammation can also result from the breakdown of cancerous tumors or from cell death at the heart's infarct. A bacterial infection is a clear example of a scenario that causes acute inflammation. Persistent inflammation is frequently caused by contact allergies, autoimmune diseases, intracellular bacterial infections, and reactions to external chemicals.

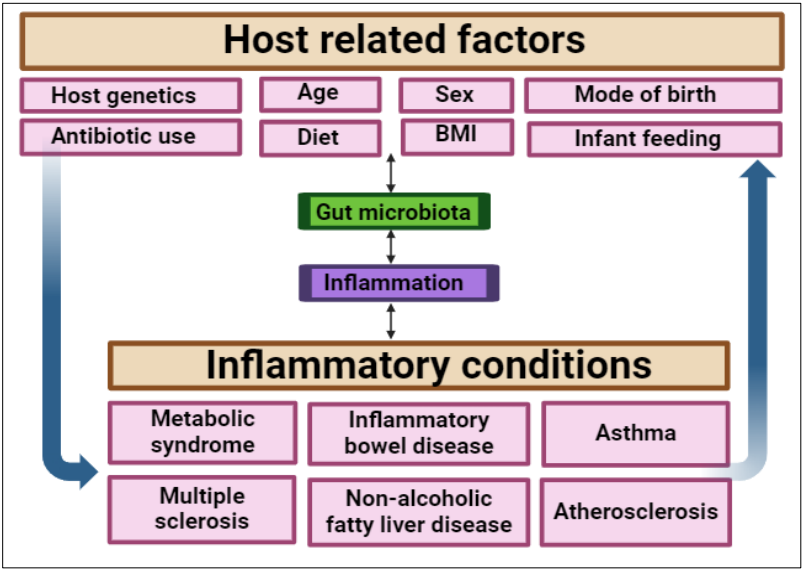


Fig 5: Links between inflammatory diseases, inflammation, and the gut microbiota

Chronic diseases can arise from pathogenic processes triggered by persistently elevated amounts of inflammatory mediators (Hakansson *et al*. 2011). Despite the fact that subacute systemic inflammation may be influenced by the microbiota, the outcome may also influence it, exacerbating the illness state. It is therefore challenging to identify a single causal route. Studies on both humans and animals have looked closely at the relationship between changed gut microbiota and systemic inflammation. It can lead to a number of illnesses, such as cancer, inflammatory bowel disease, and metabolic syndrome (Armstrong *et al*. 2018).

Conclusion

The volume of evidence showing the gut microbiota plays a major role in the development of obesity and metabolic diseases is growing. The adoption of germ-free animal models has furthered our understanding of the molecular mechanisms underlying the interplay between gut microbes and host physiology. Changes in the gut microbial ecology documented in humans and rats as a result of dietary factors, antibiotics, probiotics, or prebiotics show that the gut microbiota plays a significant modulatory role in host obesity and metabolic diseases.

Future direction

Supporting top-down analytical methodologies on an epidemiological

scale is essential for future research to better understand the roles gut bacteria play in the physiopathology of human obesity. Data from food surveys, information about pertinent environmental elements (such stress or things that affect circadian rhythms), and information about past drug or antibiotic use are all included into this process. We also hope to improve the metabolic disease risk profile and offer new perspectives on personalized medicine, where professionals may customize medication according to genetic predispositions and patient behaviors.

Author contributions

Diptika Dey is responsible for data acquisition and interpretation and the study was conceptualized, designed, and revised by Rupesh Dutta Banik.

Acknowledgement

I am extremely grateful to the higher authorities of Swami Vivekananda University for granting me permission to conduct the study.

References

1. Abrams, G. D., & Bishop, J. E. (1967). Effect of the normal microbial flora on gastrointestinal motility. *Proceedings of the Society for Experimental Biology and Medicine*, *126*(1), 301-304.
2. Alberti, K. G. M., Zimmet, P., & Shaw, J. (2005). The metabolic syndrome - a new worldwide definition. *The Lancet*, *366*(9491), 1059-1062.
3. Armstrong, H., Bording-Jorgensen, M., Dijk, S., & Wine, E. (2018). The complex interplay between chronic inflammation, the microbiome, and cancer: understanding disease progression and what we can do to prevent it. *Cancers*, *10*(3), 83.
4. Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., ... & Gordon, J. I. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the national academy of sciences*, *101*(44), 15718-15723.
5. Bruzzese, E., Volpicelli, M., Squaglia, M., Tartaglione, A., & Guarino, A. (2006). Impact of prebiotics on human health. *Digestive and Liver Disease*, *38*, S283-S287.
6. Cani, P. D., Amar, J., Iglesias, M. A., Poggi, M., Knauf, C., Bastelica, D., ... & Burcelin, R. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*, *56*(7), 1761-1772.

7. Cani, P. D., Bibiloni, R., Knauf, C., Waget, A., Neyrinck, A. M., Delzenne, N. M., & Burcelin, R. (2008). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet–induced obesity and diabetes in mice. *Diabetes*, *57*(6), 1470-1481.

8. Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., ... & Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature methods*, *7*(5), 335-336.

9. Chow, J., Lee, S. M., Shen, Y., Khosravi, A., & Mazmanian, S. K. (2010). Host–bacterial symbiosis in health and disease. *Advances in immunology*, *107*, 243-274.

10. Costello, E. K., Stagaman, K., Dethlefsen, L., Bohannan, B. J., & Relman, D. A. (2012). The application of ecological theory toward an understanding of the human microbiome. *Science*, *336*(6086), 1255-1262.

11. Després, J. P., Lemieux, I., Bergeron, J., Pibarot, P., Mathieu, P., Larose, E., ... & Poirier, P. (2008). Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. *Arteriosclerosis, thrombosis, and vascular biology*, *28*(6), 1039-1049.

12. Francescone, R., Hou, V., & Grivennikov, S. I. (2014). Microbiome, inflammation, and cancer. *The Cancer Journal*, *20*(3), 181-189.

13. Hakansson, A., & Molin, G. (2011). Gut microbiota and inflammation. *Nutrients*, *3*(6), 637-682.

14. Gill, S. R., Pop, M., DeBoy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., ... & Nelson, K. E. (2006). Metagenomic analysis of the human distal gut microbiome. *science*, *312*(5778), 1355-1359.

15. Hooper, L. V., Littman, D. R., & Macpherson, A. J. (2012). Interactions between the microbiota and the immune system. *science*, *336*(6086), 1268-1273.

16. Jiao, N., Baker, S. S., Nugent, C. A., Tsompana, M., Cai, L., Wang, Y., ... & Zhu, L. (2018). Gut microbiome may contribute to insulin resistance and systemic inflammation in obese rodents: a meta-analysis. *Physiological genomics*, *50*(4), 244-254.

17. Kahn, S. E., Hull, R. L., & Utzschneider, K. M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*, *444*(7121), 840-846.

18. Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *nature*, *444*(7122), 1027-1031.

19. Larsson, E., Tremaroli, V., Lee, Y. S., Koren, O., Nookaew, I., Fricker, A., ... & Bäckhed, F. (2012). Analysis of gut microbial regulation of host gene expression along the length of the gut and regulation of gut microbial ecology through MyD88. *gut*, *61*(8), 1124-1131.

20. Marchesi, J. R., Adams, D. H., Fava, F., Hermes, G. D., Hirschfield, G. M., Hold, G., ... & Hart, A. (2016). The gut microbiota and host health: a new clinical frontier. *Gut*, *65*(2), 330-339.

21. Neves, A. L., Chilloux, J., Sarafian, M. H., Rahim, M. B. A., Boulangé, C. L., & Dumas, M. E. (2015). The microbiome and its pharmacological targets: therapeutic avenues in cardiometabolic diseases. *Current opinion in pharmacology*, *25*, 36-44.

22. Nicholson, J. K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., & Pettersson, S. (2012). Host-gut microbiota metabolic interactions. *Science*, *336*(6086), 1262-1267.

23. Nicholson, J. K., & Wilson, I. D. (2003). Understanding'global'systems biology: metabonomics and the continuum of metabolism. *Nature Reviews Drug Discovery*, *2*(8), 668-676.

24. O'Hara, A. M., & Shanahan, F. (2006). The gut flora as a forgotten organ. *EMBO reports*, *7*(7), 688-693.

25. Parséus, A., Sommer, N., Sommer, F., Caesar, R., Molinaro, A., Ståhlman, M., ... & Bäckhed, F. (2017). Microbiota-induced obesity requires farnesoid X receptor. *Gut*, *66*(3), 429-437.

26. Piya, M. K., McTernan, P. G., & Kumar, S. J. J. E. (2013). Adipokine inflammation and insulin resistance: the role of glucose, lipids and endotoxin. *J Endocrinol*, *216*(1), T1-T15.

27. Rajilić-Stojanović, M., & De Vos, W. M. (2014). The first 1000 cultured species of the human gastrointestinal microbiota. *FEMS microbiology reviews*, *38*(5), 996-1047.

28. Rodríguez, J. M., Murphy, K., Stanton, C., Ross, R. P., Kober, O. I., Juge, N., ... & Collado, M. C. (2015). The composition of the gut microbiota throughout life, with an emphasis on early life. *Microbial ecology in health and disease*, *26*(1), 26050.

29. Shendure, J., & Ji, H. (2008). Next-generation DNA sequencing. *Nature biotechnology*, *26*(10), 1135-1145.

30. Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *nature*, *444*(7122), 1027-1031.

31. Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., ... & Gordon, J. I. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the national academy of sciences*, *101*(44), 15718-15723.

32. World Health Organization (WHO). (2016). Obesity and overweight. January 2015.

Chapter - 6

Diagnosis of Alagille Syndrome: A Genetic Disorder

Authors

Krittika Das
Department of Biotechnology, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Soumashri Mondal
Department of Biotechnology, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Suranjana Dhara
Department of Biotechnology, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Aditi Das
Department of Biotechnology, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Daizy
Department of Biotechnology, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Semanti Ghosh
Department of Biotechnology, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Chapter - 6

Diagnosis of Alagille Syndrome: A Genetic Disorder

Krittika Das, Soumashri Mondal, Suranjana Dhara, Aditi Das, Daizy and Semanti Ghosh

Abstract

Another name for Alagille syndrome (ALG) is arteriohepatic dysplasia. It is described by French pediatrician Daniel Alagille (1925- 2005). The first publication was by the American pediatrician David W. Smith and colleagues. The most prevalent cause of ALGs is a mutation in JAG1(ALGs type 1) however NOTCH2(ALGs type 2) is also occasionally mutated. ALGs is a chronic cholestasis disorder with autosomal dominant inheritance, characterized by chronic bile duct shortage, peripheral pulmonary artery stenosis, and other abnormalities. Defects in the Notch signalling system cause the complicated autosomal dominant condition known as Alagille syndrome (ALG). The liver, heart, skeleton, face, and eyes are the main body parts. JAG1(20p12) mutations account for over 90% of cases. Deletions incorporating JAG1 account for an additional 5-7% of cases and NOTCH2(1p13) mutations account for roughly 1% of cases. Type 1 (JAG1 associated) and Type2 (NOTCH2 associated) are two names for ALGs. Treatment focuses on treating congenital cardiac problems medically and surgically as well as the effects of liver illness. Its clinical features, diagnosis, and management are discussed.

Keywords: Alagille syndrome, arteriohepatic dysplasia, congenital cardiac, JAG1, NOTCH2, pulmonary artery stenosis.

1. Introduction

Arteriohepatic dysplasia, another name for Alagille syndrome, is a multisystem autosomal disorder brought on by abnormalities in the Notch signaling pathway (Ennaifer *et al*., 2016). French pediatrician Daniel Alagille initially noted this multisystem illness in 1969; the first diagnostic standards were published in 1975 (Xu *et al*., 2022). Neonatal liver disease with conjugated hyperbilirubinemia was identified as a prominent symptom of the disease when it was originally characterized in 1969 (Ponikowska *et al*., 2020). One in thirty to one in seventy thousand live babies are thought to

have ALGS (Xu *et al*., 2022). Pathogenic variations of the JAG1 gene (20p12) account for 97% of cases, while mutations in the NOTCH2 gene (1p13) account for less than 1% of cases (Ponikowska *et al*., 2020).

Alagille syndrome, which results in cholestasis, is an autosomal dominant disorder that affects multiple systems. Bile duct paucity is one of these systems. Clinical signs can include cardiovascular problems, renal anomalies, distinctive facial features, vertebral arch/other skeletal malformations, and ocular features (usually posterior embryotoxic), as the penetrance is highly diverse (Jannone *et al*., 2023). Abnormalities of the kidneys and vascular system are significant characteristics of ALGS in addition to these manifestations (Xu *et al*., 2022). However, recent advancements in genetic testing have demonstrated that clinical presentations vary, ranging from the absence of symptoms to multiorgan involvement. Tetralogy of Fallot, the most common complex congenital cardiac disease in Alagille Syndrome, very infrequently causes renal failure that requires dialysis; the literature only has a small number of isolated reports of such cases, none of which are connected to Alagille Syndrome (Ponikowska *et al*., 2020).

1.1 Genetics of alagille syndrome

More than 90% of people with ALGS who have been clinically diagnosed had mutations in JAG1, which is located on chromosome 20 (Lin *et al*., 2012), however occasionally it is deleted. Alagille syndrome is caused by a mutation of the NOTCH2 gene in 2% to 3% of cases. The genetic etiology of Alagille syndrome is unknown in about 3% of cases.

Single-pass transmembrane proteins, Jag1 and NOTCH2, have 26 and 34 exons, respectively. Through contact between NOTCH2 (receptor) and JAG1 (ligand) extracellular domain, direct communication between the two proteins is achieved. This connection requires several functional motifs, such as the extracellular EGF-like repeats on NOTCH2, the delta-state-lag2 (DSL) domain, the C2-like domain, and the epidermal growth factor-like (EGF-like) repeats on JAG1. Additionally, NOTCH2 has a sequence of Ankyrin (ANK) repeats that enable contact between the intracellular portion of NOTCH2 and transcription factors and are necessary for the propagation of signals (Gilbert *et al*., 2019).

One of the five cell surface ligands, Jagged1 (JAG1, encodes JAGGED1 cell surface protein), is mainly involved in the highly conserved Notch signalling pathway. Notch signalling is active during development and in many different organ systems, and it is essential for determining the fate of individual cells. The NOTCH intracellular domain is carried into the nucleus

via a sequence of proteolytic cleavages that are initiated by the classic JAG1-NOTCH interaction. There, it activates downstream transcription of the target gene. Numerous illnesses, notably the multisystem dominant condition Alagille syndrome, have been linked to JAG1 mutations. (Grochowski *et al*., 2016).

Three of the five classic features were identified in the original clinical description of AlGS, that are posterior embryotoxic, characteristic facial features, pulmonic stenosis, tetralogy of fallot, butterfly vertebrae. It has been shown that a significant proportion of individuals also exhibit renal and vascular abnormalities in addition to these traditional characteristics (Grochowski *et al*., 2016). The range of JAG1 mutations in ALGS includes missense, nonsense, frameshift, and splice site mutations as well as complete gene deletions. These mutations may be the source of the clinical manifestation of JAG1 haploinsufficiency (Warthen *et al*., 2006). Haploinsufficiency is the disease mechanism for ALGS, and missense mutations have occasionally been demonstrated to be harmful due to improper trafficking to the cell membrane.

The JAG1 and NOTCH2 genes encode proteins that guide cells on when to start building specific body parts during fetal development by establishing a communication channel between them. The cells in the body lack the instructions necessary to carry out their functions. This results in symptoms such as skeletal abnormalities, narrow bile ducts, and structural heart defects.

1.2 Causes

Alagille Syndrome is caused by a gene mutation. The majority of instances is due to mutations on JAG 1 gene. Alagille Syndrome is caused in 1-2% of instances by mutations in the NOTCH2 gene (Saleh *et al*., 2016). There is an autosomal dominant pattern of inheritance for these gene variations. Some individuals experience the variant at random as a result of a spontaneous genetic alteration.

In 40% of cases, ALGS is inherited from a parent; in the other 60%, it develops from other reasons. In cases when one parent is affected by ALGS, particularly if prenatal genetic testing was not done, detailed fetal ultrasonography is crucial for identifying in-utero characteristics of the condition and assessing the severity of cardiac abnormalities if the fetus has genetically confirmed ALGS (Ayoub *et al*., 2023).

1.3 Symptoms

Individual person may develop symptoms differently. Symptoms may include:

1.3.1 Cardiac Involvement

The most prevalent kind of congenital cardiac disease involves the pulmonary outflow system, with peripheral pulmonary stenosis accounting for at least two-thirds of cases.10, 15 Up to 16% of cases involve Tetralogy of Fallot, the most prevalent complicated structural abnormality.10, 15 Aortic stenosis, coarctation of the aorta, ventricular septal defect, atrial septal defect, and hypoplastic left heart syndrome have been documented as further abnormalities (Turnpenny *et al*., 2012).

1.3.2 Blood vessels alteration

Blood vessels in the head and neck can develop improperly. It is also possible for other blood arteries to shrink or change in shape. These alterations in blood arteries may be the cause of major health issues like stroke.

1.3.3 Optical involvement

The most prevalent ocular characteristic of ALGS, posterior embryotoxon, has been found in as many as 90% of cases. This is the prominent Schwalbe's ring (or line) in the center, where the uveal trabecular meshwork and corneal endothelium converge. In ALGS, a wide range of ocular abnormalities affecting the cornea, iris, retina, and optic disc may be observed (Turnpenny *et al*., 2012).

1.3.4 Renal illness

Up to 39% of people with ALGS have been shown to have both functional and structural renal abnormalities, such as renal tubular acidosis tiny hyperechoic kidneys, and renal cysts (Saleh *et al*., 2016). Renal involvement has been identified as a disease-defining characteristic of ALGS, with dysplasia accounting for 59% of abnormalities; vesicoureteric reflux (8%), renal tubular acidosis (9%), and obstructive uropathy (8%), rounding out the top five. In 2–8% of patients, renal-vascular hypertension may also develop (Ayoub *et al*., 2023).

1.3.5 Changes in spinal growth

At least 80% of individuals may have "butterfly" vertebrae, a distinctive type of segmentation abnormality. Anteroposterior radiographs show a sagittal fissure in one or more thoracic vertebrae, which is the result of the anterior vertebral arches failing to fuse (Turnpenny *et al*., 2012).

1.3.6 Growth problem

Alagille syndrome results in narrower blood vessels that blood flows can also decrease which is why oxygen, minerals, and nutrients do not reach

the cells properly. So, it affects the growth and development. However, compared to other chronic liver diseases, growth limitations are more pronounced in children with ALGS (Mitchell *et al*., 2018).

1.3.7 Yellow skin and eyes

Bilirubin is the pigment that gives bile its color. Problem may occur when liver is not able to remove bile properly. Jaundice or yellowing of the white part of the eyes can occur if bile isn't healthily exiting our body. Lack of bile might cause the stool to seem white, gray, or pale, it's also possible for urine to appear darker. (Mitchell *et al*., 2018)

1.3.8 Facial features

A prominent forehead, deep-set eyes with moderate hypertelorism, upslanting palpebral fissures, a depressed nasal bridge, a straight nose with a bulbous tip, large ears, a prominent mandible, and a pointed chin are among the mild but recognizable dysmorphic features associated with children with ALGS. Some kids have incredibly triangular faces. (Turnpenny *et al*., 2012)

1.3.9 Vascular involvement

Research has revealed irregularities in the carotid, middle cerebral, and basilar arteries, as well as reports of renovascular abnormalities, middle aortic syndrome, and Moyamoya syndrome. (Turnpenny *et al*., 2012)

1.4 Diagnosis of alagille syndrome

Based on clinical characteristics, Alagille syndrome can be diagnosed and then verified through genetic testing. Although Alagille syndrome is characterized by certain symptoms, not every patient may experience them all. A test, liver biopsy may be done to check the condition of the liver. Tests on blood are done to evaluate the liver's overall health. The utilization of genetic testing is increasing in order to detect aberrant genes linked to Alagille syndrome. Additional discoveries that could point to Alagille syndrome include:

1.4.1 Test for the heart

Alagille syndrome affects the heart and its functioning in different ways - problems with the lung's blood supply from the heart (pulmonary artery stenosis), heart structural defects such as ventricular septal defect which is a concomitant condition involving pulmonary artery stenosis, ventricular septal defect, and enlarged right ventricles, our skin turns blue due to low blood oxygen levels (cyanosis).

1.4.2 Transplanting liver

Severe pruritus, synthetic malfunction, portal hypertension, bone fractures, and growth failure are among the conditions that warrant a transplant. The patient should undergo head and abdominal imaging to detect vascular anomalies because abnormalities in the abdominal vasculature may affect the procedure's technical elements. Renal disease can be made worse by several immunosuppressive medicines, thus it needs to be assessed before transplant. (Mitchell *et al*., 2018).

1.4.3 Facial and body

Alagille syndrome bodies are born with unique physical traits that impact their faces, such as:

Among the typical triangular facial features seen in ALG patients are a high forehead, deeply set eyes, slight hypertelorism, a pointed chin, and a bulbous tip to the nose. Due to their subjective nature and interobserver variability, the use of face features in diagnostic criteria is debatable. Among the physical symptoms they experience are: The most prevalent type of vertebral bodies are called butterfly vertebrae; due to the failure of the anterior arches to fuse.

In addition, ALG patients have reduced bone density and a higher risk of fractures than would be expected from vitamin D deficiency (Mitchell *et al*., 2018). A liver biopsy that reveals fewer bile ducts than usual along with at least five of the following symptoms confirms the diagnosis of Alagille syndrome: Face forms indicative of Alagille syndrome uncommon spine and/or bone structures, such butterfly vertebrae. Unusual blood vessel or cardiac anatomy or a heart murmur, liver issues, a distinctive white ring around the cornea (Turnpenny *et al*., 2012). To determine if a person has Alagille syndrome, additional testing could be performed, such as: checking of the heart and blood vessels, spine X-rays, and eyes; ultrasonography of the abdomen. Testing for genetics and kidney function shown in Figure 1.

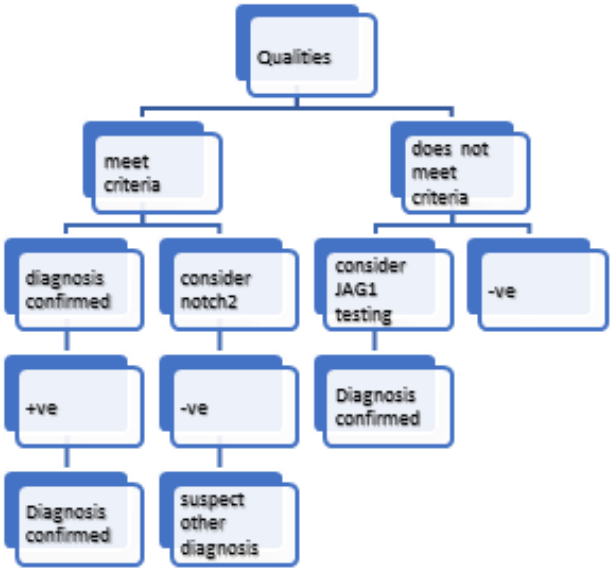


Fig 1: Suspected diagnosis of alagille syndrome

1.5 Prevention

Parents must be aware of the hazards associated with having an ALGS pregnancy because it places a heavy strain on both the fetus and the parents (as previously described). The 50% chance that the fetus may inherit the mutation from either parent should be discussed in preconception counseling. However, it is hard to predict the severity of the disease in an affected fetus due to the lack of genotype–phenotype associations. Prenatal genetic testing should be made available if pregnancy is sought after (Ayoub *et al*., 2023). Alagille syndrome has no known cure, treatment for the syndrome is symptomatic. Among the possible treatments are: Fat-soluble vitamin supplements (A, D, E, and K) may be utilized in cases when there is difficulty digesting or absorbing dietary fat and vitamins. Nutrients being sent to the patient's stomach via a feeding tube (gastrostomy tube) or a nasogastric tube (tube via our nose), taking medication (ursodeoxycholic acid) for liver illness. Severe itching may be relieved and bile flow may be increased with medication. Applying moisturizers or lotions and taking medications (such as cholestyramine, naltrexone, rifampin, and antihistamines) to ease itching difficulties are two ways that these same medications may be used to treat excessive cholesterol levels, which produce the hard, whitish nodules that grow. It may be advised to follow a high-calorie, high-protein diet to avoid malnutrition and growth failure. It might

be advised to use a feeding tube that provides significant amounts of nutrients overnight.

1.6 Conclusion

A hereditary condition called Alagille syndrome mostly affects the liver and other organs. It can cause developmental delays, deformities of the face, renal difficulties, liver problems, heart defects, and other problems. Bile duct anomalies are one of the defining characteristics; these can harm the liver and result in jaundice and other symptoms. JAG1 or NOTCH2 gene mutations are the cause of it. The goal of treatment is to control complications and symptoms.

Future developments in genetic testing, such as whole genome sequencing, to more precisely and effectively identify mutations, necessary to diagnose Alagille syndrome. Furthermore, advances in imaging methods to identify the syndrome's hallmark liver and heart abnormalities may occur. For improved management and results, early detection and intervention are essential.

References

1. Ayoub, M. D., Bakhsh, A. A., Vandriel, S. M., Keitel, V., & Kamath, B. M. (2023). Management of adults with Alagille syndrome. Hepatology International, 17(5), 1098–1112.
2. Ennaifer, R., Ben Farhat, L., Cheikh, M., Romdhane, H., Marzouk, I., & Belhadj, N. (2016). Focal liver hyperplasia in a patient with Alagille syndrome: Diagnostic difficulties. A case report. International journal of surgery case reports, 25, 55–61.
3. Gilbert, M. A., Bauer, R. C., Rajagopalan, R., Grochowski, C. M., Chao, G., McEldrew, D., Nassur, J. A., Rand, E. B., Krock, B. L., Kamath, B. M., Krantz, I. D., Piccoli, D. A., Loomes, K. M., & Spinner, N. B. (2019). Alagille syndrome mutation update: Comprehensive overview of JAG1 and NOTCH2 mutation frequencies and insight into missense variant classification. Human mutation, 40(12), 2197–2220.
4. Jannone, G., de Magnée, C., Tambucci, R., Evraerts, J., Ravau, J., Najimi, M., & Sokal, E. M. (2023). Premature senescence of the liver in Alagille patients. PloS one, 18(4), e0285019.
5. Kohut, T. J., Gilbert, M. A., & Loomes, K. M. (2021). Alagille Syndrome: A Focused Review on Clinical Features, Genetics, and Treatment. Seminars in liver disease, 41(4), 525–537.

6. Lin, H. C., Le Hoang, P., Hutchinson, A., Chao, G., Gerfen, J., Loomes, K. M., Krantz, I., Kamath, B. M., & Spinner, N. B. (2012). Alagille syndrome in a Vietnamese cohort: mutation analysis and assessment of facial features. American journal of medical genetics. Part A, 158A(5), 1005–1013.
7. Mitchell, E., Gilbert, M., & Loomes, K. M. (2018). Alagille Syndrome. Clinics in liver disease, 22(4), 625–641.
8. Saleh, M., Kamath, B. M., & Chitayat, D. (2016). Alagille syndrome: clinical perspectives. The application of clinical genetics, 9, 75–82.
9. Turnpenny, P. D., & Ellard, S. (2012). Alagille syndrome: pathogenesis, diagnosis and management. European journal of human genetics: EJHG, 20(3), 251–257.
10. Xu, F., Peng, Q., He, X., Chen, X., Jiang, S., Lu, X., & Li, N. (2022). Alagille syndrome due to a de novo NOTCH2 mutation presenting as prenatal oligohydramnios and congenital bilateral renal hypoplasia: A case report. Frontiers in pediatrics, 10, 1020536.

Chapter - 7

A Short Review of Microbiota and their Ecological Significances in the Stress of Fly Ash Contaminated Soil

Authors

Supriya Kumar Bose
Department of Biotechnology, School of Life Science, Swami Vivekananda University, Barrackpore, West Bengal, India

Prithwish Nayak
Department of Botany, Midnapore College, Paschim Medinipur, West Bengal, India

Supriti Panja
Department of Botany, Midnapore College, Paschim Medinipur, West Bengal, India

Arpita Chakraborty
Department of Biotechnology, School of Life Science, Swami Vivekananda University, Barrackpore, West Bengal, India

Authors

Keya Mandal
Department of Biotechnology, School of Life Science, Swami Vivekananda University, Barrackpore, West Bengal, India
Department of Environmental Science, Kalna College, Purba Bardhaman, West Bengal, India

Dipti Das
Department of Biotechnology, School of Life Science, Swami Vivekananda University, Barrackpore, West Bengal, India
Department of Botany, Kalna College, Purba Bardhaman, West Bengal, India

Sanjay Kar
Department of Botany, Midnapore College, Paschim Medinipur, West Bengal, India

Sabyasachi Ghosh
Department of Zoology, Kalna College, Purba Bardhaman, West Bengal, India

Chapter - 7

A Short Review of Microbiota and their Ecological Significances in the Stress of Fly Ash Contaminated Soil

Supriya Kumar Bose, Prithwish Nayak, Supriti Panja, Arpita Chakraborty, Keya Mandal, Dipti Das, Sanjay Kar and Sabyasachi Ghosh

Abstract

The disposal of fly ash (FA) is considered as an environmental pollution issue worldwide due to its limited application to manufacture bricks and cement. In India, approx. 100 million ton of fly ash per year are released from thermal power plants. But the production is anticipated to reach about 175 million ton every year in near feature. Fly ash, a by product due to burning of coal which is being dumped in ash ponds causes the degradation of cultivated land. It contains toxic heavy metals like As, Pb, Cd, Cr, U, Th, on the other hand also provides nutrients like P, N, K, S, Mn, Zn, and Fe for soil improvement. Additionally, fly ash changes various soil properties like pH, EC, density, porosity, organic carbon, water holding capacity and NPK ratio. Consequently, fly ash pollution has a diverse impact on microbial populations. Some bacteria and fungi either cannot grow in heavy metal stress or their growth rate has been enhanced significantly. This review includes a brief overview about the microbial diversity in the heterogenous metal stress of fly ash contamination. The review highlights the ecological significance of the microbiota in the stress of fly ash including prospects based on the interaction between microbes and other soil components.

Keywords: Microbiota, fly ash, metal stress, fungi, bacteria.

Introduction

Fly ash, a combustion item from burning coal in power plants is considered a global environmental pollution problem. FA which is being dumped in ash ponds causes degradation of cultivated land. The heavy metal toxicity has been depicted by different researchers due to higher level of FA pollution in the soil (Basu *et al*., 2009; Mishra & Prasad, 2017). On the other hand also FA provides alimentations like K,N, P, Zn, Fe, Mn, and S for soil improvement (Basu *et al*., 2011). Fly ash addition in the soil changes various

physicochemical parameters. Fly ash contamination also has positive or negative impacts on microbial growth in soil (Backer *et al*., 2018). Microbial diversity is regarded as an indicator of good physical condition and quality of the soil. The activity of microbes is affected by different chemical and physical characteristics of the soil (Kaur, 2017). As per the study, the overall impact on microbial activity is inconsistent in the FA-contaminated soil. Previous study has shown no adverse effect on microbial growth in the heavy metal stress (like Cd, Pb, As, Cr etc.) for fly ash contamination since, bacteria can use or tolerate those as substrates (Basu *et al*., 2011). Some research work has been revealed that Mycorrhiza and Rhizobacteria amendment in the metal contaminated soil helps to increase the growth rate and heavy metals tolerance efficacy of plants (Akhtar *et al*., 2020; Jaipal, 2018). A previous review has suggested for the implementation of PGPR and AM fungi in agriculture as low-cost commercial inputs in the field of agriculture (Backer *et al*., 2018). This review includes a brief overview about the microbial diversity in the heterogenous metal stress of fly ash contamination. The review highlights the ecological significance of the microbiota in the stress of fly ash including prospects based on the interaction between microbes and other soil components.

Effect of Fly ash on soil characters

Addition of FA in soil changes various Physicochemical parameters such as particle density, electrical conductivity, pH, bulk density, total organic carbon, total organic matter, porosity, water holding capacity, NPK ratio etc. There are numerous studies that have reported variations in soil physiochemical properties due to addition of FA to soil at various concentration. It is reported that FA has a significant effect on the dynamics of microbiota in the fly ash-amended soil. This includes soil health and soil microbes, enzyme activity, fertility, nitrogen cycling and more (Backer *et al*., 2018; Varshney *et al*., 2020).

Soil metal profile is altered due to fly ash pollution. The level of different metals like Co, Cd, Cr, Mn, Ni, Pb, etc. is gradually ameliorated with the growing amount of fly ash with the soil. The metal toxicity has been depicted by different researchers due to higher level of FA contamination in the soil (Basu *et al*., 2009; Mishra & Prasad, 2017). Further, FA provides nutrients like P, S, Fe, N, K, Zn and Mn for soil improvement (Basu *et al*., 2011). Water holding capacity is improved due to addition of fly ash to soil. The size of FA particle is similar with slit. Therefore, the bulk density (BD) of silty-clay soil is increased due to fly ash amendment whereas BD of the clay soil is decreased with usage of fly ash. Decrease in bulk density in turn

improves the porosity including moisture retention ability of the soil. Hence decreasing bulk density causes more spacing among particles increasing porosity and water-holding capacity (Dey *et al*., 2012; Panda & Biswal, 2018). Another study found that adding fly ash can also alter the bulk density, texture, and structure of the soil. By adding 50% fly ash, the permeability of clay loam soil was found to increase from 0.54 cm/hr to 2.14 cm/hr, whereas that of sandy soil decreased from 23.80 cm/hr to 9.67 cm/hr (Dhindsa *et al*., 2016).

The pH of FA varies from 4.5 to 12.0, contingent on the source coal's sulphur concentration. The fly ash produced in India is primarily of an alkaline type. Therefore, acidic soil can be neutralized by application of alkaline FA. Some research warks have been shown that application of fly ash to agricultural land instead of lime can upgrade the range of pH of the soil (Panda & Biswal, 2018; Plank *et al*., 1975). FA addition leading to increase the pH value causes decrease in electrical conductivity. Leaching of FA help to reduce concentration of soluble salt (Skousen *et al*., 2013).

Microbiota in fly ash metal stress

Microbial activity indicates the soil physical health and quality. The activity is affected by different physicochemical properties of the soil. Some fungi and bacteria can grow in metal stress in such polluted soil. Some bacteria and fungi either cannot grow in heavy metal stress or their growth rate are increased significantly. As per the previous study, the lowest number of bacteria and fungus was reported in the soil samples collected from the industrial area of Bhagwanpur (Uttarakhand). On the other hand, the highest number of bacteria and fungus were reported in the soil samples located for away from the industrial area (Kaur, 2017).

A study has revealed that the bacterial load was more at 5% than 10% and 15% amendment of FA concentration. Same result was noticed in another study where higher microbial activity was shown at the level of 8% FA amendment (Lal *et al*., 1996). Some studies have noticed that certain metals work more strongly and inhibit the activity of microorganisms with increasing concentrations of FA (Jabeen & Sinha, 2012). However, as per the study of (Page *et al*., 1979), FA also has toxic effect due to presence of metals which hinder normal metabolic processes of soil microbes.

When FA is used in soil, lower doses may lead to an increase in existing fungal and bacterial population. However, higher doses have an adverse effect on the balance of bacterial vs. fungal populations. When FA is used in conjunction with farmyard manner (FYM), gypsum and earthworms, it leads

to an increase in microbial populations with increasing dehydrogenase and alkaline phosphatase, and improved soil microbial biomass resulting in better soil microbial community (Varshney *et al*., 2020). Table 1 shows the Fly ash's impact on several microbiological community in the soil

Table 1: Effect of fly ash (FA) on soil microbial population (Varshney *et al*., 2020)

Sl. No.	Fly ash amendment	Microbial population
1	Low concentration of fly ash	*Sphingomonas* sp. 23 L was increased with high growth rate.
2	Low concentration of fly ash	*Bacillus curcas* and *Bacillus subtilis* both were increased with high growth rates.
3	FA at lower concentration	The population of arbuscular mycorrhizal fungi was increased.
4	5% fly ash amendment	*Enterobacter* sp. NBRI K28 was found to increase.
5	20% fly ash amendment	Bacteria, Actinomycetes, and fungi were found to decrease by 57, 80 and 86%
6	40% fly ash amendment	Actinomycetes and fungi were found to decrease. The population of aerobic heterotrophic bacteria was not changed remarkably.
7	50% fly ash amendment	Growth of *Azotobacter chroococcum* and Bacillus circulans were increased. Population of *Bacillus circulans* was also increased.
8	Dumping site of FA	*Paenibacillus* spp and *Bacillus* spp. were found remarkably.
9	Fly ash with FYM + soil	*Rhizobium* sp. was found to increase.

As per previous study, the overall effect of FA on the microbial activity is inconsistent in the soil. But it also has been reported that application of FA with a recommended level of farmyard manner (FYM) and lime enhanced the microbiota with their rapid reproductive potential in response to favourite changes in soil environment. Fly ash addition supplies appreciable amount of P, K, N, S, Fe, Zn and Mn for superior growth of bacteria and FYM stabilizes the pH of the soil. Presence of metals like Cd, Cr, Pb etc. in the fly ash did not affect the growth since, bacteria can use industrial wastes as substrates (Basu *et al*., 2011).

Ecological significances of microorganisms in fly ash contaminated soil

Pseudomonas, Bacillus, Azotobacter, Azospirillum, Klebsiella,

Azomonas, Mesorhizobium etc. are rhizobacteria. Some of them can grow in mental stress as well as can help many plant species to grow and develop under such stress. Plant growth-promoting bacteria can act as Phyto stimulators, Biofertilizers, Rhozoremediators, or Biopesticide (Antoun & Prévost, 2006; Jaipal, 2018).

Reduced carbon in the Soil environment including moisture enhances large scale of microbiota. Prence of the rhizomicrobiome has considerable significance to agricultural purpose. Plant cell debris and root exudates help to increase various colonization of distinctive patterns of microbes. Some bacteria can produce extensive secondary metabolites and volatile organic compounds which can help in stress tolerance including betterment in growth and development of different plant species. For example, polyamines have important physiological roles in protecting plants in such stress (Backer *et al*., 2018).

Some research work has been revealed that Mycorrhiza and Rhizobacteria amendment in the metal contaminated soil helps to increase the growth rate and heavy metals tolerance efficacy of plants. Some local strains of arbuscular mycorrhiza (AM) help to extract metals improving the soil health. Translocation of toxic heavy metals is reduced because AM fungi binds them to its tissues. Different species of 12 genera of the order Glomeromycota can remediate metal-polluted soil. These Species are Glomus, Intraspora, Archeospora etc. (Akhtar *et al*., 2020; Jaipal, 2018).

Future prospects based on the interaction between microbes and soil components

Inoculation of fungi to restore highly metal-polluted degraded land has been proposed as a new approach for reintroducing vegetation in that site (Akhtar *et al*., 2020). An effective way to improve plant growth and development would be to inoculate plants with plant-growth-promoting rhizobacteria (PGPR). Furthermore, the strategies can ameliorate in stress tolerance of the plant. The interaction between microbes and soil components has an acceptation to keep down the application of agrochemicals including synthetic fertilizers. This review has suggested for the implementation of PGPR and AM fungi in agriculture as low-cost strategies in the field of agriculture (Backer *et al*., 2018). Based on literature available, FA can be used (alone or in combination) to improve soil fertility at various dosages that in turn can significantly improve unique microbial population for better agricultural yield. Amount of FA would be used depending upon its properties, agroclimatic conditions and crop to be grown (Varshney *et al*., 2020).

Conclusion

The characteristic microbiota in the stress of fly ash has been elaborated in the sphere of the small scale of the review. Presence of heavy metals does not affect the growth since the soil microbes can utilize or tolerate the toxic elements as substrates. Therefore, a combined biotechnological approach of using FA as cost effective fertilizer with organic manner including inoculation of recommended soil microbes require to be used to reduce adverse effect of metals presence in FA. Hence this study explores that FA amendment as fertilizer is still a doubtful approach for edible crop production as it contains ecotoxic elements. So, the review recommends for more broad scale of research to elaborate the knowledge regarding interaction between microbes and soil components in the presence of fly ash for detoxification and immobilization of the hazardous elements. Then the knowledge would be more useful in the field of bioremediation of FA contaminated soil and in the agricultural context of food security.

References

1. Akhtar, O., Kehri, H. K., & Zoomi, I. (2020). Arbuscular mycorrhiza and Aspergillus terreus inoculation along with compost amendment enhance the phytoremediation of Cr-rich technosol by Solanum lycopersicum under field conditions. *Ecotoxicology and Environmental Safety*, *201*, 110869. https://doi.org/10.1016/j.ecoenv.2020.110869

2. Antoun, H., & Prévost, D. (2006). Ecology of Plant Growth Promoting Rhizobacteria. In Z. A. Siddiqui (Ed.), *PGPR: Biocontrol and Biofertilization* (pp. 1–38). Springer-Verlag. https://doi.org/10.1007/1-4020-4152-7_1

3. Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., & Smith, D. L. (2018). Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. *Frontiers in Plant Science*, *9*, 1473. https://doi.org/10.3389/fpls.2018.01473

4. Basu, M., Bhadoria, P. B. S., & Mahapatra, S. C. (2011). Influence of Soil Ameliorants, Manures and Fertilizers on Bacterial Populations, Enzyme Activities, N Fixation and P Solubilization in Peanut Rhizosphere under Lateritic Soil. *British Microbiology Resarch Journa*, *1*(1), 11–25.

5. Basu, M., Pande, M., Bhadoria, P. B. S., & Mahapatra, S. C. (2009).

Potential fly-ash utilization in agriculture: A global review. *Progress in Natural Science*, *19*(10), 1173–1186. https://doi.org/10.1016/j.pnsc.2008.12.006

6. Dey, B. S., Panigrahi, T., Panigrahi, S., & Panda, R. B. (2012). Agricultural utilisation of flyash- A way for sustainable agriculture practice and its environmental significance. *Discovery Nature*, *1*(1), 11–14.
7. Dhindsa, H. S., Sharma, R. D., & Kumar, R. (2016). Role of fly ash in improving soil physical properties and yield of wheat (Triticum aestivum). *Agricultural Science Digest - A Research Journal*, *36*(2). https://doi.org/10.18805/asd.v36i2.10626
8. Jabeen, S., & Sinha, M. P. (2012). Bacterial Population Dynamics in Fly Ash Amended Soil With and Without Amelioration by Earthworms. *Nature Environment and Pollution Technology*, *11*(1), 99–104.
9. Jaipal. (2018). *Phytoremediation of flyash contaminated soil* [Guru Jambheshwar University of Science and Technology]. http://hdl.handle.net/10603/278645
10. Kaur, M. (2017). *Phytoremediation studies on some plants species in industrial area of Bhagwanpur Uttarakhand with reference to morphometric and biochemical traits* [Forest Research Institute University]. http://hdl.handle.net/10603/175736
11. Lal, J. K., Mishra, B., Sarkar, A. K., & Lal, S. (1996). Effect of Flyash on Growth and Nutrition of Soybean. *Journal of the Indian Society of Soil Science*, *44*(2), 313–316.
12. Mishra, S., & Prasad, S. V. K. (2017). IMPACT OF COAL FLY ASH AS SOIL AMENDMENT ON PHYSICO-CHEMICAL PROPERTIES OF SOIL. *Indian J Sci Res*, *13*(2), 15–20.
13. Page, A. L., Elseewi, A. A., & Straughan, I. R. (1979). Physical and chemical properties of fly ash from coal-fired power plants with reference to environmental impacts. In F. A. Gunther & J. D. Gunther (Eds.), *Residue Reviews* (pp. 83–120). Springer New York. https://doi.org/10.1007/978-1-4612-6185-8_2
14. Panda, R. B., & Biswal, T. (2018). Impact of Fly Ash on Soil Properties and Productivity. *International Journal of Agriculture, Environment and Biotechnology*, *11(2): 275-283, April 2018*(2), 275–283.
15. Plank, C. O., Martens, D. C., & Hallock, D. L. (1975). Effect of soil

application of fly ash on chemical composition and yield of corn (Zea mays L.) and on chemical composition of displaced soil solutions. *Plant and Soil*, *42*(2), 465–476. https://doi.org/10.1007/BF00010021

16. Skousen, J., Yang, J. E., Lee, J.-S., & Ziemkiewicz, P. (2013). Review of fly ash as a soil amendment. *Geosystem Engineering*, *16*(3), 249–256. https://doi.org/10.1080/12269328.2013.832403
17. Varshney, A., Mohan, S., & Dahiya, P. (2020). Composition and Dynamics of Microbial Communities in Fly Ash-Amended Soil. In A. Varma, S. Tripathi, & R. Prasad (Eds.), *Plant Microbiome Paradigm* (pp. 231–246). Springer International Publishing. https://doi.org/10.1007/978-3-030-50395-6_12

Chapter - 8

Paving Progress, Breathing Challenges: Navigating the Complex Intersection of Aggressive Urban Development and Air Pollution

Authors

Saikat Manna
Dept of Biotechnology, Swami Vivekananda University, Kolkata, West Bengal, India

Suraj Jyote
Dept of Biotechnology, Swami Vivekananda University, Kolkata, West Bengal, India

Prerona Dutta
Dept of Biotechnology, Swami Vivekananda University, Kolkata, West Bengal, India

Anwesa Dutta
Dept of Biotechnology, Swami Vivekananda University, Kolkata, West Bengal, India

Srishti Choudhury
Dept of Biotechnology, Swami Vivekananda University, Kolkata, West Bengal, India

Sandip Sankar Ghosh
The Climate Thinker, Kolkata, West Bengal, India

Srijan Haldar
Dept of Biotechnology, Swami Vivekananda University, Kolkata, West Bengal, India

Chapter - 8

Paving Progress, Breathing Challenges: Navigating the Complex Intersection of Aggressive Urban Development and Air Pollution

Saikat Manna, Suraj Jyote, Prerona Dutta, Anwesa Dutta, Srishti Choudhury, Sandip Sankar Ghosh and Srijan Haldar

Abstract

When one or more elements introduced by humans are present in the external atmosphere to the point that they impact human health and welfare as well as atmospheric life, this is known as environmental contamination. Outdoor air pollution is a complex mixture of particles, chemicals, and biogenic compounds with well-known health impacts that come from various natural and human-caused sources. While outdoor air is a significant contributor to indoor particulate matter (PM), it is crucial to take into account other sources as well, since these might result in significantly variable particle mass and size distribution compositions inside the indoor environment. Polycyclic aromatic hydrocarbons (PAHs), a type of outdoor air pollution, raise the risk of cancer, particularly lung cancer. When fine carbon particles (PM2.5) are floating in the atmosphere, PAHs can stick to them. Particulates gather at our breathing level close to the earth. The main way they enter our bodies is through breathing. Extensive exposure to particulate matter-containing air in contaminated urban areas has been demonstrated to elevate the risk of lung cancer-related mortality by 8%, even after accounting for tobacco use. This review explores the air pollutants resulting from unbridled urban development and delves into their repercussions on human health.

Introduction

Every day, the air in metropolitan places becomes more dangerous to breathe due to constant pollution from harmful compounds. The cities have choked air. The air is unhealthy due to the smoke from industry, power plants, and cars. Other things that contribute to air pollution include chemical spills and other harmful gases.

When we urbanised our villages, we made significant progress, but there was a cost. Although we live in opulent and comfortable modern cities and towns, the state of our environment has suffered greatly as a result. Numerous issues that we encounter have been brought about by it. Fast urbanisation brought with it a web of problems, and we seem to be stuck in them as the new cities saw fast expansion. Aggressive urbanization in developing countries often leads to detrimental impacts on air quality and public health. As cities rapidly expand, there is a surge in construction activities, industrialization, and an influx of vehicles, all contributing to increased air pollution. Outdoor air pollution is a complex mixture of pollutants originating from natural and human sources including transportation, power generation, industrial activity, biomass burning, and domestic heating and cooking. The release of pollutants such as particulate matter ($PM_{2.5}$, PM_{10}), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and volatile organic compounds (VOCs) becomes pervasive. The mix of pollutants in outdoor air varies widely in space and time, reflecting the diversity of sources and the influence of atmospheric processes. These pollutants can have severe health implications, causing respiratory problems, cardiovascular diseases, and other respiratory ailments. Commonly measured air pollutants include particulate matter nitrogen dioxide, and sulfur dioxide; the concentration of particulate matter is often used as an indicator of pollution levels. Millions of people worldwide are exposed to outdoor air pollution at levels that substantially exceed existing health-based guidelines. The concentration of harmful emissions is exacerbated by inadequate urban planning, insufficient green spaces, and lax environmental regulations. Vulnerable populations, including low-income communities, often bear the brunt of this urbanization-related pollution, as they may reside in proximity to industrial zones or face limited access to healthcare. Therefore, aggressive urbanization in developing countries necessitates comprehensive strategies for sustainable urban planning, efficient public transportation, and stringent environmental policies to mitigate the adverse impacts on air quality and safeguard public health.

In this article we have discussed regarding various kinds of air pollutants that generated due to urbanisation process and pollutes the environmental condition an also highlighted the probable disease caused due to presence of these pollutants.

Air pollutant Source and problems related to aggressive urbanisation

Massive deforestation resulted from the necessity for open space to construct buildings, roads, bridges, and other infrastructure. To make room

for the constantly growing population, fields were cleared, trees were chopped down, and new spaces were created. It should go without saying that one of the main causes of pollution is tree chopping. There was a shortage of space and other natural resources like coal and water due to the high population density.

Some significant issues were brought about by the urban population's connection with the environment. The urban population's lifestyle and consumption habits drastically altered the surrounding environment. More food, energy, and water are consumed by people living in cities. Compared to rural areas, urban places have far more contaminated air. This is primarily due to the usage of cars and the growth of factories and industries that pollute the air more frequently. Nearly everything we use is powered by electricity. Cities always need more electricity, therefore more power plants are built, which pollutes the air in order to meet the demand.

The lakes, rivers and any other water bodies in urban areas is always polluted by the dump of industrial waste and sewage. The marine life faces a lot of danger. We cannot ignore that noise pollution is one of the major causes of stress related issues in urban population. More and more trees are cut down to meet the needs of urban people and in exchange very less tress are planted. The use of plastic is another major reason of degradation of environment**.**

Cause of Sudden increase in air various pollutants

Construction processes: Increasing Construction processes leads to major air pollution by two major issues. dust pollution and Emissions from Construction Machines. The former one is a very common and dominating phenomenon. Wooden dust can also be found, which is due to the grilling and drilling of the construction, gets amalgamated as particulate matter in the air during the demolition of the building. The second issue also participated in the increasing pollutants in quite an aggressive way. A lot of heavy-type vehicles are used during construction that run on diesel. There are a number of machines like the excavator, crane, bulldozers, and cement mix trailer. This construction machinery emits a large number of CO_2, SO_2, and CO into the air. The emissions from this heavy-duty machinery are worse than our usual vehicles as they have no proper emission control system and thus are a bigger concern to the environment (Natarajan N *et al*, 2022).

Increased Traffic Emissions: Automotive vehicles emit several pollutants depending upon the quality of the fuel they consume and engine efficiency. The release of pollutants from vehicles also includes fugitive

emissions of the fuel and the source and level of these emissions depending upon the vehicle type, its maintenance, etc. The major pollutants released as vehicle/fuel emissions are, carbon monoxide (CO), nitrogen oxides (NOx), photochemical oxidants, air toxics, namely benzene (C_6H_6), aldehydes, 1,3 butadiene (C_4H_6), lead (Pb), particulate matter (PM), hydrocarbon (HC), oxides of sulphur (SO_2) and polycyclic aromatic hydrocarbons (PAHs). While the predominant pollutants in petrol/gasoline driven vehicles are hydrocarbons and carbon monoxide, the predominant pollutants from the diesel based vehicles are Oxides of nitrogen and particulates (Du W *et al*, 2022).

Industrial Activities: Urbanization often leads to the establishment of industries and commercial enterprises. Industrial activities can release pollutants into the air, including particulate matter, volatile organic compounds (VOCs), and other chemicals. The type of industries present in the urbanized areas can have a direct impact on the composition of air pollutants (Choubisa SL *et al*, 2016).

Waste burning: An estimated 40 to 50 percent of the garbage is made up of carbon by mass, which means that carbon dioxide is the major gas emitted by trash burning. Those emissions are dwarfed by others sources on the global scale, such as cars and power plants, amounting to just 5 percent of total global carbon dioxide emissions. But the carbon dioxide that comes from trash burning can be a significant source in some countries and regions, and it is one not reflected in the official greenhouse gas inventories for those places.

Loss of Green Spaces: Urbanization often leads to the reduction of green spaces, trees, and vegetation. This loss can result in decreased air quality as trees play a crucial role in absorbing pollutants and producing oxygen. Lack of green cover also contributes to the urban heat island effect, which can influence local air quality (Lal RM *et al*, 2022).

Increased Energy Consumption: Urban areas typically have higher energy demands for buildings, transportation, and industries. The combustion of fossil fuels for energy production can release pollutants, including sulfur dioxide (SO_2) and nitrogen oxides, contributing to air pollution (Eswaran N *et al*, 2021).

Contribution of indoor cooking: 20% to 50% of outdoor pollution is the result of indoor cooking and heating. The process releases carbon dioxide and other greenhouse gases, as well as short-lived climate pollutants such as black carbon, another leading contributor to climate change. Scientists have

discovered that melting sea ice in the Arctic is partly caused by black carbon emissions from cookfires on the other side of the globe, swept there by ocean currents. The amounts and composition of pollutants emitted from those sources depend greatly on the cooking materials, cooking styles, and even cooking fuel. For example, charcoal is used extensively for barbecuing in most restaurants in the world because it has high heating value, is cheap compared to other types of fuels, can be easily stored, and gives a unique flavor and texture to the food. Charcoal contains various types of organic and inorganic compounds such as hydrocarbons, sulfur, water, and oxygen along and numerous trace elements. Therefore, the combustion of charcoal creates a considerable amount of airborne toxic elements both in the solid and gaseous states. The coal-tars and soot (fine black particulate matter) have been documented as human carcinogens. Sulphur present in fuels primarily in coal, petrol, kerosene, and diesel can produce sulphur dioxide gas (SO_2) during combustion. The presence of SO_2 in the air leads to irritations of the mucous membranes and the eyes, as well as chronic bronchitis. (Vardoulakis S *et al*, 2020).

Table 1: Various air pollutants contributing to emission and their respective sources

Air Pollutant	Sources	Reference
Particulate Matter (PM10)	Combustion (vehicles, industrial processes), dust	Kim K *et al*, 2021
Particulate Matter (PM2.5)	Combustion (vehicles, industrial processes), dust	Thangavel P *et al*, 2022
Nitrogen Dioxide (NO2)	Vehicle emissions, industrial combustion	Kundi M *et al*, 2020
Sulfur Dioxide (SO2)	Combustion of fossil fuels (coal, oil), industrial processes	Kandylis K 1984
Carbon Monoxide (CO)	Vehicle exhaust, industrial processes, combustion	Adach W *et al*, 2020
Volatile Organic Compounds (VOCs)	Vehicle emissions, industrial processes, solvent use	Huang YR *et al*, 2023
Ozone (O3)	Secondary pollutant from NO2 and VOCs, vehicle emissions	Schweisfurth H *et al*, 1994
Lead (Pb)	Historically from leaded gasoline, industrial processes	Williams RJ *et al*, 2018
Benzene	Vehicle exhaust, industrial processes, tobacco smoke	Mozzoni P *et al*, 2023
Formaldehyde	Construction materials, furniture, combustion processes	Zhang L *et al*, 2010

Aggressive urbanisation induced air pollution mitigation process

Urban planning and design: (Wu Z *et al*, 2023): Urbanisation is a process that responds to the population increase by changing the size, composition, and expansion of cities, which further creates long-term problems with air quality. The spatial makeup, arrangement, and density of urban land uses—referred to as urban form—will continue to change as a result of the worldwide trend towards urbanization13. Research, both empirical and theoretical, has begun to examine how urban form affects air quality. Although there is a well-established correlation between artificial surface area and air pollution, the impact of urban fragmentation on air quality has generated debate. Theoretically, compact communities encourage high residential densities with a variety of land uses, which lessens reliance on private vehicles and increases use of public transportation and walking. The small-scale urban growth

Public transportation: Throughout their entire life cycle, cars, trucks, and buses emit air pollution, including emissions from fuel manufacturing and vehicle operating. Refined fuels and their distribution, as well as the manufacture, disposal, and recycling of vehicles, are linked to additional emissions.

Industrial regulations: When hazardous materials are released into the atmosphere by industries, mines, and vehicles, it is known as industrial air pollution. Health issues like asthma, lung function decline, malignancies, and respiratory disorders can all be brought on by these contaminants. Additionally, they may cause environmental problems like acid rain and climate change. Numerous air pollutants, such as sulphur dioxide, nitrogen oxides, particulate matter, and other hazardous substances, are released into the atmosphere by industrial operations.

Vehicle emission control: Vehicles are the principal cause of India's urban air pollution problems. Vehicular emissions are caused by both combustion and evaporation. To the extent that fossil fuels continue to used, there are three general approaches that can be used to reduce emissions from combustion of fossil fuel. Precombustion controls to reduce the emission potential of the fuel itself (for example, using fuels with less sulfur or nitrogen content). Combustion controls to reduce emissions by improving the combustion process itself. Postcombustion controls to capture emissions after they have been formed but before they are released to the air. Most of the control of automobile emissions occur at the exhaust system. The approach met favored by automobile manufacturers to achieve the emission

standards has been the catalytic converter (for CO, HCs and NOx), A catalytic converter is able to oxidize hydrocarbons and carbon monoxide to carbon dioxide, while reducing NOx to N, all in the same catalyst bed. For pre-combustion controls, a number of alternatives to gasoline (petrol) are being investigated as possible fuels for the future. These include ethanol, biodisel, CNG, LPG, hydrogen and electricity Ethanol, also known as grain alcohol, has long been used as an oxygenate to reduce CO emissions, in which case the gasoline/ethanol mixture is usually referred to as gasohol. Ethanol can be blended with gasoline with a range of mixture ratios. The two mixtures common in the United States are E10 (10 percent ethanol, 90 percent gasoline, by volume) and E85 (85 percent ethanol, 15 percent gasoline).

Waste management

Waste disposal practices: The public became concerned about a lack of regulations, insufficient legislation, and the effects on the environment and human health after a number of significant and widely reported pollution accidents linked to improper waste management procedures. Consequently, numerous national and federal governments were compelled to implement novel regulatory structures to address hazardous and non-viable waste disposal practices. Waste is prioritised in a waste management hierarchy based on the most environmentally sound standards (Giusti, L. 2009).

Air quality monitoring (Singh D *et al*, 2021): The main uses of Monitoring Networks technology-based air quality monitoring system are air quality sensors and data reporting. Battery-operated air quality monitoring. As a result, these nodes have the capacity to gather enormous volumes of data regarding air quality. The monitoring node is explained in more depth in the following section. The sensor, network, and app layers of the suggested air quality monitoring system's three-tier hierarchical IoT design (Paithankar, *et al*, 2023).

Compliance and enforcement programs: These programs help owners or operators of pollutant sources to understand the requirements, as well as the actions that environmental authorities can take if the sources don't comply.

Biodiesel can be created from vegetable oils, animal fats or recycled restaurant greases. It a biodegradable, domestic, renewable energy fuel that has the potential to help reduce the need for petroleum-based transportation fuels as well as helping farmers by providing a market for excess soybean oil. As a renewable fuel whose production requires little or no fos- sil fuel, its net CO, emissions are only about one-fourth that of standard diesel. The

oxygen in biodiesel enables more complete combustion to take place, and the fewer sulfur content reduces sulfate emissions.

Biodiesel can be blended with conventional diesel in mixture strength, with the most common blend B2 (2 percent biodiesel, 98 percent standard diesel) being popular because of its supe- nor lubricity compared to ultra-low-sulfur diesel fuels. B5 and B20 blends are also frequently used. While B20 doesn't require any modifications for conventional engines to run, larger concentrations can necessitate some engine modifications to prevent operating or maintenance issues. Transesterification is a technique used to make biodiesel.

CNG (compressed natural gas) is made by compressing natural gas which is mainly composed of methane. Compared to petrol, diesel and LPG, the combustion of CNG creates fewer unwanted gases. LNG (liquefied natural gas) and CNG are frequently confused. The main distinction between LNG and CNG storage methods is that LNG is held at very low temperatures, turning into liquid, whereas CNG is stored as natural gas (mostly methane). Because CNG is such a clean fuel, it emits relatively little carbon monoxide, particulates, toxics, and reactive hydrocarbons.

LPG (Liquefied Petroleum gas) is the most popular cooking fuel. Compressed gases are mixed together and liquefied; the main constituents are either butane or propane, or a combination of the two. When the mixture of gases is maintained under pressure, it turns liquid. They revert to gaseous forms upon release of pressure, just like when the gas valve is opened. LPG is extremely flammable and volatile. Because hydrocarbons don't smell, a tiny amount of the potent odorant ethanethiol is added to help find leaks more readily.

Health Impacts due to this air pollution

In many situations, sufficient control over emissions cannot be obtained by pollution prevention approaches such as fuel or process change. In cases, the levels of the pollutants of concern in the exhaust gases or process stream must be reduced to allowable values before they are released to the atmosphere. It involves the use of pollution control equipments to remove pollutants from the gas stream before releasing it to the environment. If a pollutant is removed from the carrying gas stream, disposal of the collected material is also very important. If the collected material is truly inert, it may be disposed of in a sanitary landfill. If it is reactive and toxic then strict laws governing its disposal apply.

People who are exposed to air pollution have a variety of negative health effects. One can distinguish between two types of effects: immediate

and long-term. Diseases like pneumonia or bronchitis are examples of short-term consequences, which are transient. Along with discomfort, these can also include skin, eyes, throat, nose, or throat irritation. Anxiety, nausea, and vertigo can also be brought on by air pollution. Air pollution also includes offensive odours produced by sewers, industry, and trash disposal systems. Even if they are not as bad, some smells are awful.

Air pollution can have long-term impacts that span a lifetime or several years. Even death may result from them. Heart disease, lung cancer, and respiratory conditions like emphysema are among the long-term health benefits of air pollution. Air contamination may additionally can cause long-term harm to the liver, kidneys, brain, nerves, and other organs of people. Some scientists believe birth abnormalities are caused by air pollution. Every year, the impacts of indoor or outdoor air pollution claim the lives of over 2.5 million people globally.

Individuals respond differently to various forms of air pollution. Due to their weakened immune systems, elderly people and small children are frequently more susceptible to the effects of pollution. Air pollution exposure can exacerbate conditions like lung illness, heart disease, and asthma. Other considerations include the duration of exposure, the quantity, and kind of pollutants.

In a number of studies, the health effects of future ozone and particle matter concentrations under various climate scenarios were calculated by modelling. Most research predict a rise in ozone and fine particle-related deaths as a result of climate change; however, specific findings vary depending on the region, the scenario for climate change that is assumed, and other variables like population and background emissions (Orru H *et al*, 2017)

Conclusion

Urban air pollution is becoming a major concern since it endangers both human health and the environment. Thankfully, there are numerous easy solutions that people, groups, and governments can put into place to lessen air pollution in urban areas.

Research indicates that one of the main reasons for the depletion of natural resources is urbanisation. There is a lot of pollution in cities and towns as a result of our ongoing damage to Mother Earth. While it is impossible to undo the harm we have already caused, we can restrict future damage by taking some preventative action. It is imperative that we act now to preserve the environment and leave a better future for future generations.

This review investigates the connections between air pollution, climate change, and the health effects of air pollution. The outcomes are largely dependent upon the chosen climate change scenario and unknown future estimates of air pollution emissions. Research mostly concentrated on death; estimates about the impact on morbidity are required.

References

1. Adach W, Błaszczyk M, Olas B. Carbon monoxide and its donors - Chemical and biological properties. Chem Biol Interact. 2020 Feb 25;318:108973. doi: 10.1016/j.cbi.2020.108973. Epub 2020 Feb 6. PMID: 32035862.
2. Choubisa SL, Choubisa D. Status of industrial fluoride pollution and its diverse adverse health effects in man and domestic animals in India. Environ Sci Pollut Res Int. 2016 Apr;23(8):7244-54. doi: 10.1007/s11356-016-6319-8. Epub 2016 Feb 23. PMID: 26903127.
3. Du W, Chen L, Wang H, Shan Z, Zhou Z, Li W, Wang Y. Deciphering urban traffic impacts on air quality by deep learning and emission inventory. J Environ Sci (China). 2023 Feb;124:745-757. doi: 10.1016/j.jes.2021.12.035. Epub 2022 Jan 4. PMID: 36182179.
4. Eswaran N, Parameswaran S, Johnson TS. Biofuels and Sustainability. Methods Mol Biol. 2021;2290:317-342. doi: 10.1007/978-1-0716-1323-8_20. PMID: 34009598.
5. Giusti, L. (2009). A review of waste management practices and their impact on human health. *Waste management*, *29*(8), 2227-2239.
6. Huang YR, Yang W, Wang XY, Wang YY, Cheng Y, Wang S. [VOCs Emission Level and Emission Reduction Potential of Coating Industry in Zibo]. Huan Jing Ke Xue. 2023 Sep 8;44(9):4832-4842. Chinese. doi: 10.13227/j.hjkx.202208009. PMID: 37699802.
7. Kandylis K. Toxicology of sulfur in ruminants: review. J Dairy Sci. 1984 Oct;67(10):2179-87. doi: 10.3168/jds.S0022-0302(84)81564-7. PMID: 6389627.
8. Khan AH, López-Maldonado EA, Khan NA, Villarreal-Gómez LJ, Munshi FM, Alsabhan AH, Perveen K. Current solid waste management strategies and energy recovery in developing countries - State of art review. Chemosphere. 2022 Mar;291(Pt 3):133088. doi: 10.1016/j.chemosphere.2021.133088. Epub 2021 Nov 29. PMID: 34856242.

9. Kim K, Kwak IY, Min H. Particulate Matter 10 (PM_{10}) Is Associated with Epistaxis in Children and Adults. Int J Environ Res Public Health. 2021 Apr 30;18(9):4809. doi: 10.3390/ijerph18094809. PMID: 33946392; PMCID: PMC8124263.

10. Lal RM, Tibrewal K, Venkataraman C, Tong K, Fang A, Ma Q, Wang S, Kaiser J, Ramaswami A, Russell AG. Impact of Circular, Waste-Heat Reuse Pathways on $PM_{2.5}$-Air Quality, CO_2 Emissions, and Human Health in India: Comparison with Material Exchange Potential. Environ Sci Technol. 2022 Jul 5;56(13):9773-9783. doi: 10.1021/acs.est.1c05897. Epub 2022 Jun 15. PMID: 35706337; PMCID: PMC9261188.

11. Moshammer H, Poteser M, Kundi M, Lemmerer K, Weitensfelder L, Wallner P, Hutter HP. Nitrogen-Dioxide Remains a Valid Air Quality Indicator. Int J Environ Res Public Health. 2020 May 25;17(10):3733. doi: 10.3390/ijerph17103733. PMID: 32466201; PMCID: PMC7277805.

12. Mozzoni P, Poli D, Pinelli S, Tagliaferri S, Corradi M, Cavallo D, Ursini CL, Pigini D. Benzene Exposure and MicroRNAs Expression: *In vitro*, *In vivo* and Human Findings. Int J Environ Res Public Health. 2023 Jan 20;20(3):1920. doi: 10.3390/ijerph20031920. PMID: 36767288; PMCID: PMC9914606.

13. Natarajan, N., Vasudevan, M., Dineshkumar, S. K., Nandhini, S. S., & Balaganesh, P. (2022). Effects of air pollution on monumental buildings in India: An overview. *Environmental Science and Pollution Research*, *29*(20), 29399-29408.

14. Orru H, Ebi KL, Forsberg B. The Interplay of Climate Change and Air Pollution on Health. Curr Environ Health Rep. 2017 Dec;4(4):504-513. doi: 10.1007/s40572-017-0168-6. PMID: 29080073; PMCID: PMC5676805.

15. Paithankar, D. N., Pabale, A. R., Kolhe, R. V., William, P., & Yawalkar, P. M. (2023). Framework for implementing air quality monitoring system using LPWA-based IoT technique. *Measurement: Sensors*, *26*, 100709.

16. Schweisfurth H. Umweltschadstoff Ozon [Environmentally harmful ozone]. Dtsch Med Wochenschr. 1994 Mar 11;119(10):351-5. German. doi: 10.1055/s-2008-1058702. PMID: 8125040.

17. Singh D, Dahiya M, Kumar R, Nanda C. Sensors and systems for air

quality assessment monitoring and management: A review. J Environ Manage. 2021 Jul 1;289:112510. doi: 10.1016/j.jenvman.2021.112510. Epub 2021 Apr 4. PMID: 33827002.

18. Thangavel P, Park D, Lee YC. Recent Insights into Particulate Matter ($PM_{2.5}$)-Mediated Toxicity in Humans: An Overview. Int J Environ Res Public Health. 2022 Jun 19;19(12):7511. doi: 10.3390/ijerph19127511. PMID: 35742761; PMCID: PMC9223652.
19. Vardoulakis S, Giagloglou E, Steinle S, Davis A, Sleeuwenhoek A, Galea KS, Dixon K, Crawford JO. Indoor Exposure to Selected Air Pollutants in the Home Environment: A Systematic Review. Int J Environ Res Public Health. 2020 Dec 2;17(23):8972. doi: 10.3390/ijerph17238972. PMID: 33276576; PMCID: PMC7729884.
20. Williams RJ, Holladay SD, Williams SM, Gogal RM Jr. Environmental Lead and Wild Birds: A Review. Rev Environ Contam Toxicol. 2018;245:157-180. doi: 10.1007/398_2017_9. PMID: 29038944.
21. Wu Z, Zhao Z, Gan W, Zhou S, Dong W, Wang M. Achieving Carbon Neutrality through Urban Planning and Design. Int J Environ Res Public Health. 2023 Jan 29;20(3):2420. doi: 10.3390/ijerph20032420. PMID: 36767786; PMCID: PMC9916401.
22. Zhang L, Freeman LE, Nakamura J, Hecht SS, Vandenberg JJ, Smith MT, Sonawane BR. Formaldehyde and leukemia: epidemiology, potential mechanisms, and implications for risk assessment. Environ Mol Mutagen. 2010 Apr;51(3):181-91. doi: 10.1002/em.20534. PMID: 19790261; PMCID: PMC2839060.

Chapter - 9

Pioneering the Next Frontier: Innovative Approaches to Tackle Antibiotic Resistance

Authors

Prity Singh
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Anwesha Das
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Tiyasha Saha
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Semanti Ghosh
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Bidisha Ghosh
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Subhasis Sarkar
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Suranjana Sarkar
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Chapter - 9

Pioneering the Next Frontier: Innovative Approaches to Tackle Antibiotic Resistance

Prity Singh, Anwesha Das, Tiyasha Saha, Semanti Ghosh, Bidisha Ghosh, Subhasis Sarkar and Suranjana Sarkar

Abstract

Multi-drug resistant Staphylococcus aureus (MDR-SA) presents a formidable challenge in public health due to its resistance to conventional antibiotics. This abstract provides a comprehensive overview of MDR-SA, encompassing its epidemiology, resistance mechanisms, clinical implications, and management strategies. Staphylococcus aureus, a common commensal bacterium, can cause a range of infections, from minor skin infections to severe conditions like sepsis and pneumonia. The emergence of MDR-SA complicates treatment, heightening the risk of therapeutic failure. Resistance in S. aureus arises through diverse mechanisms, including horizontal gene transfer, target mutations, and efflux pump upregulation. Factors contributing to MDR-SA's spread include inappropriate antibiotic use and inadequate infection control measures. MDR-SA infections pose substantial clinical challenges, necessitating alternative treatment approaches such as combination therapy and antimicrobial stewardship programs. Additionally, infection prevention strategies like hand hygiene and surveillance are critical for controlling MDR-SA transmission.

Keywords: Antibiotic resistance, Epidemiology, Multi-drug resistance, *Staphylococcus aureus.*

1. Introduction

The discovery of antibiotics stands as one of the most pivotal achievements in modern medicine, having saved countless lives by not only treating infections but also preventing bacterial infections in patients with compromised immune systems, undergoing cancer chemotherapy or organ transplants. In the current era, antibiotics are a staple in medical treatment, yet their overuse has precipitated a swift emergence of resistance (Carlet *et al*., 2011). The application of antibiotics in livestock has further exacerbated

the problem by fostering antibiotic-resistant infections (Van Boeckel *et al*., 2015).

Interestingly, antibiotics are not human inventions; they are natural compounds produced by microbes like Streptomyces species or fungi to eliminate competing bacteria (Waksman *et al*., 1942; Waksman and Woodruff 1941, 1942). In response, bacteria have evolved mechanisms to neutralize these antimicrobial agents, ensuring their survival and dominance in their environments. Prolonged exposure to antibiotics has driven the evolution of resistance traits in key human pathogens, resulting in multidrug-resistant (MDR) bacteria that pose significant treatment challenges (Medina *et al*., 2016).

Antibiotic resistance encompasses various conditions where bacteria survive despite antibiotic exposure. Genetic mutations either spontaneous or induced—can confer resistance, which can spread among bacteria through processes like conjugation, transduction, or transformation. Consequently, a resistance gene that emerges under evolutionary pressure, such as antibiotic use, can proliferate, often via genetic material exchange through plasmids and transposons. Bacteria harboring multiple resistance genes are termed multidrug-resistant, clinically defined as those resistant to at least one agent from three or more antibiotic classes (e.g., β-lactamase inhibitor combinations, cephalosporins, fluoroquinolones) (Barie *et al*., 2012).

A prominent example of such resistance is Methicillin-resistant *Staphylococcus aureus* (MRSA), among others. The rapid dissemination of these antibiotic-resistant bacteria is particularly alarming, not only in healthcare settings but also within communities globally. The dangers of antibiotic resistance are significant, as infections due to resistant bacteria causes increased mortality rates. Furthermore, the economic burden on healthcare systems is substantial, marked by prolonged hospital stays and reduced productivity (Medina *et al*., 2016).

2. Origin and evolution of antibiotic resistance

The historical setting and evolution of multidrug-resistant organisms (MDROs) are inextricably linked to the distribution and abuse of antibiotics, which has resulted in antibiotic resistance. Antibiotic discovery in the twentieth century was a watershed moment in medicine, transforming the treatment of infectious illnesses and saving many lives. However, antibiotic abuse and misuse have contributed to an increase in antibiotic resistance in hospitals, communities, and the environment. Because of antibiotic abuse, microorganisms' exceptional genetic capabilities have been exploited, resulting in resistance development (Davies *et al.,* 2010). The emergence of

antibiotic-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococci (VRE), and bacteria producing extended-spectrum beta-lactamases (ESBLs), illustrates the continual battle between the development of antibiotics and the evolution of resistance. The introduction of various antibiotics has frequently been followed by the development of resistance, and the relative scarcity of new medicines has exacerbated the problem. The emergence of multidrug resistance is a complicated and continuing process impacted by antibiotic overuse and misuse, microbial genetic capabilities, and a lack of fundamental information on the matter. The formation of dominant multidrug-resistant bacterial clades has been discovered as a significant clinical and biological event, with the establishment of resistance occurring very fast after the introduction of a new antibiotic class (Landecker *et al*., 2016).

3. Virulence factors

Methicillin-resistant *Staphylococcus aureus* (MRSA) showcases its pathogenicity and resistance to host defenses through the production of various virulence factors. Among these, Panton-Valentine leukocidin (PVL) stands out, associated with severe skin and soft tissue infections (SSTIs) as well as necrotizing pneumonia. PVL's mechanism involves targeting and lysing white blood cells, resulting in tissue destruction and aiding bacterial spread. Additionally, MRSA employs several cell surface proteins to enhance adhesion to host tissues and evade immune detection. For example, protein A inhibits opsonization and phagocytosis by binding to the Fc portion of immunoglobulins. Furthermore, fibronectin-binding proteins facilitate attachment to extracellular matrix components, promoting colonization and infection. Moreover, MRSA produces exotoxins like toxic shock syndrome toxin-1 (TSST-1) and enterotoxins, contributing to conditions such as toxic shock syndrome and food poisoning through toxin-mediated mechanisms. MRSA also employs various mechanisms to resist antimicrobial agents, including the production of beta-lactamases, which hydrolyze beta-lactam antibiotics like methicillin, and efflux pumps that expel antimicrobial compounds from the bacterial cell. The acquisition of genetic elements encoding antibiotic resistance, such as the staphylococcal cassette chromosome mec (SCCmec), further enhances MRSA's ability to survive in the presence of antibiotics. Understanding the complex interactions among these virulence factors is crucial for unraveling MRSA pathogenesis and developing innovative therapeutic strategies to combat MRSA infections (Hsieh *et al*., 2023).

4. Mechanism of resistance

Resistance denotes a microorganism's diminished susceptibility to an antimicrobial drug compared to other isolates within the same species. Despite the introduction of new medications, resistance in pathogenic microorganisms is on the rise, particularly among patients undergoing prolonged treatment. Antimicrobial drugs operate by disrupting numerous metabolic pathways, including nucleotide synthesis, thereby impeding DNA/RNA synthesis, protein synthesis, and cell membrane integrity. They also hinder enzymes crucial for cell wall synthesis, such as chitin synthase. Microorganisms have evolved various mechanisms to counteract these drugs and endure their impact (Tanwar *et al*., 2014). Antimicrobial resistance mechanisms comprise reduced drug accumulation, alteration of drug targets, drug inactivation, and active efflux. Intrinsic resistance mechanisms entail limitations in drug uptake, inactivation, and efflux, whereas acquired resistance mechanisms involve modifications to drug targets, inactivation, and efflux. Gram-negative bacteria exhibit distinct resistance mechanisms compared to gram-positive bacteria due to structural disparities. While gram-negative bacteria employ all four primary mechanisms, gram-positive bacteria, lacking an LPS outer membrane, predominantly rely on restricted drug absorption and do not engage in certain forms of drug efflux (Reygaert *et al*., 2018).

Reduced drug accumulation: This involves decreasing drug permeability or increasing active drug pumping out of the cell (Jose and Cesar, 2016). The balance between antibiotic absorption and excretion determines bacterial sensitivity to a specific drug. Bacteria can develop resistance by limiting antibiotic entry through their cell membrane (Santajit and Indrawattana, 2016).

Modification of target or binding site: Bacteria can modify ribosome protection proteins or alter the binding sites of drugs, such as the penicillin-binding sites in MRSA. These structural changes can protect bacteria from antibiotics by altering protein conformations, thereby reducing their activity and hindering protein synthesis, which promotes bacterial survival and proliferation (Tang *et al*., 2014; Jose and Cesar, 2016).

Drug inactivation: Bacteria can inactivate drugs through degradation or chemical modification. β-lactamases, a broad class of enzymes, degrade drugs. Tetracycline can be hydrolyzed and inactivated by the tetX gene. Another common method of inactivation is the transfer of acetyl, phosphoryl, or adenyl groups to the drug. Numerous transferases have been

identified, with acetylation targeting drugs like aminoglycosides, chloramphenicol, streptogramins, and fluoroquinolones. Phosphorylation and adenylation are typically used to inactivate aminoglycosides (Reygaert *et al*., 2018).

Drug efflux: Active efflux involves expelling harmful chemicals and antibiotics from the cell, contributing to antibiotic resistance. Efflux systems work through an energy-dependent process, using specialized pumps within the cell wall to extrude unwanted compounds. Some efflux systems are drug-specific, while others can expel multiple drugs, contributing to multidrug resistance (MDR). Efflux pumps, found in all cell types, belong to several superfamilies, with the main facilitator superfamily and the resistance-nodulation-cell division (RND) superfamily being particularly important for bacterial resistance. These efflux mechanisms significantly impact antimicrobial resistance due to factors like gene expression, the presence of multiple efflux pumps within a species, and inducible functions (Reygaert *et al*., 2018).

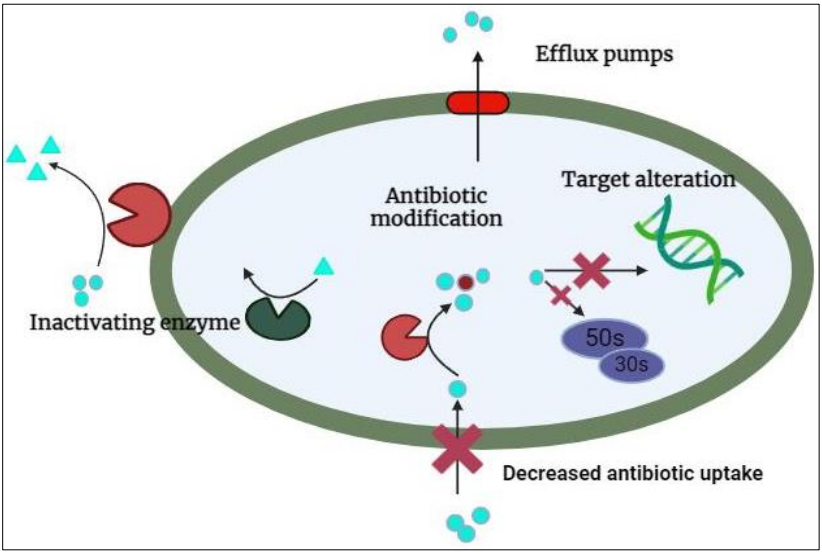


Fig 1: Mechanism of resistance development

5. MRSA

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an emerging zoonotic pathogen with profound implications for public health and veterinary medicine. Its capacity to infect the skin, mucosal membranes, and internal organs poses significant challenges, leading to severe conditions like

acne, suppurative skin infections, osteomyelitis, respiratory tract infections, endocarditis, and septicemia. Initially isolated by Alexander Ogston in 1880 during studies on septicemia and wound infections, *Staphylococcus aureus* is a Gram-positive, spherical bacterium that is often encapsulated. The majority of *S. aureus* strains (94%) produce penicillinase, rendering them highly resistant to penicillin and its derivatives. MRSA strains, specifically resistant to methicillin, are identified clinically through PCR detection of the mecA gene and cefoxitin resistance testing. The mecA gene encodes the penicillin-binding protein (PBP-2A), which underpins this antimicrobial resistance. MRSA's resistance extends to various antibiotic classes, including penicillin, macrolides, fluoroquinolones, aminoglycosides, tetracyclines, and lincosamides, setting it apart from methicillin-susceptible *S. aureus* (MSSA). MRSA infections in humans can range from pyogenic endocarditis and suppurative pneumonia to otitis media, osteomyelitis, pyogenic skin and soft tissue infections, and septic arthritis. The emergence of multidrug-resistant, virulent MRSA strains presents a significant public health challenge, necessitating stringent infection control measures and the development of novel antibiotics to combat this escalating threat (Algammal *et al*., 2020).

6. Resistance mechanism

Methicillin-resistant *Staphylococcus aureus* (MRSA) has developed a variety of mechanisms to resist the effects of antibiotics, rendering it a formidable pathogen in both healthcare and community settings. One key mechanism is the production of beta-lactamase enzymes, which break down beta-lactam antibiotics like methicillin, making them ineffective against the bacteria. Additionally, MRSA possesses the mecA gene, which encodes penicillin-binding protein 2A (PBP-2A). This protein has a reduced affinity for beta-lactam antibiotics, allowing MRSA to continue building its cell wall even in the presence of these drugs, thereby conferring resistance. Efflux pumps are also utilized by MRSA to actively expel antimicrobial agents from the bacterial cell, reducing the intracellular concentration of antibiotics and diminishing their effectiveness. Moreover, MRSA can chemically modify and inactivate antibiotics through processes such as acetylation, phosphorylation, or adenylation, further neutralizing their antimicrobial activity. Lastly, MRSA can alter its cell membrane permeability to limit the uptake of antibiotics, reducing their intracellular concentration and evading their effects. These multifaceted resistance mechanisms underscore the significant challenge posed by MRSA infections and emphasize the importance of developing novel therapeutic strategies and implementing rigorous infection control measures to combat this resilient pathogen effectively (Peacock, Paterson *et al*., 2015; Algammal *et al*., 2020).

7. Current treatment

Antibiotics are drugs prescribed to combat bacterial infections, either by killing bacteria (bactericidal) or halting their growth (bacteriostatic). They represent one of the most frequently prescribed medication groups and have been pivotal in saving numerous lives since their inception. Nonetheless, the efficacy of antibiotics faces jeopardy due to the rise of antibiotic-resistant bacteria like methicillin-resistant Staphylococcus aureus (MRSA). There are several classes of antibiotics, each with its mechanism of action and spectrum of activity. Some commonly used antibiotics for treating bacterial infections, including MRSA, include (Mahjabeen *et al*., 2022):

Beta-lactams: This class includes penicillins, cephalosporins, carbapenems, and monobactams. They work by interfering with bacterial cell wall synthesis. Examples include methicillin, oxacillin, amoxicillin, cefazolin, and imipenem.

Glycopeptides: Glycopeptides, such as vancomycin and teicoplanin, are used to treat serious infections caused by gram-positive bacteria, including MRSA. They inhibit bacterial cell wall synthesis by binding to the terminal D-alanyl-D-alanine residues of peptidoglycan precursors.

Lipopeptides: Daptomycin is a lipopeptide antibiotic used to treat MRSA and other gram-positive infections. It disrupts bacterial cell membrane function, leading to cell death.

Oxazolidinones: Linezolid is an oxazolidinone antibiotic used to treat MRSA and other gram-positive infections. It inhibits bacterial protein synthesis by binding to the 50S subunit of the bacterial ribosome.

Tetracyclines: Tetracyclines, such as doxycycline and minocycline, inhibit bacterial protein synthesis by binding to the bacterial ribosome. They are used to treat a wide range of bacterial infections, including MRSA.

Fluoroquinolones: Fluoroquinolones, such as ciprofloxacin and levofloxacin, inhibit bacterial DNA synthesis by targeting bacterial topoisomerases. They are used to treat a variety of bacterial infections, although their use is becoming increasingly limited due to the development of resistance.

8. Innovative Approaches to Treating MRSA Infections

1) Natural Extracts

a) The combination of herbal extracts from G. mangostana and Q. infectoria has demonstrated significant anti-MRSA activity.

b) Tea-tree oil (TTO) has been found by Bassett *et al*., to effectively alleviate skin irritation, suggesting its potential in managing MRSA-related skin infections.

c) It was further reported that the aqueous extract of clove and cinnamon exhibits strong bactericidal effects against E. coli and *S. aureus*, indicating its potential as a natural remedy for combating MRSA.

2) Natural drugs

a) Compounds found in nature, including curcumin, garlic, Thai longan honey, Juncus and Luzula species, Greek oregano, the Baru plant, and lichen, have demonstrated significant effectiveness against drug-resistant strains of *S. aureus*.

b) Malaysian tualang honey possesses medicinal properties, including wound healing and antibacterial activity, suggesting its potential in managing MRSA infections.

3) Synthetic chemical compounds

a) Initially, penicillin was used to treat *S. aureus* infections; however, the emergence of MRSA necessitated the development of alternative antibiotics.

b) Vancomycin has been a cornerstone in the treatment of MRSA bacteremia for over fifty years.

c) Mupirocin, a topical antibiotic, is commonly prescribed for bacterial skin infections caused by MRSA.

4) Multi-drug strategies

Combining multiple medications can offer greater benefits in treating MRSA infections. New antibacterial agents such as linezolid, tigecycline, and daptomycin have emerged for MRSA treatment. Combinations like daptomycin and fosfomycin or daptomycin and oxacillin have shown synergistic effects against MRSA strains, enhancing treatment efficacy.

5) Vaccines for MRSA treatment

Given the high antibiotic resistance of MRSA, novel therapies like vaccines are being explored. Vaccines, centyrins, and monoclonal antibodies are under development and undergoing clinical trials to induce strong immune responses against MRSA.

6) Harnessing probiotics as therapeutic agents

Probiotics offer a promising alternative to antibiotics in managing MRSA infections. These living microorganisms can restore the disrupted gut microbiome caused by antibiotic treatment. Additionally, probiotics have demonstrated various health benefits beyond gut health, including immune modulation and pathogen inhibition, making them potential candidates for MRSA treatment.

9. Prevention

Preventing methicillin-resistant *Staphylococcus aureus* (MRSA) infections necessitates a comprehensive approach that spans healthcare facilities, community settings, and individual practices. Hand hygiene stands as a fundamental measure, urging consistent handwashing with soap and water or alcohol-based sanitizers, especially pre and post patient contact. In healthcare facilities, strict adherence to infection control protocols, including routine environmental cleaning, appropriate use of personal protective equipment, and judicious antibiotic prescribing, is paramount. Surveillance for MRSA colonization among high-risk populations aids in early detection and intervention. Patient isolation and contact precautions are crucial for preventing transmission within healthcare settings. Additionally, comprehensive education and training programs ensure awareness and compliance with preventive measures among healthcare workers, patients, and visitors. Beyond healthcare, community-based interventions targeting hygiene promotion and vaccination can mitigate MRSA transmission. By implementing these multifaceted prevention strategies, healthcare facilities and public health authorities can effectively curb the spread of MRSA and reduce its burden on both individuals and communities. Continued vigilance, research, and collaboration are vital for sustaining these efforts and combating MRSA effectively (Lee *et al* 2018).

10. Future prospect

In the ongoing endeavor to combat methicillin-resistant *Staphylococcus aureus* (MRSA) and variants of antibiotic-resistant infections, the horizon appears promising, driven by innovative research, collaborative endeavors, and strategic initiatives. One pivotal avenue lies in the development of novel antibiotics, where researchers are exploring various paths such as natural product screening, antibiotic modification, and the quest for entirely new antibiotic classes. Additionally, alternative therapies, including bacteriophage therapy, monoclonal antibodies, and antimicrobial peptides, offer potential alternatives to conventional antibiotics. Strengthening

antibiotic stewardship programs and deploying rapid diagnostic tools are crucial steps toward optimizing antibiotic use and guiding precise treatment decisions. Vaccines targeting MRSA and other resistant bacteria represent promising preventive measures, while adopting a One Health approach fosters collaboration across human health, animal health, and environmental sectors to comprehensively address antibiotic resistance. By embracing these forward-looking prospects and implementing evidence-based strategies, there is optimism for mitigating the impact of antibiotic-resistant infections and preserving the efficiency of antibiotics for the future.

Conclusion

In conclusion, this paper underscores the multifaceted nature of MRSA infections, touching upon various aspects ranging from host vulnerability to antibiotic resistance and control measures. The propagation of MRSA poses a pressing public health challenge, exacerbated by its propensity for multidrug resistance. Collaboration between diverse stakeholders, including public health professionals and veterinary authorities, is imperative for effective prevention and control strategies. Continuous education and training on antibiotic sensitivity are vital for optimizing MRSA treatment approaches. Moreover, ensuring adherence to basic hygiene standards and implementing robust veterinary quarantine measures are essential components of MRSA infection prevention efforts. As MRSA emerges as a significant zoonotic infection, bridging the gap between human and animal health is crucial for mitigating its impact on both populations.

References

1. Algammal, A. M., Hetta, H. F., Elkelish, A., Alkhalifah, D. H. H., Hozzein, W. N., Batiha, G. E., ... & Mabrok, M. A. (2017). Methicillin-resistant *Staphylococcus aureus* (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. Infect Drug Resist. 2020; 13: 3255–65. *Food Microbiol*, *62*, 141-146.
2. Azeez-Akande O. Global trend of methicillin-resistant *Staphylococcus aureus* and emerging challenges for control. *African J Clin Exp Microbiol*. 2010;11.
3. Barie, P. S. (2012). Multidrug-resistant organisms and antibiotic management. *Surgical Clinics*, *92*(2), 345-391.
4. Boucher HW, Corey GR. Epidemiology of methicillin-resistant Staphylococcus aureus. Clin Infect Dis. 2008;46:S344–S349.

5. Carlet J, Collignon P, Goldmann D, Goossens H, Gyssens IC, Harbarth S, Jarlier V, Levy SB, N'Doye B, Pittet D, Richtmann R, Seto WH, van der Meer JW, Voss A (2011) Society's failure to protect a precious resource: antibiotics. Lancet 378(9788):369– 371.

6. Clegg, J.; Soldaini, E.; McLoughlin, R.M.; Rittenhouse, S.; Bagnoli, F.; Phogat, S. *Staphylococcus aureus* Vaccine Research and Development: The Past, Present and Future, Including Novel Therapeutic Strategies. Front. Immunol. 2021, 12, 705360.

7. Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and molecular biology reviews*, *74*(3), 417-433.

8. Harris, A. D. *et al*., Universal glove and gown use and acquisition of antibiotic- resistant bacteria in the ICU: a randomized trial. JAMA 310, 1571–1580 (2013).

9. Hsieh, R. C., Liu, R., Burgin, D. J., & Otto, M. (2023). Understanding mechanisms of virulence in MRSA: implications for antivirulence treatment strategies. *Expert review of anti-infective therapy*, *21*(9), 911–928.

10. Jose, M. and Cesar, A. (2016). Mechanisms of antibiotic resistance. Microbiology Spectrum 4: 10-22

11. Jyoti Tanwar, J. T., Shrayanee Das, S. D., Zeeshan Fatima, Z. F., & Saif Hameed, S. H. (2014). Multidrug resistance: an emerging crisis.

12. Kong C, Neoh HM, Nathan S. Targeting *Staphylococcus aureus* toxins: a potential form of anti-virulence therapy. Toxins. 2016;8:72.

13. Landecker, H. (2016). Antibiotic resistance and the biology of history. *Body & Society*, *22*(4), 19-52.

14. Lee, Y.C.; Chen, P.Y.; Wang, J.T.; Chang, S.C. A study on combination of daptomycin with selected antimicrobial agents: *In vitro* synergistic effect of MIC value of 1 mg/L against MRSA strains. BMC Pharmacol. Toxicol. 2019, 20, 25

15. Mahjabeen, F., Saha, U., Mostafa, M. N., Siddique, F., Ahsan, E., Fathma, S., Tasnim, A., Rahman, T., Faruq, R., Sakibuzzaman, M., Dilnaz, F., & Ashraf, A. (2022). An Update on Treatment Options for Methicillin-Resistant *Staphylococcus aureus* (MRSA) Bacteremia: A Systematic Review. *Cureus*, *14*(11), e31486.

16. Medina, E., & Pieper, D. H. (2016). Tackling threats and future

problems of multidrug-resistant bacteria. Springer International Publishing Switzerland. 14, 6.

17. Moodley, A.; Oosthuysen, W.F.; Duse, A.G.; Marais, E.; South African MSG. Molecular characterization of clinical methicillinresistant *Staphylococcus aureus* isolates in South Africa. J. Clin. Microbiol. 2010, 48, 4608–4611.
18. Nandhini, P., Kumar, P., Mickymaray, S., Alothaim, A. S., Somasundaram, J., & Rajan, M. (2022). Recent developments in methicillin-resistant *Staphylococcus aureus* (MRSA) treatment: a review. *Antibiotics*, *11*(5), 606.
19. Salgado, C. D. & Farr, B. M. What proportion of hospital patients colonized with methicillin-resistant *Staphylococcus aureus* are identified by clinical microbiological cultures? Infect. Control Hosp. Epidemiol. 27, 116–121 (2006)
20. Santajit, S. and Indrawattana, N. (2016). Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. BioMedical Research International. pp 1-8.
21. Tang, S. S., Apisarnthanarak, A. and Hsu, L.Y. "Mechanisms of β-lactam antimicrobial resistance and epidemiology of major community- and healthcare-associatedmultidrug resistant bacteria,"Advanced Drug Delivery Reviews, (78) pp. 3-13.
22. Traczewski MM, Katz BD, Steenbergen JN, Brown SD. Inhibitory and bactericidal activities of daptomycin, vancomycin, and teicoplanin against methicillin-resistant *Staphylococcus aureus* isolates collected from 1985 to 2007. Antimicrob Agents Chemother 2009;53:1735–1738.
23. Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Teillant A, Laxminarayan R (2015) Global trends in antimicrobial use in food animals. Proc Natl Acad Sci USA 112(18):5649–5654.
24. Waksman SA, Horning ES, Spencer EL (1942) The production of two antibacterial substances. Fumigacin and Clavacin. Science 96(2487):202–203.
25. Waksman SA, Woodruff HB (1942) Selective antibiotic action of various substances of microbial origin. J Bacteriol 44(3):373–384.
26. Weese JS, van Duijkeren E. Methicillin-resistant *Staphylococcus aureus* and Staphylococcus pseudintermedius in veterinary gmedicine. Vet Microbiol. 2010;140:418–429.

27. World Health Organization. WHO Guidelines on Hand Hygiene in Health Care. World Alliance for Patient Safety (WHO Press, Geneva, 2009).

Chapter - 10

Nanoparticle and Antibiotic: Combinatorial Therapy for Biofilm Prevention

Authors

Sulogna Mitra
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, India

Bidisha Ghosh
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Suranjana Sarkar
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, India

Subhasis Sarkar
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, India

Chapter - 10

Nanoparticle and Antibiotic: Combinatorial Therapy for Biofilm Prevention

Sulogna Mitra, Bidisha Ghosh, Suranjana Sarkar and Subhasis Sarkar

Abstract

Microbes form surface adherent community called biofilm. The mechanism that different bacteria employ to forms biofilms vary, frequently depending on environmental conditions. Antimicrobial loaded nanoparticles alone or combined with other substances could reserve the biofilm formations and block the bacterial activity of nanomaterials. Some group of microorganisms are helped to develop resistance to antibiotics as bacterial biofilms are difficult to destroy via antibiotics. Among the new development of combinatorial therapy, we can positively see that the probability of use of nanoparticles as antimicrobial agents is greatly explored. Some bacterial activity includes to kill the pathogens without effecting another organ. The mechanism of action employed by different nanomaterials reduce biofilm formation and improve antibiotic therapy. Combinatorial therapy has developed that nanoparticles can get the significant interest in present days. It can help to develop the new generation of antibacterial agents. The pathway of this therapy provides the extensive antibacterial actions but it is critical for successful treatment of various infections.

Keywords: Nanoparticles, combinatorial, antimicrobial, infection, antibiotic therapy.

Introduction

A biofilm is any syntropic grouping of microorganisms that develops on the surface of microbial cells. Biofilms, which resemble water and transportation pathways, are three-dimensional biopolymer frameworks with a variety of heterogeneous layers. Ultrafine unit particles with one or more dimensions at the nanoscale, ranging from 1 to 100 nm, are called nanoparticles (NPs). Initially, even while it discusses how NPs may break up biofilm formations, more could be said about the precise ways that NPs interact with biofilms and how those interactions affect their antimicrobial

activity. It might also go over the drawbacks and difficulties that come with using NPs in therapeutic contexts, like worries about toxicity, stability, and possible resistance development. The introduction should also discuss the state of the field's research at the moment, emphasizing any knowledge gaps or contradictory results that call for greater study. This critical study would open up new avenues for future research and give a more nuanced grasp of the subject. The high surface-area-to-volume ratio of the nanoparticles is consistent with their antibacterial activity. This is so because tiny particles interact with bacteria more and have better antibacterial activity because they have the largest surface area. Antibacterial agents have recently been delivered via innovative nanotechnology-based antimicrobials to destroy planktonic bacteria, species resistant to antibiotics, and biofilm structures. Compounds' anti-biofilm action was found to be improved when it was combined with the special qualities of nanoparticles or functionalized surfaces. Nanoparticles could target specific components of the biofilm matrix and readily enter the structure of the biofilm.

Biofilm formation and antibiotic resistance

The process by which bacteria go from free-swimming planktonic form to a sessile form that forms biofilms is known as biofilm formation. Multidrug-resistant (MDR) bacteria play a crucial role in biofilm development, which is the main cause of chronic infections and other illnesses related to healthcare [Morris and Cerceo *et al*. 2020]. Preventing the production of biofilm is the primary method of preventing infections on medical equipment [Tran *et al*., 2020]. In biofilm formation, there are five steps which are as follows:

1. Initial attachment
2. Irreversible attachment
3. Maturation I
4. Maturation II
5. Dispersion

Biofilms have a complicated role in antimicrobial resistance (AMR) and have the potential to greatly increase resistance. For instance, when examined in a planktonic form, 100% of isolates in a research looking at Staphyloccus epidermis drug resistance in biofilms were susceptible to the antibiotic vancomycin [Puvaca *et al*., 2021]. Three pathways in particular are significant for the antibiotic resistance of bacteria in biofilms:

1. Resistance at the Biofilm Surface

2. Resistance within Biofilm Microenvironments
3. Resistance of Bacterial "Persister" Cells

The process of biofilm development protects bacteria from desiccation, the host's immune system, and the oxidation of antibiotics and biocides. Certain strains of S. aureus can produce significant infections that are challenging to treat and may be resistant to one or more medications [Kovacevic *et al*., 2021].

Combination antimicrobial therapies on bacterial biofilm - New therapeutic strategies

A possible method to both spread and destroy biofilms or, in certain situations, even prevent biofilm formation is the combination of biofilm dispersal agents and antibiotics. Combining an antibiotic and an antibiofilm agent seems to be generally more effective than using either drug alone for treating biofilms *in vitro* and, in certain rare instances, *in vivo* [Sophia *et al*., 2022). Rarely do biofilm dispersal agents naturally have antibacterial properties [Figure 1].

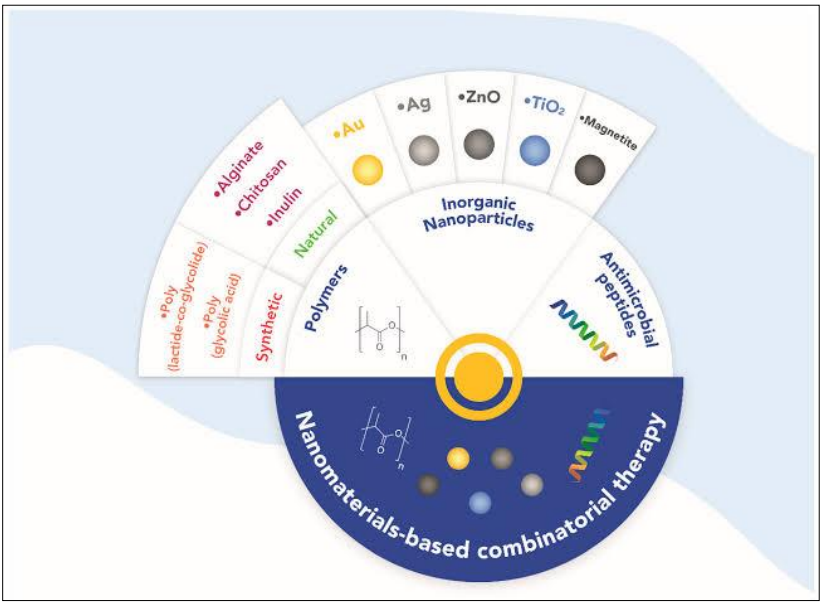


Fig 1: Combinatorial therapies used in the treatment of antibiotic-resistant bacteria

Therefore, in order to effectively disperse and remove biofilms, their prospective application as biofilm treatment techniques necessitates augmentation with an efficacious antimicrobial agent [Flemming and

Rumbaugh, 2018]. When an efficient antibiotic or antimicrobial is mixed with a dispersal agent, the combination treatment kills the cells that reside in the biofilm and disperses the remaining cells, thereby stopping further spread. Antimicrobial resistance is being prevented, reduced, or reversed by a variety of antimicrobial agents, including synthetic and natural polymers, inorganic nanoparticles, and antimicrobial peptides [Angel *et al*., 2022).

Nanoparticle based combinatorial treatments

Gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), titanium oxide nanoparticles (TiO2NPs), and zinc oxide nanoparticles (ZnONPs) are a few of the most significant inorganic nanoparticles. Antibiotics, polymers, and antimicrobial peptides can be used to treat INPs in a way that reduces the need for therapeutic doses while still having a synergistic impact [Krychowiak *et al*. 2018] [Table 1].

Table:1

Combinatorial treatments of effective of anti-micro bial agents	
Endotracheal tubes	Usage of AGNPs and surface engineered AUNPs
Wound dressings	Usage of Nano bandages with various metal nanoparticles (Ag, Zn, Au)
Oral implants	Usage of T: implants with Zno-NPs, Nano adhesive filings
Contact Lenses	Usage of Zn-CuO, Silica gel coating Hydrogel with AgNPs
Pacemakers	Usage of AgNPs, Polycationic NPs, PEG based NPs
Drug delivery	Usage of Liposomes, Polymeric nanoparticles, micro emulsions etc.

There are two ways to combine INPs and polymers: either the nanoparticles are integrated into the polymer matrix or the polymer is used as a coating for the INPs.

Combinatorial approaches wherein the primary constituent of potent antibacterial drugs is inorganic nanoparticles-

Gold nanoparticles: The physiochemical, optical, and low toxicity features of AuNPs make them very interesting. It can be functionalized with various polymers and protective agents for antimicrobial peptides and antibiotics [Angel *et al*., 2022).

Silver nanoparticles: Silver's use is a result of its antimicrobial qualities. Certain cell lines have shown AgNPs to be cytotoxic, according to certain study. As a result, combinations containing extra antimicrobial treatments have been suggested to increase AgNPs' activity and decrease their cytotoxicity [Liao *et al*. 2019]. AgNPs and bacteriocins together provide an efficient therapy for ESKAPE pathogens [Sidhu *et al*. 2020].

Zinc oxide nanoparticles: ZnO NPs have drawn a lot of interest

because of their effective bacteriocidal action against germs that are resistant to multiple drugs (MDR). In *E. coli*, ZnO NPs and ampicillin worked in concert (Angel *et al*., 2022).

Titanium oxide nanoparticles: TiO2 NPs are frequently utilized in the treatment of water and air due to their strong catalytic activity. TiO2 NPs combined with various polymers can be utilized to combat MDR bacteria [Harun *et al*., 2020]. In order to combat *E. coli* and S. aureus, Zhang *et al*. produced a nanocomposite of polytetrafluorethylene (PTFE) and TiO2-NPs.

Conclusion and Future perspective

Antibiotics and NPs combined in specific ratios can prevent drug resistance in bacteria or push them back toward drug susceptibility. The field of combinatorial treatment development is diverse. The cytotoxicity effects, production costs, and efficacy of combination tactics provide challenges for their clinical implementation. Even though antibiotics have proven to be an effective tool in the fight against pathogenic bacteria, new treatments are desperately needed to combat pathogens that have evolved defense mechanisms. Ultimately, the research emphasizes how important biofilms are to antimicrobial resistance (AMR) and how promising nanoparticles (NPs) are in treating infections linked to biofilms. NPs have the potential to improve the effectiveness of antibacterial treatments due to their high surface-area-to-volume ratio and capacity to target certain elements of the biofilm matrix. Translating these discoveries into clinically useful medicines, however, still presents difficulties. More investigation is required to improve the transport and dosage of NPs and to clarify the mechanisms behind their interaction with biofilms. Furthermore, for NPs to be widely used, it is imperative that safety issues and possible environmental effects are addressed. It is imperative that future perspectives concentrate on creating multidisciplinary strategies that combine clinical care, nanotechnology, and microbiology to successfully address illnesses connected to biofilms. For these developments to be translated into workable solutions for healthcare settings, cooperation between researchers, physicians, and regulatory bodies is essential.

References

1. Adeniji O. O., Nontongana N., Okoh J.C., Okoh A.I. The potential of antibiotics and nanomaterial combinations as therapeutic strategies in the management of multidrug-resistant infections: A Review: Int.J.Mol.Sci.2022,23(23),15038; https://doi.org/10.3390/ijms232315038.

2. Bari A.K., Balalekar T.S., Poojary A., Rohra S. Combination drug strategies for biofilm eradication using synthetic and natural agents in KAPE pathogens. Front. Cell.Infect. Microbiol., 17 April 2023; volume 13. https://doi.org/10.3389/fcimb.2023.1155699.

3. Brar A., Majumder S., Navarro M.Z., Benoit-Biancamano M.O., Ronholm J., George S. Nanoparticle-Enabled Combination Therapy Showed Superior Activity against Multi-Drug Resistant Bacterial Pathogens in comparison to Free Drugs. Nanomaterials 2022,12(13), 2179; https://doi.org/10.3390/nano12132179.

4. Harun N.H., Mydin R.B.S.M.N., Sreekantan S., Saharudin K.A., Basiron N., Seeni A. The bactericidal potential of LLDPE with Tio_2/ZnO nanocomposites against multidrug resistant pathogens associated with hospital acquired infections.

5. Hawas S., Verderosa A.D., Totsika M. Combination Therapies for Biofilm Inhibition and Eradication: A Comparative Review of Laboratory and Preclinical Studies. Front.Cell. Microbiol.,25 February 2022.

6. Hemeg H.A. Nanomaterials for alternative antibacterial therapy. Int J. Nanomed. 2017;12:8211-8225. doi:10.2147/IJN.S132163.

7. J. Biomater. Sci. Polym. Ed. 2020;31:1757-1769. doi;10.1080/09205063.2020.1775759.

8. Kovacevic Z., Radionovic M., Cabarkapa I., Kladar N., Bozin B. Natual agents against bovine mastitis pathogens. Antibiotics. 2021;10(2):p:205. doi: 10.3390/antibiotics10020205.

9. Krychowiak M., Kawiak A., Narajczyk M., Borowik A., Krolicka A. Silver Nanoparticles Combined with Naphthoquinones as an effective Synergistic Strategy against Staphylococcus aureus. Front. Pharmacol. 2018;9;816. doi:10.3389/fphar.2018.00816.

10. Leon- Buitimea A., Garza-Cardenas C. R., Roman-Garcia M.F., Ramirez-Diaz C.A., Ulloa-Ramirez M., Morones-Ramirez J.R. Nanomaterials-Based combinatorial Therapy as a strategy to combat Antibiotic Resistance. Antibiotic (Basel). 2022 Jun;11(6):794. doi: 10.3390/antibiotics11060794.

11. Leon-Buitimea A., Garza-Cardenas C.R., Roman-Garcia M.F., Ramirez-Diaz C.A., Ulloa-Ramirez M., Morones-Ramirez J.R. Nanomaterials-Based Combinatorial Thearpy as a Strategy to Combat Antibiotic

Resistance. Antibiotics (Basel). 2022 Jun; 11(6):794. doi: 10.3390/antibiotics11060794.

12. Liao C., Li Y., Tjong S.C.Bactericidal and Cytotoxic Properties of Silver Nanoparticles. Int.J.Mol.Sci.2019;20:499. doi: 10.3390/ijms20020449.
13. Ma L., Kohli M., Smith A. Nanoparticles for combination drug therapy. ACS Nano. 2013;7:9518-9525. doi: 10.1021/nn405674m.
14. Malaekeh-Nikouei B., Bazzaz B.S.F., Mirhadi E., Tajani A.S., Khameneh B. The role of nanotechnology in combating biofilm-based antibiotic resistance. Journal of Drug Delivery Science and Technology. Volume 60, December 2020: 101880. https://doi.org/10.1016/j.jddst.2020.101880.
15. Puvaca N., de Llanos Frutos R. Antimicrobial resistance in Escherichia coli strains isolated from humans and pet animals. Antibiotics. 2021;10(1):p.69. doi: 10.3390/antibiotics10010069.
16. Rather M.A., Gupta K., Mandal M. Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies. Braz J Microbiol. 2021 Dec; 52(4):1701-1718. doi: 10.1007/s42770-021-00624-x.
17. Sidhu P.K., Nehra K. Bacteriocin-capped silver nanoparticles for enhanced antimicrobial efficacy against food pathogens. IET Nanobiotechnol.2020;14:245-252. doi: 10.1049/iet-nbt.2019.0323.
18. Spirescu V.A., Chircov C., Grumezescu A.M., Andronescu E. Polymeric Nanoparticles for Antimicrobial Therapies: An up-to-date Overview. Polymers. 2021; 13:724. doi: 10.3390/polym13050724.
19. Thambirajoo M., Maarof M., Lokanathan Y., Katas H., Ghazalli N.F., Tabata Y., Fauzi M.B. Potential of Nanoparticles Integrated with Antibacterial Properties Biofilm and antibiotic resistance. Antibiotics (Basel).2021 Nov;10((11):1338. doi:10.3390/antibiotics10111338.
20. Tran H.M., Tran H., Booth M.A., Fox K.E., Nguyen T.H., Tran N., Tran P.A. Nanomaterials for Treating Bacterial Biofilms on Implantable Medical Devices: Nanomaterials (Basel).2020 Nov;10(11):2253.doi: 10.3390/nano10112253.
21. Worthington R.J., Melander C. Combination approaches to combat multidrug-resistant bacteria. Trends Biotechnol. 2013; 31:177-184. doi: 10.1016/j.tibtech.2012.12.006.