About the Book

The Living Laboratory: Biotechnology & Microbial Frontiers is a book that explores the intersection of biotechnology and microbial science, offering an in-depth look at how microorganisms play a pivotal role in advancing biotechnology and shaping scientific progress. This work delves into the cutting-edge developments in the study and manipulation of microbes, demonstrating their importance in various fields such as medicine, agriculture, and environmental science.

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

ISBN 9789361353659
9 789361 353659
₹ 797 US$ 16

The Living Laboratory: Biotechnology & Microbial Frontiers — AkiNik Publications

The Living Laboratory

Biotechnology & Microbial Frontiers

Dr. Priyankar Pal

AKINIK PUBLICATIONS

The Living Laboratory

Biotechnology & Microbial Frontiers

Editor

Dr. Priyankar Pal

Assistant Professor, Department of Biotechnology, School of Life Sciences,
Swami Vivekananda University, 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. Priyankar Pal*

Publication Year: *2025*

Edition: *1st*

Pages: *159*

Paperback ISBN: *978-93-6135-365-9*

EBook ISBN: *978-93-6135-634-6*

Book DOI: *https://doi.org/10.22271/ed.book.3211*

Price: *₹797/-*

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)*

Contents

S. No.	Chapters	Page Nos.
1.	**Role of Microbial Consortium in Waste Water Treatment** *(Abu Jishan, Subhasis Sarkar, Suranjana Sarkar and Bidisha Ghosh)*	01-12
2.	**Comparative Analysis of Culture-Dependent and Culture-Independent Techniques for Elucidating Microbial Diversity** *(Anuska Biswas and Debjit De)*	13-25
3.	**Insecticides and their Toxicological Impact: A Comprehensive Overview** *(Anindita Nandy, Palash Kumar Pal, Priya Roy, Nimai Chandra Saha and Priyajit Banerjee)*	27-49
4.	**Extracts of Pomegranate - Role in Fighting Cancer** *(Soham Banik, Archita Dhara, Arpita Das, Tiyasa Manna, Prakriti Patra, Aditya Das, Ananyo Mazumdar and Pritha Pal)*	51-63
5.	**Advancements in Gene Therapy for Leukemia: From Bench to Bedside: A Review** *(Tanya Das, Sovan Patra, Mayisha Sultana, Tuhin Mukherjee, Souradeep Banerjee and Priyankar Pal)*	65-84
6.	**Antibiotic Resistance and Novel Therapeutic Approaches** *(Swastika Datta, Mushkan, Tanisha Bhowmick, Dipti Das, Tiyasha Saha and Rupesh Dutta Banik)*	85-102
7.	**AI in Eco-Friendly Sustainable Agriculture** *(Ramu Samanta and Semanti Ghosh)*	103-114
8.	**Antibiotics and Prawn Culture: A Mini Review** *(Sandipan Taki, Trisheeta Koley, Sayani Saha, Debabrata Paria and Srijan Haldar)*	115-122

9. Antibiotics Targeting the Cell Wall of Gram-Negative Bacteria **123-138**

(Aritra Chatterjee, Sumita Mal, Priyajit Banerjee, Bidisha Ghosh, Subhasis Sarkar and Suranjana Sarkar)

10. Sustainable Strength: Exploring the Potential of Jute Fiber **139-159**

(Chandan Das, Bidisha Ghosh, Suranjana Sarkar and Subhasis Sarkar)

Chapter - 1

Role of Microbial Consortium in Waste Water Treatment

Authors

Abu Jishan
Dept. of Biotechnology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barrackpore, Barasat, Kolkata, West Bengal, India

Subhasis Sarkar
Dept. of Microbiology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barrackpore, Barasat, Kolkata, India

Suranjana Sarkar
Dept. of Microbiology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barrackpore, Barasat, Kolkata, India

Bidisha Ghosh
Dept. of Biotechnology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barrackpore, Barasat, Kolkata, West Bengal, India

Chapter - 1

Role of Microbial Consortium in Waste Water Treatment

Abu Jishan, Subhasis Sarkar, Suranjana Sarkar and Bidisha Ghosh

Abstract

Wastewater resources must be managed, cleaned, and reused because freshwater supplies are limited and environmental preservation is imperative. The high concentration of nitrogen, phosphorus, micronutrients, and other organic compounds in wastewater from homes, businesses, and dairy farms contributes to eutrophication in natural water bodies. Due to microalgae's ability to use these nutrients, bioremediation and biomass generation can be accomplished financially. Microalgae and bacteria can create a complimentary consortium for effective wastewater treatment and nutrient recovery because of their diversity. The co-culture of microalgae and bacteria, which often comprise a unique community known as the microalgal-bacterial consortium (MABC), is facilitated by the physiological activities of both microalgae and bacteria working in concert. In this consortium, heterotrophic bacteria are supplied with oxygen (O_2) from micro-algal photosynthesis for the purpose of organic carbon (OC) oxidation. The carbondioxide (CO_2) that is subsequently created is then sent back to the microalgae for photosynthesis. The fundamental synergistic relationship in MABC is that microalgae may grow faster and produce more biomass in common synergistic relationships than in pure algal environments. Bacteria and microalgae can interact symbiotically or competitively. Microalgae and bacteria engage in symbiotic relationships that involve nutrition exchange, cell-to-cell communication, and chemical compound stimulation. Additionally, microalgae have the ability to detect vitamins, siderophores, and quorum-sensing signal molecules produced by bacteria lead to symbiotic interactions that promote bioactivity, tolerance, and elimination. The effectiveness of wastewater treatment is increased when microalgae and bacteria have a symbiotic connection.

Keywords: Bacterial consortium, biomass, eutrophication, microalgae, nutrient recovery, wastewater.

Introduction

Due to the increased need for necessities like food, water, and clothing brought on by population growth, industrialization is growing quickly (Khan, S. et al., 2021). Solids, soluble or insoluble organic and inorganic contaminants, heavy metals, microbes, and other substances are all present in wastewater. These contaminants could be harmful or carcinogenic (Ibrahim, S. et al., 2020). Their existence, therefore, raises major health concerns and implies water poisoning. Unfortunately, home wastewater and textile industry wastewater are deposited into water bodies, where they release large amounts of toxic wastewater that pose serious environmental risks, resulting in increased polycyclic aromatic hydrocarbon (PAHs) concentrations. The microbial method known as "bioremediation" uses microorganisms to naturally produce new, resistant strains of themselves while converting a variety of toxic substances into less dangerous forms. The enzymatic system is responsible for the mechanism by which the xenobiotic chemical is broken down by microbes (Khan, S. et al. 2021). The most effective way to address the issue without negatively impacting the ecological system is through bioremediation, which is also environmentally benign. The low molecular weight (LMW) PAHs, such as naphthalene (NAP), phenanthrene (PHN), anthracene (AN), and fluorene (FLU), and the high molecular weight (HMW) PAHs, such as pyrene (PY), benzo(e)pyrene (B(e)P) and benzo(k)fluoranthene (B(k)F) were both effectively destroyed by the consortium. The primary biocatalysts for the biodegradation of hydrocarbons are microorganisms. Application of extremophilic bacterial strains in the treatment procedure to go around environmental constraints like salinity and temperature. In a continuous stirred tank reactor, the bacterial consortia effectively decomposed the PAHs in effluent from petroleum refineries, removing 94 ± 3.8% of COD. The PAH-degrading halothermophilic consortium contained *Ochrobactrum halosaudia* strains AJH1, AJH2, and AJH3, as well as Pseudomonas aeruginosa strain AJH3 (Arulazhagan Pugazhendi, et al. 2017).

Consortia cultivation

Individually cultivated microorganisms from various environments were tested for their capacity to bioremediate wastewater. Using particular media like Jensen's media, carboxymethylcellulose (CMC), Pikovskaya's (PKV), nitrogen removal, COD reduction, sodium dodecyl sulfate (SDS) and polychlorinated biphenyl (PCB) degradation, cellulolytic and proteolytic activity were investigated, respectively. Selected as possible candidates for

consortia designing and employed for wastewater treatment (flask and reactor study) based on the screening activities and good efficiency of nutrient removal in the flask experiment (Jha, V. et al., 2021). Effective bacteria such as *Lysinibacillus xylanilyticus*, *Planococcus maritimus*, *Fictibacillus nanhaiensis*, *Escherichia coli*, and *Acinetobacter pittii* were isolated from their respective sources. Molasses was used as the medium to create the microbial community, and it was cultured at 37°C for three days at a p H of 3.8.(Mahilarasi, A., et al. 2019). Proteus vulgaris NCIM-2027 and *Micrococcus glutamicus* NCIM-2168, members of the bacterial consortium GR, were able to quickly decolorize and degrade several different reactive dyes, including the widely used sulfonated reactive dye Green HE4BD.The decolorization activity of Consortium GR is significantly higher than that of the individual strains. The optimal physicochemical parameters were found to increase the effectiveness of dye degradation and decolorization. The use of inexpensive co-substrates, such as agricultural waste extracts, may improve the consortium GR's decolorization capabilities. Semi-synthetic medium containing various carbon and nitrogen sources (1% each), such as glucose, sucrose, peptone, urea, beef extract, lactose, casein, and yeast extract, was utilized to investigate the impact of these sources on the decolorization of Green HE4BD. Moreover, 1 g of rice husk, rice straw, bagasse powder, and wood shavings were combined with 100 ml of distilled water each and autoclaved at 1210C for 20 minutes in order to make the procedure economically possible. Subsequently, consortium GR introduced 10 m L of extract from each agricultural waste to a semi-synthetic medium to investigate the impact on Green HE4BD's decolorization ability (Saratale, et al. 2010).

Dye degradations

Developing countries' textile industries (TIs) have a significant impact on the global economy. The wastewater from the textile industry (TIWW) is distinguished by its high p H, dark hue, total dissolved solids (TDS), total suspended solids (TSS), total organic carbon (TOC), and biochemical and chemical oxygen demands (BOD and COD). Toxic metal ions, organic compounds, and a variety of resistant coloring pollutants (RCPs/dyes) are also present in TIWW. RCPs are the primary cause of environmental pollution and health risks among them. Asthma, eczema, allergies, immunosuppression, hypersensitivity, and intercalation with DNA helical structure and RNA duplex are all brought on by TIWW, which is also partitioned to the lipid membrane of live cells. *Brevibacillus laterosporus*,

for instance, demonstrated 67% decolorization of effluent from the textile sector in 48 hours. Therefore, compared to pure/single strains, consortium treatment is a more sustainable and effective method of degrading wastewater contaminants. Because bacterial strains work better together than they do individually, the bacterial consortium demonstrated greater remediation efficiency. For instance, 98% of the Navacron Ruby dye was decolored by a bacterial consortium consisting of *Pseudomonas aeruginosa*, *Enterococcus faecium*, and *P. aeruginosa* (Kishor, et al. 2022). The production of an active microbial consortium (mixed liquor suspended solids, or MLSS) by the gradual adaptation of a microbial community (such as activated sludge from an effluent treatment plant) to harmful or resistant substances would be an intriguing endeavor. This biomass might be very helpful to speed up the decolorization process, and the technique might end up being appropriate for usage on a big scale. The general purpose of the microbial consortium's anaerobic adaptation for decolorization. It has been discovered that a microbial community's ability to adapt to hazardous or resistant substances can significantly speed up the decolorization process. a comparable impact on the biodegradation process when microbial cultures adjust. It seems sense to believe that an extracellular enzyme is responsible for the decolorization activity seen. One can interpret anaerobic azo dye reduction as involving either an indirect reaction with reduced enzyme cofactors or a direct enzymatic reaction. The enzyme transfers the reducing equivalents from the oxidation of the organic substrate (glucose) to the azo dyes, as per the direct enzymatic azo dye reduction process. The azo dyes are reduced by enzymatically reduced electron carriers in the indirect method. An early description of the decolorization technique was similar. Before then, there's a chance that –N=N– will partially reduce into –NH–NH– functionality through a two-electron, two-proton reduction process. (Dafale, et al., 2008).

Petrochemicals degradation

One of the sectors with the quickest growth is the petrochemical sector. This sector is crucial to the production of a wide range of chemicals and the expansion of the economy. The petrochemical sector produces hazardous waste that includes both organic and inorganic substances, making it a hazardous set of industries (Fulekar, et al., 2017). Saline wastewater is frequently regarded as an effluent with at least 10,000–15,000 mg L^{-1} of Total Dissolved Solids (TDS) and high levels of organic components. High salinity has traditionally been a defining feature of wastewater discharged

from businesses like textile, petrochemical, pesticide, organic peroxide, pharmaceutical, and tannery. Excessive salt in wastewater not only hinders the metabolic processes of heterotrophic bacteria involved in biological wastewater treatment, but it also lowers the remediation process's effectiveness. Various environmentalists have tried to treat saline wastewaters by isolating and enriching salt-tolerant microbes. The presence of resistant organics, such as aromatic and aliphatic hydrocarbons as well as chlorinated hydrocarbons, limits the direct biological treatment of saline petrochemical wastewater and results in low BOD5/COD ratios in these industrial wastewaters. The investigation involved the use of a consortium including three isolated salt-tolerant bacteria, namely *Kocuria turfanesis, Halomonas alkaliphila*, and *Pseudomonas balearica*, to treat a saline petrochemical wastewater with a BOD5/COD ratio of less than 0.1. From petrochemical wastewater containing mineral salt media with a salinity of 3%, certain bacteria were identified (Ahmadi, M., et al. 2017). Owing to the lack of suitable bacterial strains, petroleum hydrocarbon breakdown under harsh circumstances including high salinity, temperature, and p H was challenging. The effectiveness of an extremophilic bacterial consortia in treating wastewater from petroleum refineries and biodegrading various petroleum hydrocarbons under harsh conditions is described in depth in the current work. The optimal conditions for the breakdown of petroleum hydrocarbons were found to be 8% salinity, p H 10, and 60°C. Under ideal, harsh conditions, the consortium saw the whole breakdown of Low Molecular Weight (LMW) petroleum hydrocarbons (200 ppm) in 8 days, including anthracene, phenanthrene, fluorene, and naphthalene. The extremophilic consortium demonstrated 93%, 60%, 55%, and 51% degradation of high molecular weight (HMW) hydrocarbons, such as pyrene (100 ppm), benzo(e)pyrene (20 ppm), benzo(k)fluoranthene (20 ppm), and benzo(a)pyrene (20 ppm), at optimal severe conditions. Fluorene (61%) was mineralized by the extremophilic consortia upto 24% in highly salinized conditions. Under harsh conditions, the addition of yeast extract significantly sped up the biodegradation process. Within the consortium in charge of treating wastewater from petroleum refineries and breaking down petroleum hydrocarbons were the extremophilic strains *Ochrobactrum* (30%), *Bacillus* (26%), *Marinobacter* (22%), Pseudomonas (10%), Martelella (5%), Stenotrophomonas (4%) and Rhodococcus (3%). Only a few bacterial strains -*Ochrobactrum*, *Bacillus*, *Marinobacter*, and *Rhodococcus* were dominant at various high severe environments. Numerous reports have indicated that Ochrobactrummaydegradepetroleumhydrocarbonsinsalineandthermophilicen

vironments the ability of Bacillus as a prospective strain to degrade hydrocarbons under harsh conditions and the efficiency of Marinobacter to degrade various petroleum hydrocarbons under saline conditions and function as an indicator of oil reserves in the marine environment. Prior investigations have shown Marinobacter's effectiveness as a hydrocarbon degrader in saline environments (Al-Mur, et al. 2021).

Pesticides degradation

Around the world, synthetic chemical compounds are employed to meet home, food, and feed needs. These substances include, among others, steroids, antibiotics, and insecticides. Pesticides are used in a variety of home and agricultural applications to lessen the pest burden. Long-term exposure to these chemicals has negative effects on the environment, other living things, and humans. Many pesticide classes, including carbamates, organophosphates, organochlorines, and pyrethroids, are widely employed to control agricultural pests and disease vectors in homes and public areas. Pesticides' toxicity to biological systems, persistence, mobility, timing of application, and formulation all affect how hazardous they are to the environment. According to the study's findings, lindane from polluted soil may be degraded by applying bioaugmentation to a bacterial consortium and biostimulation at the same time. Pesticides can be consumed by bacterial strains from the genera *Arthrobacter*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, *Rhizobium*, and *Sphingomonas* as a source of carbon, nitrogen, and phosphorus. Conversely, *Phanerochaete chrysosporium*, Pleurotus ostreatus, *Aspergillus niger, Fusarium proliferatum, Candida sp., Trametes versicolor, Cunninghamella elegans,* and *Penicillium* sp. have been identified as the fungi involved in pesticide bioremediation. A bacterial consortium made up of 21 different species had been shown to degrade atrazine,2,4- dichlorophenoxyacetic acid (2,4-D), carbofuran, diazinon, and glyphosate more efficiently. Using the consortium (MC-BSPK) comprising *Bacillus* sp. MCB, *Serratia* sp. MC-S, *Pseudomona* ssp. MC-S and *Klebsiella* sp. MC-K, chlorpyrifos, and 3,5,6-trichloro-2-pyridinol were totally decomposed in 9 days. It was discovered that a combination of *Pseudomonas putida*, *Stenotrophomonas maltophilia*, and *Staphylococcus warneri* proved efficient in breaking down chlorpyrifos (Bhatt, et al. 2021).

Heavy metals degradation

Hazardous and difficult to biodegrade, heavy metals in industrial effluent have the potential to enrich the food chain. This will cause major environmental issues on a global scale. The drawbacks of conventional

physicochemical heavy metal cleanup techniques include secondary contamination and exorbitant expenditures. A group of bacteria and microalgae exhibits symbiotic benefits. Through photosynthesis, microalgae absorb carbon dioxide and change inorganic materials into organic ones while releasing oxygen. Carbon dioxide is released when oxygen-consuming bacteria eat organic materials and oxygen. For the purpose of forming a consortium, these two require symbiosis to occur. Small size, large specific surface area, fast growth and metabolism, high reproductive potential, high biological activity, high adsorption capacity, environmental adaptability, ubiquity in the aquatic environment, affordability, and ease of availability are the characteristics of microalgae and bacteria. There are many different sources from which species can be acquired and screened, including places contaminated with heavy metals (Zhao, et al. 2023). The microbial combinations are able to totally eliminate the chromium ions. Recovering the used biomass after the heavy metal removal process is one of the primary obstacles in using biomass for heavy metal removal on an industrial scale. In order to prevent contamination of the outlet effluent in this work, the immobilized biomass was employed. To make the collection procedure easier and to keep the immobilized biomass distinct from the treated solution (industrial waste), it was hung in a tissue that resembled a tea bag. The pretreatment of the utilized biomass involved immersing it in a mildly acidic solution, which improved its capacity for biosorption and eliminated any byproducts that would have been harmful to the environment. It was discovered that the most potential microbes to be effective in removing lead ions were Cr56FSU, bakery yeast, Pb26BSP, Pb3BA, and Cr15BW, among others. The most potential microbes to be effective in removing chromium ions were Cr3BA, Cr5FA, Pb26BSP, Cr15BW, and Pb11BW. With P values <0.05 at a 95% confidence level, every organism that was chosen demonstrated a statistically significant impact on eliminating the targeted heavy metal ions, with the exception of the isolate Cr56FSU, which had a *P*-value of 0.054, which was quite close to being significant (Migahed, et al. 2017).

Future perspective

Technology has advanced to the point that it is now feasible to create and engineer microbial consortia, increasing microbial productivity and yield above and beyond what is achievable with a pure culture. The development of diverse biotechnological instruments has made it feasible to enhance the intended roles of microbial strains. As a result, we can enhance the

capabilities of microbial strains inside consortiums. In contrast to axenic cultures, consortium members can deteriorate more quickly when they collaborate. A microbial consortium aids in lowering the amount of environmental contaminants that are resistant to removal. The increase of pollutants is always detrimental to the ecosystem. Different metabolic pathways are used to break down different types of contaminants. Still, it is rare for one strain to be able to break down multiple pesticides at once. Using mixed microbial cultures is the way to get each strain to degrade more quickly and potently. Thus, according to the planned stages, the synthetic microbial consortia can breakdown the contaminants more quickly thanks to technical developments. These consortiums adapt to their natural surroundings and do no harm to the ecosystems. Further research is necessary to build and comprehend the diversity and functionality of microbial consortia in the future. To further build a microbial consortium with distinct activities, databases of food requirements, metabolites, community interactions, and enzymes that catalyze the stages can be developed. By taking these actions, the research of axenic cultures enters a new microbial era that will facilitate the development of consortia's potential applications in various disciplines as well as bioremediation. Microbial consortia can now be used in the biotechnology industry more readily thanks to the quick development of modern technologies. Furthermore, the breakdown of large-scale pesticides that contaminate numerous locations worldwide may benefit from the creation of synthetic microbial consortia with useful applications.

Conflict of interest

The authors have no conflict of interest.

Acknowledgement

The authors would like to thank Swami Vivekananda University for their constant support.

References

1. Ibrahim S, El-Liethy MA, Elwakeel KZ, Hasan MAEG, Al Zanaty AM, Kamel MM. Role of identified bacterial consortium in treatment of Quhafa Wastewater Treatment Plant influent in Fayuom, Egypt. Environ Monit Assess. 2020;192(1):1-10.
2. Khan S, Bhardwaj U, Iqbal HM, Joshi N. Synergistic role of bacterial consortium to biodegrade toxic dyes containing wastewater and its simultaneous reuse as an added value. Chemosphere. 2021;284:131273.

3. Arulazhagan Pugazhendi AP, Huda Qari HQ, Basahi JMA, Godon JJ, Jeyakumar Dhavamani JD. Role of a halothermophilic bacterial consortium for the biodegradation of PAHs and the treatment of petroleum wastewater at extreme conditions.
4. Jha V, Dafale NA, Hathi Z, Purohit H. Genomic and functional potential of the immobilized microbial consortium MCSt-1 for wastewater treatment. Sci Total Environ. 2021;777:146110.
5. Mahilarasi A, Jaianand K, Rameshkumar K, Balaji P, Veeramanikandan V. Formulation of effective microbial consortium and its application for industrial wastewater treatment. J Drug Deliv Ther. 2019;9(4-s):111-117.
6. Saratale RG, Saratale GD, Chang JS, Govindwar SP. Decolorization and biodegradation of reactive dyes and dye wastewater by a developed bacterial consortium. Biodegradation. 2010;21:999-1015.
7. Kishor R, Raj A, Bharagava RN. Synergistic role of bacterial consortium (RKS-AMP) for treatment of recalcitrant coloring pollutants of textile industry wastewater. J Water Process Eng. 2022;47:102700.
8. Dafale N, Rao NN, Meshram SU, Wate SR. Decolorization of azo dyes and simulated dye bath wastewater using acclimatized microbial consortium–biostimulation and halo tolerance. Bioresour Technol. 2008;99(7):2552-2558.
9. Fulekar MH. Microbial degradation of petrochemical waste-polycyclic aromatic hydrocarbons. Bioresour Bioprocess. 2017;4(1):1-16.
10. Ahmadi M, Jorfi S, Kujlu R, Ghafari S, Soltani RDC, Haghighifard NJ. A novel salt-tolerant bacterial consortium for biodegradation of saline and recalcitrant petrochemical wastewater. J Environ Manag. 2017;191:198-208.
11. Al-Mur BA, Pugazhendi A, Jamal MT. Application of integrated extremophilic (halo-alkalo-thermophilic) bacterial consortium in the degradation of petroleum hydrocarbons and treatment of petroleum refinery wastewater under extreme conditions. J Hazard Mater. 2021;413:125351.
12. Bhatt P, Bhatt K, Sharma A, Zhang W, Mishra S, Chen S. Biotechnological basis of microbial consortia for the removal of pesticides from the environment. Crit Rev Biotechnol. 2021;41(3):317-338.

13. Zhao D, Cheah WY, Lai SH, Ng EP, Khoo KS, Show PL, Ling TC. Symbiosis of microalgae and bacteria consortium for heavy metal remediation in wastewater. J Environ Chem Eng. 2023;11(3):109943.
14. Migahed F, Abdelrazak A, Fawzy G. Batch and continuous removal of heavy metals from industrial effluents using microbial consortia. Int J Environ Sci Technol. 2017;14:1169-1180.

Chapter - 2

Comparative Analysis of Culture-Dependent and Culture-Independent Techniques for Elucidating Microbial Diversity

Authors

Anuska Biswas
Department of Microbiology, 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 - 2

Comparative Analysis of Culture-Dependent and Culture-Independent Techniques for Elucidating Microbial Diversity

Anuska Biswas and Debjit De

Abstract

The term “Metagenomics” is evaluated in this review because it provides a thorough and an advanced overview of the culture-independent or metagenomic methodologies to identify the microbial world. Over 99 percent of microorganisms are known to be uncultivable. Metagenomics provides the door for the unculturable approaches and their advancement. The use of conventional methods for microbe culture results in the unknown status of many microorganisms. The field of metagenomics is essential to the search for previously unidentified microbial species. The study of the intricate genomes of microbial habitats is the goal of the relatively new and fast expanding science of metagenomics. A significant portion of the microbiome is left uncultured, despite the fact that traditional methods of culturing are crucial for the isolation and research of microorganisms of interest in microbiology. The procedures take a lot of time, and for a variety of reasons, they are not always correct. Thus, in order to separate and investigate the selected bacterium, the metagenomic method is required. To sum up, uncultivated bacteria are common in a variety of settings, metagenomic investigations regularly uncover novel genes and proteins that are absent from farmed microbes. Compared to conventional techniques that concentrate on cultured organisms, metagenomics is a more promising strategy because of its innate capacity to find unique sequences. Since its inception, metagenomics has been able to identify a large number of new genes, many of which code for compounds with substantial potential for use in medicinal applications, such as biocatalysts.

Keywords: Metagenomics, cultured methods, uncultured method, sequencing, next generation sequencing.

1. Introduction

Microbiology has experienced a transformation during the last 25 years that has altered microbiologists' view of microorganisms and how to study them. The realization that most microorganisms cannot be grown readily in pure culture forced microbiologists to question their belief that the microbial world had been conquered [1]. Microbialecology mainly depended on traditional culturing methods, which involved isolation of microorganisms in a lab setting in order to study them, before to the development of metagenomics. This approach was not easy and reliable, as many microbes could not be cultivated in a lab setting. The identification of the DNA double helix in 1953 and the creation of the Polymerase Chain Reaction (PCR) by Kary Mullis in the 1980s marked major advancements in the field of microbiology [3]. Through the use of PCR, researchers were able to amplify particular DNA sequences, which made it easier to study genes from organisms that could not be cultured.

The word "metagenomics" was first used in the early 2000s to refer to a new technique that directly analyzed genetic material from environmental samples, without the need for culturing. Jo Handelsman and colleagues conducted a study in which they studied soil samples and discovered a wide variety of microbial life, proving that microbial biodiversity had not yet been fully explored by conventional culturing techniques [4].

This new strategy became possible due to the availability of new high through put sequencing technologies brought in by various companies to assist researchers with high purchase, quick, and cheap sequencing of large quantity of DNA.

In the middle of 2000s up to the 2010s, there was a growing use of metagenomic approaches and its diversified fields including marine microbial ecology, soil, and human microbiology. Probably the most profound effect generated by metagenomics was in the research of the human microbiome where the interactions between microbial communities and human health appeared to be much more intricate. Bioinformatics tools necessary for metagenomics analysis were also emerging in this period; they contributed to the study of metagenomics by providing means for taxonomic classification, functional annotation of genes and proteins to foster the understanding of the avalanche of data beginning to be produced.

To date, metagenomics still offers a vast improvement in the field of microbial research as it achieved synthesis with other disciplines such as

metatranscriptomic and metabolomic techniques to provide a more elaborate outlook of microbial consortia and functions. The field is also availing itself to improvement in computational techniques and artificial intelligence which helps in data evaluation and analysis. Genomic analysis at the level of communities of organisms is further growing in its applications in medicine, environmental sciences, industry and business and extending our understanding of the function of microorganisms in different settings.

2. Pros and cons of culture methods

In microbiology, culture techniques have many advantages. For example, they can be used to separate and identify particular microorganisms from mixtures, which help in- depth research into their traits and behaviors. These techniques are important for antibiotic sensitivity testing, which guides the selection of appropriate treatments, and they help in the quantitative analysis of microbial populations. Furthermore, microorganisms can be cultured to create a controlled environment for genetic modification and growth, which is necessary for a variety of research applications.

These approaches do have some disadvantages, though. The use of microorganisms for studying certain species is limited because not all of them can be cultivated in a laboratory. It can take a lot of time and work to cultivate something like Most clinical pathogens grow easily over 24 to 48 h in plate media but several bacterial species require a much longer time [5]; long incubation times and very careful handling are frequently needed to avoid contamination [6]. Additionally, the costs and knowledge required for culturing can be high, and there are continuous risks associated with working with pathogenic microorganisms. Overall, while culture methods are fundamental to microbiology, their limitations necessitate careful consideration in their application.

3. Uncultured methods

Unculture methods in microbiology may be defined as a number of procedures that can be employed to learn more about microorganisms that cannot be successfully cultured in a standard laboratory. Many attempts have been made to develop efficient methods of isolating uncultured microorganisms [7]. These techniques are crucial for discovering the broad range of microbial ecosystems in different habitats like soil, water and our body because many microorganisms have particular kinds of nutrition and growth prerequisites or depend upon synergy with other organisms [8]. Some of the techniques include metagenomics where the environmental samples

are directly subjected to DNA sequencing without taking the necessity of culturing the microbes individually. This approach has been critical in identification of new microbial species that may exist in specific ecosystems and their probable uses. Another important method is Fluorescent In Situ Hybridization. Fluorescence in situ hybridization (FISH) is a molecular cytogenetic technique that allows the localization of a specific DNA sequence or an entire chromosome in a cell [10]. FISH permits for microorganism visualization in all of their natural habitats using specific ready probes accompanied by fluorochromes which can hybridize to the corresponding specific r RNA of the target organism. This makes possible the evaluation of the microbial impacts and their localization in real context. In addition, stable isotope probing (SIP) identifies a connection between the metabolic activity and unique microorganisms in the environment by enriching the DNA samples with isotopically labelled substrates which aids in identifying the role of these microorganisms in the biogeochemical processes of the given environment [11]. In general, the unculture methods have transformed microbial ecology and evolution that has assisted in establishing the part of microorganisms in ecosystem well-being and illness. These techniques do not only enhance our capability as to organization identification and analysis of super organisms of microorganisms but also bring out other opportunities in biotechnology, medicine and the environment, affirming the significance of these normally mediocre organisms towards the sustenance of existence in this planet.

a) Metagenomics

Metagenomics is indeed turning the traditional microbiology upside down since the microorganisms in their natural environments can be studied at once without isolating them [12]. Metagenomics is the investigation of samples taken from environment without isolation of the organism. The fact that microbial life is very diverse becomes a problem when using cultivation as a method of identification since most microorganisms cannot exist in artificial environment. This is the reason why this method is most useful. In the field of metagenomics, researchers are able to examine the tangled microbial populations in every environment possible-be it soil, water from the sea, or the human intestines, and more significantly, be able to study more about their compositions, functions, and roles in the various ecosystems. Using molecular data one can detect previously described and undescribed species and thus better understand the dynamics and organization of these communities.

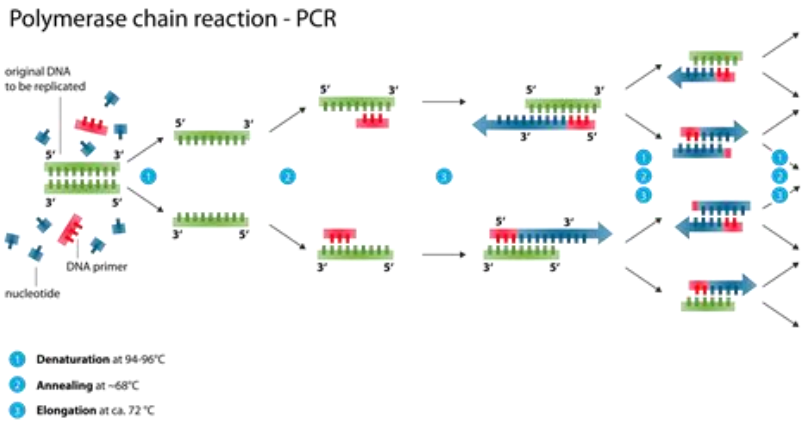
b) Amplicon sequencing

Amplicon sequencing is a targeted approach to the sequencing of certain interest areas in the genome through polymerase chain reaction (PCR) amplification of a particular DNA fragment many times. Amplicon sequencing is more sensitive than culturing, identifying more bacteria per sample than culturing [13]. Such an approach is vital in order to guide research in varied fields like genetics, cancer, and microbiology toward regions known to have biological significance, such as gene variants, mutations, or microbial diversity. The first step is the production of appropriate primers specific to the target site that could just be a couple of hundred base pairs.

The primers enable several rounds of denaturation, annealing, and extension through annealing to the DNA template with selective amplification of areas of interest.

Amplicons generated might be purified and sequenced using high-throughput sequencing technologies leading to massive quantities.

This power is truly in the resolution information provided for the individual loci, which is very important for complex genomes or communities. In a paradigm, amplicon sequencing is often more widely applied within microbial ecology for examining the16S r RNA gene-a very conserved region within bacteria that enables the identification and taxonomy of microbial species found within the sample. This technique shines light into the dynamics, diversity and structure of microbiomes in a variety of settings- ranging from environmental samples to human microbiomes. In addition, the latest advancements in sequencing techniques allow for the possibility of producing superb amplicons, which can then be purified and sequenced using high-throughput sequencing, thereby generating huge amounts of data that could then be analyzed fora number of applications, ranging from variant calling to studying microbial communities.



However, there are disadvantages in amplicon sequencing. Primers significantly determine the success of this approach, because their effectiveness and specificity are very important; otherwise, nonspecific amplification may cause false results and reduced ability to replicate. It also causes problems in results interpretation, especially in homogeneous areas. Therefore, good scrutiny and validation of results are essential. Scientists usually solve these problems by using numerous bioinformatics pipelines and tools in handling the sequencing data with tasks such as variant calling, alignment, and quality control checks. Taking everything into account, amplicon sequencing is a powerful approach for extracting accurate genetic information from selected regions. It will contribute much information that can be used in the investigation of the improvement of our understanding of complex biological systems and boost progress in areas such as environmental science and personalized medicine [14].

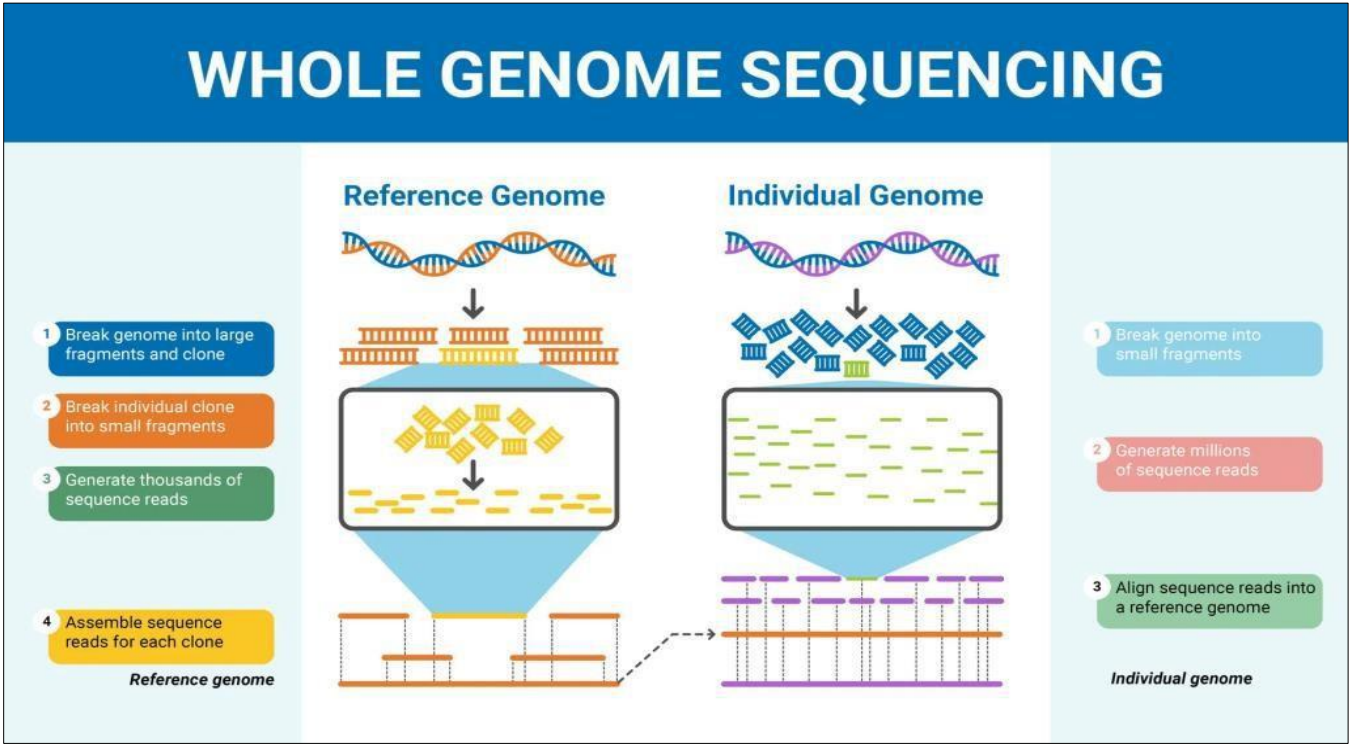
Whole genome sequencing

Whole genome sequencing, or WGS, is the comprehensive determination of an organism's genome by complete DNA sequencing of all its genes as well as non-coding regions, carried out through high-throughput sequencing technologies to generate a large volume of data, enabling researchers to analyze the whole genetic makeup in a single experiment. Whole genome sequencing (WGS) is a cutting-edge technique used to determine the complete DNA sequence of an organism’s genome in one comprehensive analysis. Unlike targeted sequencing methods that focus on specific regions of the genome, WGS provides a full view of all the genetic material, including both coding (exons) and non-coding (introns, regulatory sequences) regions. By analyzing every base pair in the genome, WGS

captures a wide array of genetic variations, such as Single Nucleotide Polymorphisms (SNPs), insertions, deletions, copy number variations, and large structural rearrangements, many of which may be missed by other methods. Since 2008, when next-generation sequencing (NGS) approaches entered the research setting, there has been a significant decline in sequencing costs. These approaches allow either the whole genome (via Whole-Genome Sequencing (WGS) or parts of it (via Whole-Exome Sequencing (WES) or targeted panels) to be sequenced faster, at great depth and increasing sensitivity, and this decrease in costs has made the clinical application of WES and WGS more feasible.[15]

This powerful tool has revolutionized genomic research and clinical diagnostics. It is particularly valuable for identifying genetic mutations associated with rare hereditary diseases, complex disorders like cancer, and drug responses. In personalized medicine, WGS can inform treatment decisions by revealing an individual's genetic predispositions to diseases and how they might respond to certain therapies. Its utility extends to prenatal screening, infectious disease tracking, and evolutionary studies, providing insights into how populations evolve, adapt, and migrate over time.

The cost of WGS has decreased significantly since its inception, making it more accessible for large-scale projects like the Human Genome Project and initiatives aimed at cataloging genetic diversity across populations. Nevertheless, the vast amount of data generated by WGS poses challenges for data storage, analysis, and interpretation, requiring advanced bioinformatics tools and expertise. As technology continues to improve, WGS is expected to play an increasingly vital role in healthcare, agriculture, and environmental sciences, offering the potential to unlock new discoveries and innovations [16].


WHOLE GENOME SEQUENCING
Reference Genome
Individual Genome
1 Break genome into large fragments and clone
2 Break individual clone into small fragments
3 Generate thousands of sequence reads
4 Assemble sequence reads for each clone
Reference genome
1 Break genome into small fragments
2 Generate millions of sequence reads
3 Align sequence reads into a reference genome
Individual genome

c) Conclusion

Metagenomics has altered the way problems are solved by microbiologists, revised what a genome means, and increased the speed at which genes are identified. Ever since the revolution of metagenomics, its practical benefits to biotechnology seem high nevertheless. The functional assays have used the enzyme and antibiotic libraries generated from different settings to screen for new activity. Understanding the function and structure of microbial populations is crucial for a myriad of uses, the most important of which include medicine, agriculture and industry [17].

The capability of genomics of metagenomic libraries is however bound by the development of techniques that are key in library construction and analysis. For example, in terms of sequence-based approaches, the turn-around time and the cost for nucleotide sequencing will be restrictions which will increasingly become of little importance to courts as the sequencing technology becomes better. The De novo sequence-based functional assignment will also be aided by efficient workflows suited for detecting protein structure homology rather than primary sequence alone. Some advances that will enhance the management and manipulation of large collections will incorporate modern analytic methods to efficiently reconstruct multiple genomes from large sequence datasets and cost-effective oligonucleotide arrays for library screening or that effortlessly

References

1. Olsen, G. J., N. R. Pace, M. Nuell, B. P. Kaine, R. Gupta, and C. R. Woese. 1985. Sequence of the 16S rRNA gene from the thermoacidophilic archaebacterium Sulfolobus solfataricus and its evolutionary implications. J. Mol. Evol. 22:301-307. [PubMed] [Google Scholar]
2. https://eurofinsgenomics.eu/en/eurofins-genomics/material-and-methods/amplicon-sequencing/
3. Mullis, K. B. 1990. The unusual origin of the polymerase chain reaction. Scientific American, 262(4): 56–61, 64–65. [PubMed] [Google Scholar]
4. https://scholar.google.com/citations?view_op=view_citation&hl=en&user=e53g1HYAAAAJ&citation_for_view=e53g1HYAAAAJ:u-x6o8ySG0s C
5. Chapin, K. C. 2007. Principles of stains and media, p. 182–191. In Murray, P. R., Baron, E. J., Jorgensen, J. H., Landry, M. L., Pfaller, M.

A. (eds.), Manual of Clinical Microbiology, 9th ed. ASM Press, Washington, DC. [Google Scholar]

6. Ghodbane, R., Raoult, D., Drancourt, M. 2014. Dramatic reduction of culture time of Mycobacterium tuberculosis. Sci Rep 4:4236. doi:10.1038/srep04236. [PMC Free Article] [PubMed] [CrossRef] [Google Scholar]
7. Alain, K., Querellou, J. 2009. Cultivating the uncultured: limits, advances, and future challenges. Extremophiles 13:583–594. doi:10.1007/s00792-009-0261-3. [PubMed] [CrossRef] [Google Scholar]
8. Vartoukian, S. R., Palmer, R. M., Wade, W. G. 2010. Strategies for culture of 'unculturable' bacteria. FEMS Microbiol Lett 309:1–7. doi:10.1111/j.1574-6968.2010.02000.x. [PubMed] [CrossRef] [Google Scholar]
9. Microbial interactions: Ecology in a molecular perspective. Braz. J. Microbiol. (2016)
10. https://sequencing.com/education-center/whole-genome-sequencing
11. Parra, I., & Windle, B. 1993. High-resolution visual mapping of stretched DNA by fluorescent hybridization. Nature Genetics, 5, 17–21. doi:10.1038/ng0993-17.
12. Kleiner, M., Wentrup, C., Holler, T., Lavik, G., Harder, J., Lott, C., et al. 2015. Use of carbon monoxide and hydrogen by a bacteria–animal symbiosis from seagrass sediments. Environ Microbiol., 17:5023–5035. [PubMed] [Google Scholar]
13. Dutilh, B. E. 2014. Metagenomic ventures into outer sequence space. Bacteriophage, 4:e979664. doi:10.4161/21597081.2014.979664. [PubMed] [Google Scholar]
14. Dowd, S. E. et al. 2008. Survey of bacterial diversity in chronic wounds using pyrosequencing, DGGE, and full ribosome shotgun sequencing. BMC Microbiol., 8, 43.
15. Deurenberg, R. H. et al. 2017. Application of next-generation sequencing in clinical microbiology and infection prevention. J. Biotechnol., 243, 16–24.
16. Davies, S. C. 2016. Annual Report of the Chief Medical Officer: Generation Genome. Department of Health, London. [Google Scholar]

17. Zhao, S., Prenger, K., Smith, L., Messina, T., Fan, H., Jaeger, E., Stephens, S. Rainbow: A tool for large-scale whole-genome sequencing data analysis using cloud computing. BMC Genomics. [Google Scholar] [CrossRef] [PubMed]

18. Alneberg, J., Bjarnason, B. S., de Bruijn, I., Schirmer, M., Quick, J., Ijaz, U. Z., et al. 2014. Binning metagenomic contigs by coverage and composition. Nat. Methods, 11, 1144–1146. doi:10.1038/nmeth.3103. [PubMed] [CrossRef] [Google Scholar]

19. Andrews, M. C., Duong, C., Gopalakrishnan, V., Iebba, V., Chen, W. S., Derosa, L., et al. 2021. Gut microbiota signatures are associated with toxicity to combined CTLA-4 and PD-1 blockade. Nat. Med., 27, 1432–1441. doi:10.1038/s41591-021-01406-6. [PubMed] [CrossRef] [Google Scholar]

Chapter - 3

Insecticides and their Toxicological Impact: A Comprehensive Overview

Authors

Anindita Nandy

Department of Biotechnology, School of Life Science, Swami Vivekananda University, Sewli Telinipara, Malir Math, Bara Kanthalia, North 24 Parganas, West Bengal, India

Departmentof Zoology, Diamond Harbour Women's University, Sarisha, South 24 Parganas, West Bengal, India

Palash Kumar Pal

Department of Biotechnology, School of Life Science, Swami Vivekananda University, Sewli Telinipara, Malir Math, Bara Kanthalia, North 24 Parganas, West Bengal, India

Priya Roy

Departmentof Zoology, Diamond Harbour Women's University, Sarisha, South 24 Parganas, West Bengal, India

Nimai Chandra Saha

Director of Public Instruction (DPI), Education Directorate, Higher Education Department, Govt. of West Bengal, Bikash Bhavan, Salt Lake, Kolkata, West Bengal, India

Priyajit Banerjee

Department of Biotechnology, School of Life Science, Swami Vivekananda University, Sewli Telinipara, Malir Math, Bara Kanthalia, North 24 Parganas, West Bengal, India

Chapter - 3

Insecticides and their Toxicological Impact: A Comprehensive Overview

Anindita Nandy, Palash Kumar Pal, Priya Roy, Nimai Chandra Saha and Priyajit Banerjee

Abstract

Pesticides are compounds usually used to manage unwanted growth of pests, particularly insects, weeds and restrict development of different plant diseases. However, in most of the cases, pesticides have been evident to be toxic to other higher-order organisms involved in the food chain. Although there are three different types of pesticides, in the present treatise we will focus on the toxic effects of diverse insecticides only since it is the most predominating candidate among the pesticides in exerting non-specific toxicity to other organisms. Insecticides are characterized as chemical compounds applied to eradicate unwanted pests responsible for damaging crops. However, uncontrolled use of such insecticides and their concomitant contamination to nearby surface waterbodies may cause severe health issues in different aquatic and mammalian species, even may led to death of the organism. Despite of the fact that numerous researchers are attempting alternative strategies to combat the situation, but poor understanding of the chemical behaviour is actually affecting the population balance in the ecosystem. Usually, organophosphates, organochlorines, pyrethroids and necotenoides are used as common insecticides. Interestingly, insecticide induced toxicities have been reported to inhibit growth rate, act as an endocrine disruptor, cause spinal abnormalities, and tissue damage in the major organs, including brain, liver, spleen and kidney, of the organism. As a result, cognitive dysfunction, genetic abnormalities and developmental complications are common in insecticide-exposed individuals. Thus, it is high time to take protective and effective measures to minimize the contamination and side effects of the insecticides.

Keywords: Insecticide, hepatotoxicity, organochlorine, organophosphate, pyrethroid, neonicotinoids.

1. Introduction

Biological agents or synthetic compounds are employed as pesticides to attract, seduce, kill, or minimize pests. They are mostly applied in agricultural fields to eradicate weeds and protect crops from insects, bacterial, and fungal diseases while they are growing, as well as to shield food from other biological pollutants like rats and mice while it is being stored [1].

Typically, pesticides are divided into two categories: synthetic and biological. While synthetic pesticides are produced through industrial methods, natural compounds are extracted and used to prepare biological pesticides, for example, plant extracts (pyrethrin from chrysanthemum plants or azadirachtin from neem). Depending upon the type of pest to be controlled, pesticides can also be classified into three major categories: insecticides (for insects) (Table 1), herbicides (for weeds), and fungicides (for fungus).

Broad-spectrum pesticides are usually applied to get rid of multiple pests at a time, while narrow-spectrum pesticides are applied to minimize a particular species [2]. The use of an enormous quantity of insecticides (~2 million tons/year) globally makes the situation more critical. The United States and Argentina are the next two countries that produce the most pesticides, behind China. The demand for agricultural products like pesticides has increased due to overpopulation and overexploitation [3].

Pesticides can affect an individual both directly or indirectly. Usually, ingestion through drinking water and food and inhalation through air and dust are the primary routes of indirect insecticide contamination in humans. However, people working in agricultural fields, factories, etc., are directly exposed to insecticide-induced toxicities. Exposure to indirect pesticides may happen more often than direct pesticide application [4].

Pesticides are known to induce detrimental effects on human health in particular regions. Certain substances may simply cause minor skin irritations, while others may have an impact on the liver or lungs. Some have the potential to cause cancer, while others may be harmful to the reproductive system or alter hormones. There are several neurotoxic insecticides. Specifically, pesticides cause neurotoxicity in humans by upsetting the nervous system of the insect they kill, basically by paralyzing the organism and restricting its further movement. There have also been a few chemicals from different pesticide classes linked to neurotoxic effects [5].

The term ‘neurotoxicity’ refers to direct or secondary impacts of compounds that cause harm to an animal's or human's nervous system. Many

chemicals are employed as experimental instruments to disrupt or harm the nervous system of animals, and many more can cause neurotoxic illness in humans. Some directly affect brain cells, while others obstruct metabolic processes that the nervous system is particularly reliant on. While some cause maldevelopment or harm to the adult nervous system, others interfere with brain function. Changes might come and go quickly, progress gradually over a few days or weeks and then regress over several months or years, or result in long-term impairments [6].

Severe hepatic tissue damage (hepatotoxicity) is usually caused due to exposure to toxic chemicals referred to as hepatotoxins or hepatotoxicants that include industrial chemicals, natural chemicals, herbal products, overuse of medicines, etc. [7-9].

Pesticides are known to serve as vital tools for both public health and agriculture. However, the truth is that pests and non-target species, including humans, frequently have the same molecular targets when it comes to insecticides. This is especially the case with pyrethroid, organophosphate, and neurotoxic organochlorine insecticides. In a similar vein, miticides (killing mites) and acaricides (killing ticks and other pests) that block mitochondrial complexes are becoming more widely utilized.

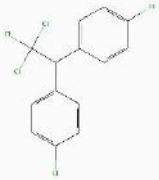
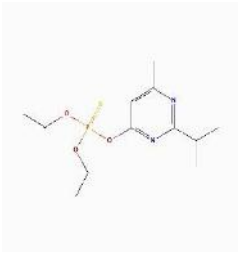
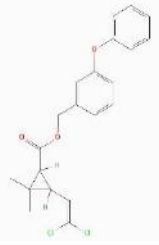
Several herbicides and fungicides have been shown to affect the mammalian brain, despite the fact that they theoretically shouldn't have common targets with mammals [10]. The main pesticide classes that have been suspected or evidenced to cause neurotoxicity and hepatotoxicity, as observed in diverse experimental or clinical records, will be the subject of this review.

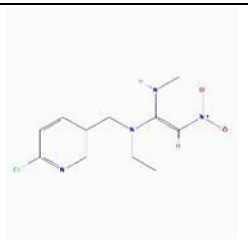
2. Organochlorine insecticides

Chlorinated substances often employed as insecticides are known as organochlorines (OC). These substances are highly persistent in the environment and are classified as persistent organic pollutants, or POPs. Though most developed nations forbid them, OC insecticides were formerly effectively used to combat typhus and malaria [11]. Due to their prolong chemical half-life, they undergo slow degradation in the air, water, soil, and living things after being discharged into nature; thus, their accumulation increases over time within the contaminated natural body or, organism. This is why, they become vulnerable to protracted transboundary vast-distance air pollution (LTAP) where they get transported by both anthropogenic activity and atmospheric movement across vast distances. A significant contributor

to LTAP is even the body weight of migrating bird flocks. Due to their slow degradation property, OCPs are responsible for their biomagnification in the higher order organism of the food chain. Dichlorodiphenyltrichloroethane (DDT), for instance, can be permanently sequestered into the body's soft tissue compartments, primarily the adipose tissues, where it can stay for about 50 years. From there, it can separate into the bloodstream and enter plasma or serum lipids before being actively secreted into breast milk, which serves as mammal's primary route of elimination [12].

Table 1: Details of different types of insecticides

Insecticide class	Example	Structure	Target	Effects
Organochlorine	DDT Lindane		Continuous opening of sodium channel Functional Restriction of GABA and voltage-dependent chloride channels	Tremors Hyper-excitability Seizures
Organophosphate	Diazinon Chlorpyrifos Mipafox		Functional Restriction of Ach E	Cholinergic syndrome Peripheral axonopathy
Pyrethroid	Deltamethrin Allethrin		Continuous opening of sodium channels	Hyper-excitability

Neonicotinoids	Nitenpyram- imidacloprid Dinotefuran Clothianidin		Inhibition of Ach E receptor	Paralysis

Dichlorodiphenyltrichloroethane (DDT)

DDT-induced neurotoxicity

The insecticidal qualities of DDT (dichlorodiphenyltrichloroethane) were discovered in 1939, after it was originally synthesized in 1874. However, due to this compound's chemical properties, which include accumulation and bioconcentration in the lipid systems of all animal species and the possibility of negative effects on both people and wild animals, its usage has been prohibited in many nations since the 1970s [13].

DDT is known to target a particular neuronal ATPase responsible for controlling the physiological levels of different ionic components in the nerve membrane, such as K+, Na+, and Ca++. It is also evident to cause interruption during the closure and opening of the Na+ and K+ channels, respectively. Furthermore, it has been proposed that DDT inhibits the transit of Ca++, an essential component required for the regulation of neurotransmitter release. Together, these processes work to sustain the electrical signal propagation through the neuron membrane, which intensifies the release of transmitters and causes hyperexcitability, tremors, and convulsions, which are symptoms of central nervous system excitation [14].

Prolonged exposure to DDT at acute concentrations is observed to promote motor disturbances, unregulated movements, vulnerability to fear emotions, and over-excitation to external factors. Collectively, all these physiological complications lead to slight to severe body tremors, and such symptoms occur long (2–3 days) after being exposed to DDT. Ultimately, shortness of breath followed by respiratory congestion leads to the death of the individual [15].

DDT consumption causes symptoms in people when it exceeds 10 mg/kg of body weight. The first signs of DDT poisoning at greater dosages are paresthesia in the same region and on the tongue, then hyperesthesia in the mouth and lower face. Vertigo, trembling in the limbs, hyperexcitability,

disorientation, and vomiting may ensue, although convulsions are limited to cases of severe poisoning [16]. However, the possibility of long-term DDT exposure has sparked worries about a range of possible harmful health effects.

Despite the fact that DDT has been suspected to cause neurotoxic effects in humans, no conclusive data is available through human trials. Workers who sprayed DDT were shown to have cognitive impairment in two investigations, but for both studies, there were no accessible DDT or its metabolite dichlorodiphenyldichloroethylene (DDE) readings. According to some reports, the brains of seven individuals undergoing Alzheimer's disease (AD) were found to possess higher levels of DDT in comparison to the control values.

DDT-induced hepatotoxicity

DDT has been shown to be hepatotoxic and carcinogenic in animals, as evidenced by increased liver weights, hepatic hypertrophy, phenobarbital-like stimulation of microsomal enzymes, and the production of hepatocellular tumors. Numerous epidemiological studies have been carried out, and the findings suggest either favorable or unfavorable relationships between DDT exposure and the development of human tumors [21].

Despite the fact that pesticide-induced toxicity has been reported in several mammalian organs, the liver seems to be the most vulnerable one. According to epidemiological studies, exposure to DDT impacted liver fat levels and was linked to liver cancers. The incidence of human liver cancer and DDT blood concentrations were shown to be significantly correlated in many investigations.

Animal experiments revealed that prolonged exposure to DDT resulted in elevated liver weights, hepatocellular enlargement, liver fibrosis, production of unwanted enzymes, enormous oxidative load, metabolic problems, and the induction of hepatocellular tumors. Additionally, gap junctional intercellular communication was decreased by chronic DDT exposure. Acute exposure to the DDT combination may cause an increase in liver cytochrome P450 levels, lipid peroxidation, and the liver cell cycle to advance [22].

Preventive strategies

Based on a mammalian study, it was suggested that bioaccumulation level of DDT and DDE may be correlated with the quantity of dietary intake

of protein and fat. Moreover, enhanced dietary protein intake was found to decrease DDT concentration in the liver; however, DDT level was enhanced following dietary fat consumption. However, such observations are important to overcome the side effects of DDT in a natural way. Despite of the fact that lipid solubility and oestrogenic properties of DDT is suggested to be involved in lipid metabolism but no further information is available till date, thereby warranting more experimental evidences [23].

Endosulfan

Endosulfan-induced neurotoxicity

Endosulfan, also known as hexachloro-hexahydro-methano-benzodioxathiepinoxide ($C_9H_6O_3Cl_6S$), is a cyclodiene class organochlorine (OC) pesticide with a molecular weight of 407 [24]. It is an ester of cyclic sulfurous acid. Endosulfan is still one of the most often-used agricultural pesticides, especially in poorer nations, despite having potentially fatal toxic consequences. The popularity of endosulfan lies in its greater effectiveness, easy availability due to cost efficiency, and stable composition [25].

Regrettably, endosulfan has been linked to toxicity and mortality on the Indian subcontinent [26]. Absorption of endosulfan can happen through skin contact, ingestion, inhalation, or transplacental routes. Within the first six hours of exposure, histopathological studies have reported damaging effects on key organs. Among the different cyclodienes, some notable ones include chlordane, heptachlor, aldrin, dieldrin, and endosulfan.

On the other hand, lindane is defined as a gamma isomer of hexachlorohexane that may alter chloride currents by interacting with GABA receptors, which causes neuronal excitement [30]. Additionally, endosulfan-exposed mice show severely disrupted synaptic trafficking events and synaptogenesis, leading to stunted central nervous system development. These effects negatively influence behavior, memory, and learning.

Furthermore, maternal exposure to organochlorines has been associated with autism, a developmental condition influenced by both environmental and genetic factors. According to Roberts et al., infants born to mothers exposed to organochlorines (OCs) during early trimesters had a higher chance of developing autism. They demonstrated a link between endosulfan, DDT, and dicofol a substance that shares chemical similarities with DDT and prenatal exposure [33].

Endosulfan-induced hepatotoxicity

Endosulfan exposure has always been associated with the promotion of diverse histopathological damages, such as tissue deformation and necrosis in the liver [34]. According to research, fish exposed to endosulfan exhibited higher circulatory levels of different systemic markers but decreased levels of different functional proteins. It was concluded that endosulfan raises ALT and AST enzyme levels and destroys liver tissue [35].

Dieldrin was found in detectable amounts in both control and Parkinson's disease (PD) patient brains; however, PD patients' brains, caudate nucleus to be particular, exhibited higher concentrations of dieldrin when compared to the control values. The same scenario in PD patient brains was found in the case of all the other studied organochlorine compounds; thus, it may be argued that uncontrolled neuronal death may be a resultant of enhanced organochlorine accumulation in the brain [36].

3. Organophosphate insecticides

According to Costa (2018), the name "Organophosphate" (OP) describes a class of synthetic organic molecules linked to either a sulfur or an oxygen atom through a covalent bond, including pentavalent phosphorus. The initial use for OP compounds was as pesticides in the early 1900s. In the case of organophosphates, acetylcholinesterase (AChE) seems to be the prime target in exerting their toxic effects [37].

Organophosphate induced physiological complications

Researching the mechanisms underlying OPs' neurotoxic effects has gained significant attention as their usage in agriculture grows. All areas of the brain are susceptible to OPs, but the hippocampus, which is the primary area controlling memory and learning, is particularly vulnerable. OPs are absorbed by skin contact, the respiratory and digestive systems, or both. They reach the tissues and are swiftly absorbed. High-level exposure to OP poisoning is known to have neurological consequences.

On the other hand, persistent and decreased exposure to OPs may lead to the development of several types of neurotoxicity [38]. In humans, a wide range of neuronal, heart, lung, intestinal, sensory, and nerve conduction-associated diseases are also evident as outcomes of OP-induced toxicity. A few of such consequences of OPs primarily involve profuse secretion, shortness of breath, and cardiac pressure, which might be fatal depending on the degree of exposure.

On the other hand, survivors of acute OP exposure may have prolonged neurological and mental effects such as cognitive impairments, inability to process brain information and decision-making, and memory loss. These effects may involve motor dysfunction, depression, and mood disorders. Long-term OP exposure has always been found to develop neurologic and psychiatric disorders, such as poor motor function, anxiety, depression, psychotic symptoms, concentration problems, and difficulties in information processing. Collectively, all these signs and diseases have been defined as chronic OP-induced neuropsychiatric diseases (COPIND) [39].

Pathways involved in organophosphate induced toxicity

Exposure to pesticides may lead to the generation of reactive radicals, Reactive Nitrogen Species (RNS), and Reactive Oxygen Species (ROS), collectively which may affect the health of the liver in any mammal. A wealth of evidence indicates that pesticides involve the uncontrolled generation of free radicals mediated by the emergence of intracellular oxidative stress, thus exerting their toxic effect on the target cell/tissue [41-47].

As oxidants, OPs weaken the protective functions of enzymatic antioxidants, particularly superoxide dismutase, catalase, and glutathione cycle-associated enzymes. A study in the Hep G2 cell line reported enhanced membrane lipid distortion upon malathion, methylparathion, and parathion-induced oxidative stress. A similar observation was noted in the hepatic tissue of rats exposed to dimethoate, chlorpyrifos, chlorfenvinphos, and metathiathion, and in fish subjected to diazinon exposure [48-62, 41].

Many hepatic complications, such as chronic hepatitis, ischemia traumas, steatosis, age-related issues, and inflammatory distortions, are primarily caused by mitochondrial oxidative damage. Numerous investigations have demonstrated the involvement of mitochondria in hepatotoxicity induced by OPs. For instance, by impairing cellular antioxidant defenses, prolonged exposure to dichlorvos and chlorpyrifos and acute exposure to malathion might enhance the mitochondrial release of cytochrome C and thereby increase its concentration in the cytosol, which in turn triggers caspase-3.

In the liver, diazinon can decrease endoplasmic chaperones that have anti-apoptotic qualities, enhance the level of a pro-apoptotic protein disulfide isomerase, and increase the Bax/Bcl-2 ratio, thus promoting the enhancement of caspase-9 and caspase-3 proteins that eventually initiate the apoptotic pathways. A study in OP-exposed individuals documented

complete suppression of atropine metabolism along with functional inactivation of hepatic microsomal enzymes being the cause of this. In vitro, bethanin has successfully blocked lipid peroxidation and heme breakdown. It also lessens the hepatotoxicity that carbon tetrachloride causes in fish models [63].

4. Pyrethroid insecticides

The six insecticidal components of pyrethrum extract, or natural pyrethrins, are the source of a synthetic pesticide generally referred to as a "pyrethroid." Many pyrethroid structures and their applications in home, veterinary, and agricultural pest management have been made possible through extensive research conducted by government, academic, and agrochemical research institutions. Presently, the range of current natural and commercial insecticides refers to the "pyrethroids," which involve compounds that are conceptually removed from the pyrethrins [64].

Pyrethroid induced physiological complications

Pyrethroids have historically been divided into two subclasses T and CS depending upon their capability of inducing whole-body tremor and choreoathetosis with salivation, respectively, in rats when administered intravenously or orally at doses that are almost deadly.

T-syndrome can be characterized by aggression, hypersensitivity to external stimuli, and the development of short tremors that extend throughout the whole body. An increase in core body temperature was linked to tremor-related hyper muscular activity. Pawing and burrowing behaviors were the first signs of the CS syndrome. Later symptoms included excessive saliva secretion, tremors throughout the whole body that progressed to sinuous writhing known as choreoathetosis, an enhanced fright reaction, and finally clonic convulsions.

It was determined that increased salivation and wetness of the ventral body surface, which results in heat loss, was the cause of the animals with CS syndrome's decreased core body temperature. Salivation and choreoathetosis usually go hand in hand, although in a few instances, T-syndrome - a tremor accompanied by salivation was also noted [65].

Pyrethroid induced hepatotoxicity

The liver is essential to the detoxification process of pyrethroids. Because of this, pyrethroids have a propensity to accumulate and become hepatotoxic, impairing normal liver functions [66].

Primary evidence of pyrethroid-induced liver toxicity has been observed

in several investigations where alteration in liver weight has been considered a marker. Liver weight after pyrethroid exposure was found to achieve a noteworthy level. Alpha-cyano derivative-induced stress-induced lipid accumulation and rapid cell proliferation appear to be the primary causes of this rise [67, 68].

Oral administration of pyrethroids (Deltamethrin) at 1.5 and 35 mg/kg was demonstrated to cause severe toxicity in the liver and kidney tissues of rats. It was further found that Deltamethrin possesses the capability of reducing the intracellular antioxidant capacity by modulating the rate of lipid peroxidation. Additionally, variations in the activities of catalase and SOD have been calculated. Hepatotoxicity may be indicated by the increased activity of ALT and aspartate aminotransferase and the reported reduction in GSH concentration. Pyrethroid-induced hepatotoxicity is prevented by glutamine [69]. It has also been noted that spirulina has preventive properties against poisoning in rats.

Preventive strategies

The most typical symptom of pyrethroid toxicity is skin paresthesia. Treatment for this is as easy as lathering the affected area with oil or vitamin E lotion. When given topically to the skin between 29 hours prior to and 15 minutes following the pyrethroid, vitamin E proved efficacious and provided protection for almost five hours [72]. Despite the report that using vitamin E along with corn oil or Vaseline can provide significant relief, the underlying mechanism through which vitamin E exerts protection is still not understood [73, 74]. Vitamin E accumulates in high amounts in the membrane and reticulum, which might be the cause of this protection.

Neonicotinoid insecticides

Neonicotinoids (NEO) are a cornerstone of pest control, having been identified in the 1990s as a novel class of insecticides upon their discovery. Because of their systemic activity, these agonists of postsynaptic nicotinic acetylcholine receptors shield important crops (such as corn, soybean, wheat, sugar beet, grapes, and orchards) against harm caused by a remarkably wide variety of phytophagous insects [78].

Neonicotinoid induced physiological complications

NEO pesticides can have a variety of harmful consequences on the brain, and the degree of damage usually depends on the age of the individual at which it was exposed. Further studies demonstrated that exposure to NEO

pesticides during early stages of development may affect the expression of diverse essential development-associated genes responsible for neuronal development in infants. As a result, decreased neurogenesis and altered neuronal shape and distribution may result in incomplete development or brain hypoplasia.

NEO pesticides are well documented to cause cognitive impairments and motor dysfunction in mammals, and such consequences do not depend upon the age of the individual or the period of exposure. NEO-mediated changes in the levels of different neurotransmitter systems, responsible for cognitive and behavioral functions, may be the cause of these effects.

Another mechanism that is impacted by NEO pesticide exposure is neurotransmission. It is hypothesized that the cholinergic neurotransmitter system in animals would be most impacted by these pesticides since they cause neurotoxicity by acting on nAChRs. This component is essential to regulate cognitive functions, including memory, attention, and learning, and its disruption is linked to the onset of a number of neurodegenerative illnesses.

Neonicotinoid induced hepatotoxicity

Among the different target organs of NEO, liver seems to be the most susceptible one as it serves as a vital organ for metabolism and the removal of foreign pollutants in mammal. Extensive investigations in mice and rats regarding the toxic effects of NEO on the liver and its role in promoting the development of hepatocarcinoma revealed structural damage, malfunction in enzyme activity and tissue necrosis. For instance, Yang et al. discovered that oral imidacloprid treatment in male mice may result in hepatic oxidative stress, problems related to the metabolism of hepatic bile acid, abnormalities related to enterohepatic circulation, and impairment of the function of the gut barrier. Therefore, extensive epidemiological investigations are desperately required to clarify the detrimental effects of liver damage linked to internal NEO exposure [88].

Preventive strategies

A number of active ingredients, such as vitamins, non-enzymatic antioxidant (GSH), caffeic acid phenethyl ester (CAPE), curcumin, etc prevent systemic toxicity brought on by neonicotinoid-induced damage by blocking apoptosis, lipid peroxidation, and ROS signaling pathways [89].

5. Conclusion

Collectively, it appears that apart from affecting or, killing specific pests, a certain insecticide may exert detrimental side effects to other non-target species. Such incidence indicates the probable involvement of diverse signaling molecules responsible for the development of the lethal effects of any insecticide. Thus, it is high time to develop species specific insecticide to nullify its unwanted side affects on any other organism. In such regard, natural insecticides seem to be relatively safer in comparison to the artificially synthesized ones. However, carefully designed and extensive studies regarding the molecular pathways of toxicity are required to develop such insecticides.

References

1. Lushchak, V. I., Matviishyn, T. M., Husak, V. V., Storey, J. M., & Storey, K. B. (2018). Pesticide toxicity: a mechanistic approach. EXCLI Journal, 17, 1101.
2. Kumar, R., Sankhla, M. S., Kumar, R., & Sonone, S. S. (2021). Impact of pesticide toxicity in aquatic environment. Biointerface Research in Applied Chemistry, 11(3), 10131-10140.
3. Sule, R. O., Condon, L., & Gomes, A. V. (2022). A common feature of pesticides: oxidative stress—the role of oxidative stress in pesticide-induced toxicity. Oxidative Medicine and Cellular Longevity, 2022(1), 5563759.
4. Alavanja, M. C., Hoppin, J. A., & Kamel, F. (2004). Health effects of chronic pesticide exposure: cancer and neurotoxicity. Annual Review of Public Health, 25(1), 155-197.
5. Costa, L. G., Giordano, G., Guizzetti, M., & Vitalone, A. (2008). Neurotoxicity of pesticides: a brief review. Frontiers in Bioscience, 13(4), 1240-1249.
6. Spencer, P. S., & Lein, P. J. (2014). Neurotoxicity.
7. Richardson, J. R., Fitsanakis, V., Westerink, R. H., & Kanthasamy, A. G. (2019). Neurotoxicity of pesticides. Acta Neuropathologica, 138, 343-362.
8. Navarro, V. J., & Senior, J. R. (2006). Drug-related hepatotoxicity. New England Journal of Medicine, 354(7), 731-739.
9. Willett, K. L., Roth, R. A., & Walker, L. (2004). Workshop overview:

hepatotoxicity assessment for botanical dietary supplements. Toxicological Sciences, 79(1), 4-9.

10. Papay, J. I., Clines, D., Rafi, R., Yuen, N., Britt, S. D., Walsh, J. S., & Hunt, C. M. (2009). Drug-induced liver injury following positive drug rechallenge. Regulatory Toxicology and Pharmacology, 54(1), 84-90.
11. Jayaraj, R., Megha, P., & Sreedev, P. (2016). Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. Interdisciplinary Toxicology, 9(3-4), 90-100.
12. Mrema, E. J., Rubino, F. M., Brambilla, G., Moretto, A., Tsatsakis, A. M., & Colosio, C. (2013). Persistent organochlorinated pesticides and mechanisms of their toxicity. Toxicology, 307, 74-88.
13. ATSDR, T. (2002). Profile for DDT, DDE and DDD. U.S. Department of Health and Human Services, Public Health Service, 497, 17-26.
14. Harada, T., Takeda, M., Kojima, S., & Tomiyama, N. (2016). Toxicity and carcinogenicity of dichlorodiphenyltrichloroethane (DDT). Toxicological Research, 32(1), 21-33.
15. Ecobichon, D. J., & Joy, R. M. (1993). Pesticides and neurological diseases. CRC Press.
16. Ray, D. E., & Fry, J. R. (2006). A reassessment of the neurotoxicity of pyrethroid insecticides. Pharmacology & Therapeutics, 111(1), 174-193.
17. Eskenazi, B., Chevrier, J., Rosas, L. G., Anderson, H. A., Bornman, M. S., Bouwman, H., ... & Stapleton, D. (2009). The Pine River statement: human health consequences of DDT use. Environmental Health Perspectives, 117(9), 1359-1367.
18. Rogan, W. J., & Chen, A. (2005). Health risks and benefits of bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT). The Lancet, 366(9487), 763-773.
19. Misra, U. K., Nag, D., & Murti, C. K. (1984). A study of cognitive functions in DDT sprayers. Industrial Health, 22(3), 199-206.
20. de Joode, B. V. W., Wesseling, C., Kromhout, H., Monge, P., Garcia, M., & Mergler, D. (2001). Chronic nervous-system effects of long-term occupational exposure to DDT. The Lancet, 357(9261), 1014-1016.
21. Turusov, V., Rakitsky, V., & Tomatis, L. (2002). Dichlorodiphenyltrichloroethane (DDT): ubiquity, persistence, and risks. Environmental Health Perspectives, 110(2), 125-128.

22. Liu, X., & Song, L. (2022). Quercetin protects human liver cells from o, p'-DDT-induced toxicity by suppressing Nrf2 and NADPH oxidase-regulated ROS production. Food and Chemical Toxicology, 161, 112849.

23. Jaga, K., & Duvvi, H. (2001). Risk reduction for DDT toxicity and carcinogenesis through dietary modification. The Journal of the Royal Society for the Promotion of Health, 121(2), 107-113.

24. Coutselinis, A., Kentarchou, P., & Boukis, D. (1976). Separation and identification of the insecticide "Endosulfan" from biological materials. Forensic Science, 8(3), 251-254.

25. Yadla, M., Yanala, S. R., Parvithina, S., Chennu, K. K., Annapindi, N., & Vishnubhotla, S. (2013). Acute kidney injury in endosulfan poisoning. Saudi Journal of Kidney Diseases and Transplantation, 24(3), 592-593.

26. Peshin, S. S., Srivastava, A., Halder, N., & Gupta, Y. K. (2014). Pesticide poisoning trend analysis of 13 years: a retrospective study based on telephone calls at the National Poisons Information Centre, All India Institute of Medical Sciences, New Delhi. Journal of Forensic and Legal Medicine, 22, 57-61.

27. Jindal, A., & Sankhyan, N. (2012). Endosulfan poisoning resulting from skin exposure. The Indian Journal of Pediatrics, 79, 1104-1104.

28. Neild, G. H., Viswanathan, S., Jayakrishnan, K. S., & Vijan, V. (2011). Endosulfan and black urine. NDT Plus, 4(5), 353.

29. Keifer, M. C., & Firestone, J. (2007). Neurotoxicity of pesticides. Journal of Agromedicine, 12(1), 17-25.

30. Lafuente, A., & Pereiro, N. (2013). Neurotoxic effects induced by endosulfan exposure during pregnancy and lactation in female and male rat striatum. Toxicology, 311(1-2), 35-40.

31. Lee, I., Eriksson, P., Fredriksson, A., Buratovic, S., & Viberg, H. (2015). Developmental neurotoxic effects of two pesticides: Behavior and neuroprotein studies on endosulfan and cypermethrin. Toxicology, 335, 1-10.

32. Kanthasamy, A. G., Kitazawa, M., Kanthasamy, A., & Anantharam, V. (2005). Dieldrin-induced neurotoxicity: relevance to Parkinson's disease pathogenesis. Neurotoxicology, 26(4), 701-719.

33. Neild, G. H., Viswanathan, S., Jayakrishnan, K. S., & Vijan, V. (2011). Endosulfan and black urine. NDT Plus, 4(5), 353.

34. Uboh, F. E., Asuquo, E. N., & Eteng, M. U. (2011). Endosulfan-induced hepatotoxicity is route of exposure independent in rats. Toxicology and Industrial Health, 27(6), 483-488.

35. Guignet, M., & Lein, P. J. (2019). Neuroinflammation in organophosphate-induced neurotoxicity. In Advances in Neurotoxicology (Vol. 3, pp. 35-79). Academic Press.

36. Farkhondeh, T., Mehrpour, O., Forouzanfar, F., Roshanravan, B., & Samarghandian, S. (2020). Oxidative stress and mitochondrial dysfunction in organophosphate pesticide-induced neurotoxicity and its amelioration: A review. Environmental Science and Pollution Research, 27, 24799-24814.

37. Naughton, S. X., & Terry Jr, A. V. (2018). Neurotoxicity in acute and repeated organophosphate exposure. Toxicology, 408, 101-112.

38. Roberts, E. M., English, P. B., Grether, J. K., Windham, G. C., Somberg, L., & Wolff, C. (2007). Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. Environmental Health Perspectives, 115(10), 1482-1489.

39. Bandyopadhyay, U., Das, D., & Banerjee, R. K. (1999). Reactive oxygen species: Oxidative damage and pathogenesis. Current Science, 658-666.

40. Karami-Mohajeri, S., Ahmadipour, A., Rahimi, H. R., & Abdollahi, M. (2017). Adverse effects of organophosphorus pesticides on the liver: A brief summary of four decades of research. Arhiv za Higijenu Rada i Toksikologiju, 68(4), 261-275.

41. Sahin, I. Ç., Onbasi, K., Sahin, H., Karakaya, C. E. N. G. İ. Z., Ustun, Y. U. S. U. F., & Noyan, T. E. V. F. İ. K. (2002). The prevalence of pancreatitis in organophosphate poisonings. Human & Experimental Toxicology, 21(4), 175-177.

42. Muniz, J. F., McCauley, L., Scherer, J., Lasarev, M., Koshy, M., Kow, Y. W., ... & Kisby, G. E. (2008). Biomarkers of oxidative stress and DNA damage in agricultural workers: A pilot study. Toxicology and Applied Pharmacology, 227(1), 97-107.

43. Bagchi, D., Bagchi, M., Hassoun, E. A., & Stohs, S. J. (1995). In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. Toxicology, 104(1-3), 129-140.

44. Yamano, T., & Morita, S. (1992). Hepatotoxicity of trichlorfon and dichlorvos in isolated rat hepatocytes. Toxicology, 76(1), 69-77.

45. López, O., Hernández, A. F., Rodrigo, L., Gil, F., Pena, G., Serrano, J. L., ... & Pla, A. (2007). Changes in antioxidant enzymes in humans with long-term exposure to pesticides. Toxicology Letters, 171(3), 146-153.

46. Bagchi, D., Balmoori, J., Bagchi, M., Ye, X., Williams, C. B., & Stohs, S. J. (2002). Comparative effects of TCDD, endrin, naphthalene and chromium(VI) on oxidative stress and tissue damage in the liver and brain tissues of mice. Toxicology, 175(1-3), 73-82.

47. Vidyasagar, J., Karunakar, N., Reddy, M. S., Rajnarayana, K., Surender, T., & Krishna, D. R. (2004). Oxidative stress and antioxidant status in acute organophosphorous insecticide poisoning. Indian Journal of Pharmacology, 36(2), 76-79.

48. Banerjee, B. D., Seth, V., Bhattacharya, A., Pasha, S. T., & Chakraborty, A. K. (1999). Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicology Letters, 107(1-3), 33-47.

49. Akhgari, M., Abdollahi, M., Kebryaeezadeh, A., Hosseini, R., & Sabzevari, O. (2003). Biochemical evidence for free radical-induced lipid peroxidation as a mechanism for subchronic toxicity of malathion in blood and liver of rats. Human & Experimental Toxicology, 22(4), 205-211.

50. Kovacic, P. (2003). Mechanism of organophosphates (nerve gases and pesticides) and antidotes: Electron transfer and oxidative stress. Current Medicinal Chemistry, 10(24), 2705-2709.

51. Sharma, Y., Bashir, S., Irshad, M., Nag, T. C., & Dogra, T. D. (2005). Dimethoate-induced effects on antioxidant status of liver and brain of rats following subchronic exposure. Toxicology, 215(3), 173-181.

52. Nili-Ahmadabadi, A., Tavakoli, F., Hasanzadeh, G. R., Rahimi, H. R., & Sabzevari, O. (2011). Protective effect of pretreatment with thymoquinone against Aflatoxin B1-induced liver toxicity in mice.

Daru: Journal of Faculty of Pharmacy, Tehran University of Medical Sciences, 19(4), 282.

53. Rahimi, H. R., Agin, K., Shadnia, S., Hassanian-Moghaddam, H., & Oghazian, M. B. (2015). Clinical and biochemical analysis of acute paint thinner intoxication in adults: A retrospective descriptive study. Toxicology Mechanisms and Methods, 25(1), 42-47.

54. Ma, J., Liu, Y., Niu, D., & Li, X. (2015). Effects of chlorpyrifos on the transcription of CYP3A cDNA, activity of acetylcholinesterase, and oxidative stress response of goldfish (Carassius auratus). Environmental Toxicology, 30(4), 422-429.

55. Verma, R. S., & Srivastava, N. (2003). Effect of chlorpyrifos on thiobarbituric acid reactive substances, scavenging enzymes and glutathione in rat tissues.

56. Messarah, M., Amamra, W., Boumendjel, A., Barkat, L., Bouasla, I., Abdennour, C., ... & Feki, A. E. (2013). Ameliorating effects of curcumin and vitamin E on diazinon-induced oxidative damage in rat liver and erythrocytes. Toxicology and Industrial Health, 29(1), 77-88.

57. Üner, N., Sevgiler, Y., Durmaz, H., & Piner, P. (2007). In vivo alterations in glutathione-related processes, lipid peroxidation, and cholinesterase enzyme activities in the liver of diazinon-exposed Oreochromis niloticus. Toxicology Mechanisms and Methods, 17(6), 317-324.

58. Oruc, E. (2011). Effects of diazinon on antioxidant defense system and lipid peroxidation in the liver of Cyprinus carpio (L.). Environmental Toxicology, 26(6), 571-578.

59. Ahmadian, E., Khosroushahi, A. Y., Eghbal, M. A., & Eftekhari, A. (2018). Betanin reduces organophosphate-induced cytotoxicity in primary hepatocyte via an anti-oxidative and mitochondrial-dependent pathway. Pesticide Biochemistry and Physiology, 144, 71-78.

60. Soderlund, D. M., Clark, J. M., Sheets, L. P., Mullin, L. S., Piccirillo, V. J., Sargent, D., ... & Weiner, M. L. (2002). Mechanisms of pyrethroid neurotoxicity: Implications for cumulative risk assessment. Toxicology, 171(1), 3-59.

61. Soderlund, D. M. (2012). Molecular mechanisms of pyrethroid insecticide neurotoxicity: Recent advances. Archives of Toxicology, 86, 165-181.

62. Bhushan, B., & Saxena, P. N. (2017). Hepatotoxicity under stress of type II pyrethroids in mammals: A mechanistic approach. International Journal of Toxicology, 9, 194-203.

63. Singh, V. K., Dixit, P., & Saxena, P. N. (2005). Cybil-induced hepato-biochemical changes in Wistar rats. Journal of Environmental Biology, 26(4), 725-727.

64. Bhushan, B., Saxena, P. N., & Saxena, N. (2013). Biochemical and histological changes in rat liver caused by cypermethrin and beta-cyfluthrin. Archives of Industrial Hygiene and Toxicology, 64(1), 57-67.

65. Gündüz, E., Ülger, B. V., İbiloğlu, İ., Ekinci, A., Dursun, R., Zengin, Y., ... & Güloğlu, C. (2015). Glutamine provides effective protection against deltamethrin-induced acute hepatotoxicity in rats but not against nephrotoxicity. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research, 21, 1107.

66. Abdel-Daim, M. M., Abuzead, S. M., & Halawa, S. M. (2013). Protective role of Spirulina platensis against acute deltamethrin-induced toxicity in rats. PLOS One, 8(9), e72991.

67. Chrustek, A., Hołyńska-Iwan, I., Dziembowska, I., Bogusiewicz, J., Wróblewski, M., Cwynar, A., & Olszewska-Słonina, D. (2018). Current research on the safety of pyrethroids used as insecticides. Medicina, 54(4), 61.

68. Malley, L. A., Cagen, S. Z., Parker, C. M., Gardiner, T. H., Van Gelder, G. A., & Rose, G. P. (1985). Effect of vitamin E and other amelioratory agents on the fenvalerate-mediated skin sensation. Toxicology Letters, 29(1), 51-58.

69. Ray, D. E., & Fry, J. R. (2006). A reassessment of the neurotoxicity of pyrethroid insecticides. Pharmacology & Therapeutics, 111(1), 174-193.

70. Tucker, S. B., Flannigan, S. A., & Smolensky, M. H. (1983). Comparison of therapeutic agents for synthetic pyrethroid exposure. Contact Dermatitis, 9(4).

71. Kagan, V. E., Bakalova, R. A., Serbinova, E. E., & Stoytchev, T. S. (1990). Fluorescence measurements of incorporation and hydrolysis of tocopherol and tocopheryl esters in biomembranes. In Methods in Enzymology (Vol. 186, pp. 355-367). Academic Press.

72. Machlin, L. J. (1991). Vitamin E. In Handbook of Vitamins (L. J. Machlin, ed.).

73. Aldana, L., Tsutsumi, V., Craigmill, A., Silveira, M. I., & De Mejia, E. G. (2001). α-Tocopherol modulates liver toxicity of the pyrethroid cypermethrin. Toxicology Letters, 125(1-3), 107-116.

74. Ali, Z. Y. (2012). Neurotoxic effect of lambda-cyhalothrin, a synthetic pyrethroid pesticide: Involvement of oxidative stress and protective role of antioxidant mixture. New York Science Journal, 5, 93-103.

75. Romero, A., Ares, I., Ramos, E., Castellano, V., Martínez, M., Martínez-Larrañaga, M. R., ... & Martínez, M. A. (2015). Evidence for dose-additive effects of a type II pyrethroid mixture. Environmental Research, 138, 58-66.

76. Karthi, S., & Shivakumar, M. S. (2015). The protective effect of melatonin against cypermethrin-induced oxidative stress damage in Spodoptera litura (Lepidoptera: Noctuidae). Biological Rhythm Research, 46(1), 1-12.

77. Lin, Y. S., Chen, W. Y., & Liang, W. Z. (2022). Investigation of cytotoxicity and oxidative stress induced by the pyrethroid bioallethrin in human glioblastoma cells: The protective effect of vitamin E and its underlying mechanism. Chemical Research in Toxicology, 35(5), 880-889.

78. Jactel, H., Verheggen, F., Thiéry, D., Escobar-Gutiérrez, A. J., Gachet, E., Desneux, N., & Neonicotinoids Working Group. (2019). Alternatives to neonicotinoids. Environment International, 129, 423-429.

79. Christen, V., Rusconi, M., Crettaz, P., & Fent, K. (2017). Developmental neurotoxicity of different pesticides in PC-12 cells in vitro. Toxicology and Applied Pharmacology, 325, 25-36.

80. Kimura-Kuroda, J., Nishito, Y., Yanagisawa, H., Kuroda, Y., Komuta, Y., Kawano, H., & Hayashi, M. (2016). Neonicotinoid insecticides alter the gene expression profile of neuron-enriched cultures from neonatal rat cerebellum. International Journal of Environmental Research and Public Health, 13(10), 987.

81. Kagawa, N., & Nagao, T. (2018). Neurodevelopmental toxicity in the mouse neocortex following prenatal exposure to acetamiprid. Journal of Applied Toxicology, 38(12), 1521-1528.

82. Nakayama, A., Yoshida, M., Kagawa, N., & Nagao, T. (2019). The neonicotinoids acetamiprid and imidacloprid impair neurogenesis and alter the microglial profile in the hippocampal dentate gyrus of mouse neonates. Journal of Applied Toxicology, 39(6), 877-887.

83. Burke, A. P., Niibori, Y., Terayama, H., Ito, M., Pidgeon, C., Arsenault, J., ... & Hampson, D. R. (2018). Mammalian susceptibility to a neonicotinoid insecticide after fetal and early postnatal exposure. Scientific Reports, 8(1), 16639.

84. Tanaka, T. (2012). Reproductive and neurobehavioral effects of clothianidin administered to mice in the diet. Birth Defects Research Part B: Developmental and Reproductive Toxicology, 95(2), 151-159.

85. Tanaka, T. (2012). Effects of maternal clothianidin exposure on behavioral development in F1 generation mice. Toxicology and Industrial Health, 28(8), 697-707.

86. Hampel, H., et al. (2018). The cholinergic system in Alzheimer's disease. Brain, 141(7), 1917-1933.

87. Salim, S. (2017). Oxidative stress and the central nervous system. Journal of Pharmacology and Experimental Therapeutics, 360(1), 201-205.

88. Chen, Y., Yu, W., Zhang, L., Cao, L., Ling, J., Liao, K., ... & Jin, H. (2023). First evidence of neonicotinoid insecticides in human bile and associated hepatotoxicity risk. Journal of Hazardous Materials, 446, 130715.

89. Xu, X., Wang, X., Yang, Y., Ares, I., Martínez, M., Lopez-Torres, B., ... & Martinez, M. A. (2022). Neonicotinoids: Mechanisms of systemic toxicity based on oxidative stress-mitochondrial damage. Archives of Toxicology, 96(6), 1493-1520.

Chapter - 4

Extracts of Pomegranate - Role in Fighting Cancer

Authors

Soham Banik
UG Student, Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Archita Dhara
UG Student, Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Arpita Das
UG Student, Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Tiyasa Manna
UG Student, Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Prakriti Patra
UG Student, Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Aditya Das
UG Student, Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Ananyo Mazumdar
UG Student, Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Pritha Pal
Assistant Professor, Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

Chapter - 4

Extracts of Pomegranate - Role in Fighting Cancer

Soham Banik, Archita Dhara, Arpita Das, Tiyasa Manna, Prakriti Patra, Aditya Das, Ananyo Mazumdar and Pritha Pal

Abstract

"Pomegranate" explains the fruit of the deciduous tree which is Punica granatum, which belongs to the Lythraceae family of trees. Numerous accounts state that it started in Iran and moved throughout the ancient Mediterranean region and Northern India. It is now currently grown for fruit in North and Tropical Africa, North America, South America, and also Europe, and as decorative trees and bushes. Several researchers have been interested in the pomegranate fruit because of its distinct chemical makeup, high in flavonoids and antioxidant tannins. The two main components of pomegranate peel, which are punicalagin and ellagic acid, are found to have potent anti-proliferative properties for inhibiting cancer cell carcinogenesis. In many animal models, the potential cancer therapeutic effects of polyphenol-rich fractions extracted from pomegranate fruits have been investigated. Pomegranate leaves consist of glycosides of apigenin, which is a flavone having anxiolytic and progestinic properties, as well as some special tannins. It was also demonstrated that pomegranate fruit, seed, and peel extracts specifically suppress the growth of cancer cells found in the prostate and lungs, with no side effects on healthy cells. In recent years, research has also shown that pomegranate extracts partially suppress the growth of cancer cells associated with the breast, prostate, colon, and lung. This review mainly focuses on the aspects of different parts of pomegranate contributing to anti-tumor properties.

Keywords: flavonoids, punicalagin, ellagic acid, carcinogenesis, glycosides, prostate, lung.

Introduction

Cancer is the disease caused by unrestricted proliferation of cells in our body. Even though it is generally believed that cancer has a hereditary origin, studies in the last few years have described that a multitude of

environmental and epigenetic factors play a crucial role in the occurrence and spreading of the disorder. Due to its unknown cause and some of the risk factors, including use of tobacco, intake of alcohol, improper diet, obesity, and smoking, cancer is globally called a lifestyle illness [Khan N. et al, 2010].

The pomegranate, or Punica granatum, is a part of the deciduous family of trees which is Lythraceae. According to many stories, it was initially planted in Iran in modern times and then subsequently spread over the Mediterranean region and northern India. It is now grown for fruit and also as ornamental trees and bushes across North America, South America, North and Tropical Africa, and also Europe. Pomegranate is a berry that is spherical, consisting of 200–1400 white to deep red or purple seeds covered in a thick reddish peel.

Ellagic acid and punicalagin are the main two components of pomegranate peel [Syed et al, 2013]. They contain a high concentration of hydrolysable tannins and anthocyanins. As a result, pomegranate seeds are edible and have potent anti-inflammatory and antioxidant qualities [Sharma et al, 2017]. Anthocyanins from pomegranate showed comparatively higher antioxidant activity compared to the known antioxidants in red wine and green tea [Sharma et al, 2017].

In many medical systems, pomegranate has been used as a treatment or cure for a variety of ailments. Pomegranates were considered a full pharmacy in their own right in ancient Indian Ayurvedic medicine. In addition to its antiparasitic properties, it was suggested that it be used to treat ulcers and diarrhea. Another traditional medical system, the Unani medical system, states that pomegranates are beneficial in the treatment of diabetes.

The numerous research papers on pomegranate's health benefits that have been released in recent years show how much interest the scientific community has today for pomegranate's potential as a remedy. The pomegranate tree's leaves, bark, and roots are among the other plant parts that are abundant in chemical components with medicinal qualities in addition to the fruit itself.

Chemical constituents of pomegranate fruit

The seeds make up ~3% of the fruit weight of pomegranate and its juice contribute about 30% while the peel contains the internal network of membranes. The seed oil, its major isomer contains conjugated linolenic acid and punicic acid. Other components are sterols, steroids, and cerebrosides

[Kaufman M & Wiesman Z, 2007]. The antioxidant activity of the pomegranate juice is greater than certain well-recognized antioxidants of red wine and green tea, and the reason is its polyphenolic content [Gil MI et al, 2000]. White to deep purple seeds inserted into a white spongy membrane, covered by a thick reddish skin, make up the pomegranate fruit and is enriched with bioactive constituents which include phenolics, proanthocyanidin compounds, flavonoids, and ellagitannins [Sharma et al, 2017].

It also consists of minerals, non-metals like nitrogen and phosphorus, and certain metals such as potassium, calcium, magnesium, sodium, and complex polysaccharides [Lansky E.P & Newman R.A, 2006].

The peel of mesocarp is made up of a variety of compounds, including ellagitannins, gallagyl esters, hydroxybenzoic acids, hydroxycinnamic acids, anthocyanins, gallotannins, and dihydroflavonol. Dihydrokaempferol-hexoside, brevifolin carboxylic acid, valoneic acid bilactone, cyanidin–pentoside–hexoside, and vanillic acid 4-glucoside are the only ones that have been previously described.

Pomegranates are primarily composed of phenols known as ellagitannins. The content of punicalagin ranges from 4 to 565 mg/L in the juice and from 11 to 20 g/kg in the peel and mesocarp [Syed et al, 2013]. Due to their high concentration of hydrolysable tannins (pedunculagin, punicalin, punicalagin, gallagic acid, ellagic acid, and also glucose esters) and anthocyanins (cyanidin-3,5-diglucoside, pelargonidin-3,5-diglucoside, delphinidin-3-glucoside, delphinidin-3,5-diglucoside, pelargonidin-3-glucoside, cyanidin-3-glucoside) [Sharma et al, 2017], numerous researchers have exhibited interest in the pomegranate fruit because of its distinctive chemical makeup, which is high in flavonoids and antioxidant tannins.

Pomegranate seeds exhibit potent antioxidant and anti-inflammatory qualities. Thus, pomegranate extracts have shown to specifically stop the growth and development of lung, prostate, breast, and colon cancer cells while showing no such damage to healthy working cells [Sharma et al, 2017].

Role of pomegranate extracts in prostate cancer prevention:

The second leading cause of cancer-related mortality among men in the United States is prostate cancer (PCa). As of the most recent data, there were 180,890 new PCa diagnoses and 26,120 estimated mortality cases in the United States [Siegel R.L. et al, 2016].

The effects of pomegranate on prostate cancer (PCa) have been investigated and documented in animal models, cell culture systems, and in a human phase II clinical trial, both in vitro and in vivo. Studies using in vivo and in vitro experiments were conducted to examine the effects of several pomegranate preparations, including oils, pericarp polyphenols, and fermented juice polyphenols, on the proliferation of human PCa cells [Adhami et al, 2009]. Every preparation was demonstrated to inhibit the growth of human PCa LNCaP, PC-3, and DU 145 cells, but normal prostate epithelial cells responded far less strongly.

Both the triggering of apoptosis and changes in the distribution of the cell cycle were responsible for these effects. According to reports, pomegranate fruit's peel and juice contain significant amounts of ellagic acid, caffeic acid, luteolin, and punicic acid, which lower the invasive potential of PC-3 cells. When caffeic acid, luteolin, and punicic acid were combined in equal amounts and administered at the same gross dose, the results showed an unparalleled suppression of PC-3 cell invasion.

Fermented PJ polyphenols, PSO, and pomegranate pericarp polyphenols were used to treat human PCa cells. This resulted in decreased cell proliferation, an increase in G2/M phase cells, and the induction of apoptosis [Sharma et al, 2017]. The regulation of CDK serves as the main mechanism for PFE's pro-apoptotic and anti-proliferative effects. When PFE was used to treat PC-3 cells, cell growth was suppressed, and apoptosis occurred.

The study found that pomegranate extracts essentially downregulated cyclins D1, D2, and E, as well as cdk2, cdk4, and cdk6, while increasing the regulation of p21 and p27, inducing apoptosis in PC-3 cells. This was followed by an increase in cleaved PARP, a decrease in Bcl-2, and an overexpression of Bax.

Additionally, athymic nude mice implanted with CWR22Rv1 cells showed a sufficient suppression in tumor growth upon oral administration of PFE in drinking water, which included a reduction in serum PSA output [Malik A. et al, 2005].

Following this, ellagic acid, a substance found in pomegranate juice, was added to LNCaP cells. This resulted in the cleaving of the caspase-3 enzyme, and the Bax/Bcl-2 ratio seemed to increase. Ellagic acid treatment resulted in a downregulation in the expression of cyclin D1 and cdk1, while the expression of p21 and p27 showed an upregulation.

According to these findings, ellagic acid can be used as a chemotherapeutic drug to treat PCa [Naiki-Ito A. et al, 2015].

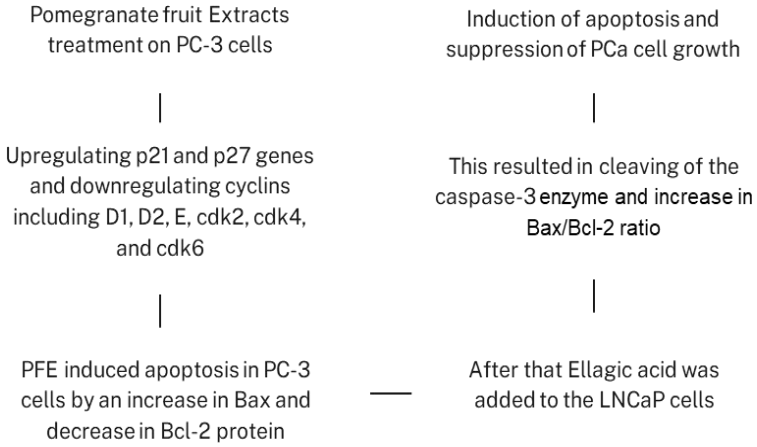


Fig 1: Mechanism of Inhibition of PCa Cells: An experiment carried out in a thymicnude mice

Role of pomegranate extracts in lung cancer prevention

In studies as of 2016, the leading cause of cancer-related mortality worldwide is the Lung Cancer. Lung and bronchus cancers in both sexes are predicted to produce 224,390 new cases and 158,080 fatalities in the United States in the year 2016, according to statistics [Siegel R.L. et al, 2016]. Recent studies have shown that Pomegranate fruit extracts can be an appropriate measure to stop lung cancer cells from growing in culture. Human lung cancer A549 cells viability was remarkably reduced by PFE treatment, although normal human bronchial epithelial cells were barely affected. A549 cells, when treated with PFE, underwent a dose-dependent pause in the G0/G1 phase of the cell cycle, followed by a downregulation in the expression of downstream cell cycle regulatory proteins and an upregulation in WAF1/p21 and KIP1/p27. PFE treatment also showed suppression of different signaling pathways, including the MAPK PI3K)/Akt, and NF-κB pathway [Khan N. et al, 2007]. Punicalagin, which is extracted from the pomegranate husk, was found to have potent anti-proliferative properties against lung cancer cells and significant antioxidant properties by inhibiting the buildup of oxidative DNA products. Both cancer cell lines i.e. A549 and H1299 showed similar response to the tested compounds. Basically, in a Benzopyrene-induced lung cancer model, it was demonstrated that punicalagin and ellagic acid expressed potent anti-

mutagenic and anti-proliferative properties [Zahin M. et al, 2014]. Pomegranate peel's anti-proliferative characteristics against lung cancer, were assessed in a recent study. The investigation's findings demonstrated that pomegranates possess anti-proliferative properties outside of their edible fruit. It was also discoveredthat pomegranate leaf extract (PLE) suppressedthe growthofmice Lewislung carcinoma cell line LL/2 as well as non-small cell lung cancer cell lines (A549, H1299). H1299 cell migration and invasionwerefoundtobereducedafter PLE treatment, indicating that PLE isuse fulin reducing metastasis [Li Y. et al, 2016].

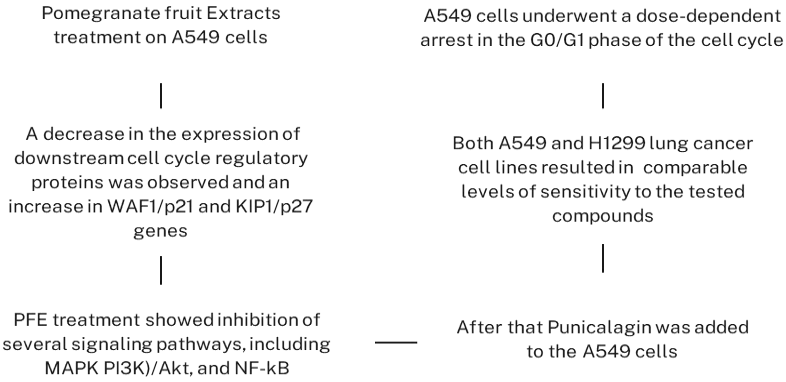


Fig 2: Mechanism of inhibition of A549 Cells: An experiment carried out in B(a)P induced lung cancer model

Role of pomegranate extracts in colon cancer prevention

As of 2016, the third leading cause of cancer-related deaths for both men and women in the United States is the Colorectal Cancer. It was estimated that around 134,490 new cases of colorectal cancer were identified in the US in 2016 [Siegel R.L. et al, 2016]. Ellagitannin and urolithin, present in pomegranate inhibits the growth of colon cancer cells. This occurs by disrupting the cell cycle and blocking the MAPK pathway [Zhao Y. et al, 2014]. Starting in 2000, researchers conducted in vitro studies using human colon cancer cell lines to investigate the notable anticancer properties of Ellagic acid against colorectal cancer. Ellagic acid led to the suppression of growth in colon cancer SW480 cells by causing DNA damage, which inturn activated p53 and p21 while reducing the levels of insulin-like growth factor-II(IGF-II) [Narayanan B.A. et al., 2001]. The apoptosis triggered by Ellagic acid demonstrated a decrease in adenosinetriphosphate (ATP) production. The colorectal cancer Caco-2 cell line was used to explore the apoptosis induction by Ellagic acid. The exposure of these cells to the

polyphenolic compound resulted in a poptos is by decreasing Bcl-XL levels, releasing cytochromec from the mitochondria, activating initiator caspase-9, and subsequently leading to the activation of effector caspase-3 [Larrosa M. et al, 2006]. Some research has indicated that isolated individual polyphenols from pomegranate juice may diminish the overall anti- proliferative effects, highlighting the necessity of various compounds for achieving chemical synergy and significant outcomes in contrast to singular purified agents. HT-29 cancer cells were treated with pomegranate juice and was found to inhibit TNFα-induced COX-2 protein expression, as well as reducing AKT activity and NFκB DNA binding [Adams L.S. et al, 2006]. These investigations emphasize the significance of pomegranate juice that reduced inflammatory signaling pathways in colon cancer cells [Sharma et al, 2017].

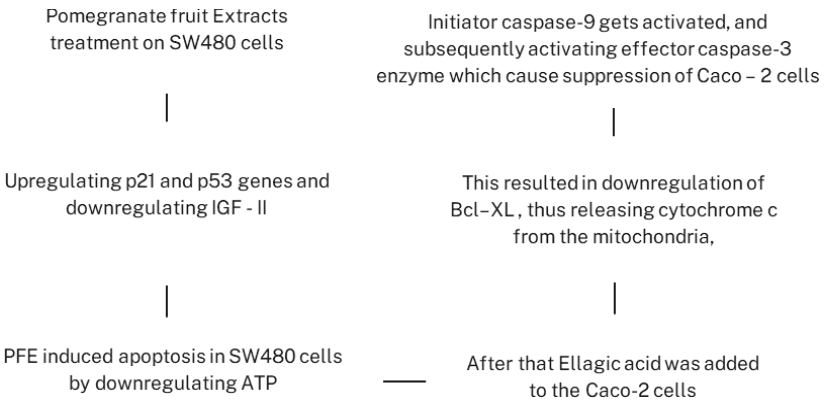


Fig 3: Mechanism of inhibition of Caco-2 cells: Observations of different *in vitro* experiments carried out

Conclusion

Pomegranate fruit extracts are recognized as potential chemopreventive or chemotherapeutic agents due to their ability to modify signal transduction pathways, leading to effects such as anti-proliferative, antitumorigenic, and anti-inflammatory outcomes [Schust J. et al., 2006]. Recently, the use of dietary agents for cancer prevention has emerged as a promising area in oncology, attracting considerable interest from both researchers and clinical professionals. Plant extracts have demonstrated effective cancer suppression despite being cost-effective and readily accessible [Sharma et al, 2017]. Pomegranate juice has demonstrated noteworthy outcomes in a phase II human clinical trial for prostate cancer; however, similar clinical investigations are needed for other types of cancers, including colon cancer, breast cancer, etc. [Adhami et al., 2010]. Furthermore, two key components

of pomegranate, punicalagin and ellagic acid, have been shown to be effective in preventing various cancers by interrupting the repetitive cell cycle of cancer cells and promoting apoptosis in those cells.

Conflict of interest

There is no conflict of interest related to the study.

Acknowledgement

I would like to express my special thanks of gratitude to our honorable Chairman Sir, V.C. Sir, COO Sir, Director Sir of School of Life Sciences and other higher authorities of Swami Vivekananda University. I am also thankful to our respected HOD of the Department of Microbiology and Biotechnology, School of Life Sciences, Swami Vivekananda University for giving me such wonderful opportunity to carry out this study.

References

1. Adams, L.S., Seeram, N.P., Aggarwal, B.B., Takada, Y., Sand, D. & Heber, D. (2006). Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells. *J Agric Food Chem*, 54(3):980-5. doi: 10.1021/jf052005r. PMID: 16448212.
2. Adams, L.S., Zhang, Y., Seeram, N.P., Heber, D. & Chen, S. (2010). Pomegranate ellagitannin-derived compounds exhibit antiproliferative and antiaromatase activity in breast cancer cells *in vitro*. *Cancer Prev Res (Phila)*, 3(1):108-13. doi: 10.1158/1940-6207.CAPR-08-0225. PMID: 20051378; PMCID: PMC2805471.
3. Adhami, V.M., Khan, N. & Mukhtar, H. (2009). Cancer chemoprevention by pomegranate: laboratory and clinical evidence. *Nutr Cancer*, 61(6):811-5. doi:10.1080/01635580903285064.PMID:20155621; PMCID: PMC2989797.
4. Ceci, C., Lacal, P.M., Tentori, L., De Martino, M.G., Miano, R. & Graziani, G. (2018). Experimental Evidence of the Antitumor, Antimetastatic and Antiangiogenic Activity of Ellagic Acid. *Nutrients*, 10(11):1756. doi: 10.3390/nu10111756. PMID: 30441769; PMCID: PMC6266224.
5. Dai, Z., Nair, V., Khan, M. & Ciolino, H.P. (2010). Pomegranate extract inhibits the proliferation and viability of MMTV-Wnt-1 mouse

mammary cancer stem cells in vitro. *Oncol Rep*, 24(4):1087-91. PMID: 20811693.

6. Kaufman, M. & Wiesman, Z. (2007). Pomegranate oil analysis with emphasis on MALDI-TOF/MS triacylglycerol fingerprinting. *J Agric Food Chem*, 55(25):10405-13. doi: 10.1021/jf072741q. Epub 2007 Nov 16. PMID: 18004807.
7. Khan, N, Afaq, F. & Mukhtar, H. (2010). Lifestyle as risk factor for cancer: Evidence from human studies. *Cancer Lett*, 293(2):133-43. doi: 10.1016/j.canlet.2009.12.013. Epub 2010 Jan 18. PMID: 20080335; PMCID: PMC2991099.
8. Khan, N., Hadi, N., Afaq, F., Syed, D.N., Kweon, M.H. & Mukhtar, H. (2007). Pomegranate fruit extract inhibits prosurvival pathways in human A549 lung carcinoma cells and tumor growth in athymic nude mice. *Carcinogenesis*, 28(1):163-73. doi:10.1093/carcin/bgl145.Epub2006Aug18.PMID:16920736.
9. Lansky, E.P. & Newman, R.A. (2007). *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *J Ethnopharmacol*, 109(2):177-206. doi: 10.1016/j.jep.2006.09.006. Epub 2006 Sep 10. PMID: 17157465.
10. Larrosa, M., Tomás-Barberán, F.A. & Espín, J.C. (2006). The dietary hydrolysable tannin punicalagin releases ellagic acid that induces apoptosis in human colon adenocarcinoma Caco-2 cells by using the mitochondrial pathway. *J Nutr Biochem*,17(9):611-25. doi:10.1016/j.jnutbio.2005.09.004.Epub2005 Oct 11. PMID: 16426830.
11. Li, Y., Yang, F., Zheng, W., Hu, M., Wang, J., Ma, S., Deng, Y., Luo, Y., Ye, T. & Yin, W. (2016). *Punica granatum* (pomegranate) leaves extract induces apoptosis through mitochondrial intrinsic pathway and inhibits migration and invasion in non-small cell lung cancer in vitro. *Biomed Pharmacother*, 80:227-235. doi: 10.1016/j.biopha.2016.03.023. Epub 2016 Mar 28. PMID: 27133061.
12. Malik, A., Afaq, F., Sarfaraz, S., Adhami, V.M., Syed, D.N. & Mukhtar, H. (2005). Pomegranate fruit juice for chemoprevention and chemotherapy of prostate cancer. *Proc Natl Acad Sci U S A*, 102(41):14813-8. doi: 10.1073/pnas.0505870102. Epub 2005 Sep 28. PMID: 16192356; PMCID: PMC1253570.
13. Naiki-Ito, A., Chewonarin, T., Tang, M., Pitchakarn, P., Kuno, T.,

Ogawa, K., Asamoto, M., Shirai, T. & Takahashi, S. (2015). Ellagic acid, a component of pomegranate fruit juice, suppresses androgen-dependent prostate carcinogenesis via induction of apoptosis. *Prostate*. 75(2):151-60. doi: 10.1002/pros.22900. Epub 2014 Oct 4. PMID: 25284475.

14. Narayanan, B.A. & Re, G.G. (2001). IGF-II downregulation associated cell cycle arrest in colon cancer cells exposed to phenolic antioxidant ellagic acid. *Anticancer Res*, 21(1A):359-64. PMID: 11299762.

15. Rahman, M.M., Islam, M.R., Akash, S., Hossain, M.E., Tumpa, A.A., Abrar Ishtiaque, G.M., Ahmed, L., Rauf, A., Khalil, A.A., Al Abdulmonem, W. & Simal-Gandara, J. (2023). Pomegranate-specific natural compounds as onco-preventive and onco-therapeutic compounds: Comparison with conventional drugs acting on the same molecular mechanisms. *Heliyon*, 9(7):e18090. doi: 10.1016/j.heliyon.2023.e18090.PMID:37519687;PMCID:PMC10372646.Schust,J.,Sperl,B.,Hollis,

16. A., Mayer, T.U. & Berg, T. (2006). Stattic: a small-molecule inhibitor of STAT3 activation and dimerization. *Chem Biol*, 13(11):1235-42. doi: 10.1016/j.chembiol.2006.09.018. PMID: 17114005.

17. Sharma, P., Mc Clees, S.F. & Afaq, F. (2017). Pomegranate for Prevention and Treatment of Cancer: An Update. *Molecules*, 22(1):177. doi: 10.3390/molecules22010177. PMID: 28125044; PMCID: PMC5560105.

18. Siegel, R.L., Miller, K.D. & Jemal, A. (2016). Cancer statistics. *CA Cancer J Clin*, 66(1):7-30. doi: 10.3322/caac.21332. Epub 2016 Jan 7. PMID: 26742998.

19. Syed, D.N., Chamcheu, J.C., Adhami, V.M. & Mukhtar, H. (2013). Pomegranate extracts and cancer prevention: molecular and cellular activities. *Anticancer Agents Med Chem*, 13(8):1149-61. doi: 10.2174/18715206113130800003. PMID: 23094914; PMCID: PMC4052369.

20. Torre, L.A., Bray, F., Siegel, R.L, Ferlay, J., Lortet-Tieulent, J. & Jemal, A. (2012). Global cancer statistics. *CA Cancer J Clin*, 65(2):87-108. doi: 10.3322/caac.21262. Epub 2015 Feb 4. PMID: 25651787.

21. Zahin, M., Ahmad, I., Gupta, R.C. & Aqil, F. (2014). Punicalagin and ellagic acid demonstrate antimutagenic activity and inhibition of

benzo[a]pyrene induced DNA adducts. *Biomed Res Int*, 2014:467465. doi: 10.1155/2014/467465. Epub 2014 May 14. PMID: 24949451; PMCID: PMC4052943.

22. Zhao, Y., Miao, G., Li, Y., Isaji, T., Gu, J., Li, J. & Qi, R. (2014). Micro RNA-130b suppresses migration and invasion of colorectal cancer cells through down regulation of integrin β1. *PLoS One*, 9(2):e87938. doi: 10.1371/journal.pone.0087938. PMID: 24498407; PMCID: PMC3912181.

Chapter - 5

Advancements in Gene Therapy for Leukemia: From Bench to Bedside: A Review

Authors

Tanya Das
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Sovan Patra
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Mayisha Sultana
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Tuhin Mukherjee
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Souradeep Banerjee
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Priyankar Pal
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Kolkata, West Bengal, India

Chapter - 4

Advancements in Gene Therapy for Leukemia: From Bench to Bedside: A Review

Tanya Das, Sovan Patra, Mayisha Sultana, Tuhin Mukherjee, Souradeep Banerjee and Priyankar Pal

Abstract

A potential treatment for hereditary hematopoietic diseases is gene therapy. Gene therapy is basically a method that modifies a person's cell genetic material to treat or prevent disease.

Although gene therapy has numerous advantages, there is a danger of insertional oncogenesis. This can be reduced by adding a suicide gene, such as thymidine kinase from the Herpes simplex virus, to the therapeutic vector. Leukemia is a form of cancer that affects the bone marrow and blood, mainly in infants and adults. It is mainly characterized by the uncontrollably high over production of white blood cells. There are mainly six types of leukemia, but acute lymphoblastic leukemia and chronic myeloid leukemia are the most common ones that can be treated with gene therapy due to their greater likelihood of recovery.

Viral or non-viral vectors are used to transfer a functioning gene into the patient's cells. The inability of these vectors to transduce nondividing cells is a serious drawback. Utilizing innovative retroviral vectors produced from lentiviruses, like the Human Immunodeficiency Virus (HIV), may be able to solve this issue.

Keywords: Lymphoblastic leukemia, gene therapy, lentiviruses, therapeutic effect, retroviral vectors.

Introduction

Leukemia, a terrible blood carcinoma, claims hundreds of lives annually. It is a type of cancer that affects the blood and bone marrow.

June, C.H., & Sadelain, M. (2018). Many types of leukemia exist. Specific types of leukemia are prevalent in children, while other forms

primarily affect adult blood-forming tissues in the body, such as bone marrow and the lymphatic system.

White blood cells, which are massive defenders against infections, typically divide and proliferate in a coordinated manner in response to the body's demands. However, in persons with leukemia, the bone marrow creates an inordinate amount of white blood cells.

Levine, B.L., Miskin, J., Wonnacott, K., & Keir, C. (2017). Despite advances in chemotherapy and radiation therapy, treatment outcomes remain unsatisfactory, especially for high-risk patients (Maude et al., 2014). Gene therapy can target specific cancer cells without harming healthy tissues, which makes it an intriguing strategy

Grupp et al. (2013). There are mainly four types of leukemia: Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML), Chronic Lymphocytic Leukemia (CLL), and Chronic Myeloid Leukemia (CML).

Davila et al. (2014). The entire 3 billion nucleotides of the human genome have been decoded, which was considered an unimaginable task a few years ago. The estimated number of genes ranges between 35,000 and more than 100,000.

The science of gene therapy is based on the insertion of genes to correct a deficiency or halt the progression of disease, consequently improving the quality of life. Therefore, genes are required. Instantaneously, we have tens of thousands on hand. Although gene therapy holds considerable potential for achieving this goal, transferring genetic information into higher organisms remains a massive technical problem.

Leukemia is treated using several kinds of gene therapy techniques, including gene editing therapies such as:

1. **CRISPR-Cas9** – mostly corrects genetic mutations in leukemia cells.
2. **TALEN** – edits certain genes.
3. **ZFN** – pertains to gene sequences.

Gene therapy strategies for leukemia

Gene editing

Genetics and medicine have changed drastically because of the revolutionary technology known as gene editing (Jinek et al., 2012). Gene editing has made it possible to precisely alter the DNA sequence, creating

previously unheard-of possibilities for the treatment of genetic illnesses, increased crop yields, and the advancement of synthetic biology (Doudna & Charpentier, 2012; Sternberg et al., 2014).

A major turning point in the history of gene editing was reached in 2012 with the discovery of the CRISPR-Cas9 system, which provided an effective, adaptable, and scalable gene-editing tool. CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated protein 9) is a bacterial defense system that enables accurate editing of DNA sequences. It is modeled after a naturally occurring bacterial system in which the enzyme Cas9 and CRISPR RNA (crRNA) cooperate to protect against viral infections (Dunbar et al., Science 359, 175, 2018).

Mechanism of CRISPR-Cas9: Grupp et al. (2013)

1) Detection of the target**:** gRNA finds the specific DNA sequence

↓

2) DNA cutting: Cas9 cuts DNA by creating a double-stranded break.

↓

3) Repair and editing: Cells are repaired and enhanced, implementing necessary modifications. Another types of gene editing are:

Transcription Activator-Like Effector Nuclease (TALEN) Miller et al. (2011)

TALEN is a programmable DNA-binding protein that uses a non-specific DNA cleavage domain to cause double-stranded breaks (DSBs) at precise places. Cermak et al. (2011). TALEN was developed before CRISPR-Cas9; however, it has certain similarities. Reyon et al. (2012). It has more specificity than CRISPR-Cas9. Maude et al. (2014).

Mechanism of TALEN

1. Identify a target site: Custom TALEN attaches to a specific DNA sequence.

↓

2. Dimerization: Two TALEN molecules bind together and place Fok Idomains.

↓

3. DNA cleavage: Fok I cuts DNA, resulting in DSB.

↓

4. Repair: Cells repair DSBs using non-homologous end joining (NHEJ) or homologous recombination (HR).

Zinc Finger Nucleases (ZFNs)

ZFNs are designed enzymes that can make precise alterations to DNA sequences. They were among the first gene editing tools, before CRISPR-Cas9and TALEN. It mainly identifies specific segments of DNA. The key objective of the Fok I Cleavage Domain is to cut DNA with non-specific endonuclease.

Mechanism of (ZFNs)

1. ZFD (Zinc Finger Domain): Identifies particular DNA sequences. Porteus & Baltimore (2003)

↓

2. The major function of the Fok I Cleavage Domain is to cut DNA with non-specific endonuclease. Bibi Kova et al. (2003).

↓

3. Selection of the target site: ZFD attaches to a certain DNA sequence. Cathomen & Joung (2008)

↓

4. Dimerization: Fok I domains are positioned by the union of two ZFNs.

↓

5. DNA cleavage: Double-Stranded Breaks (DSBs) are produced when Fok I cuts DNA.

↓

6. Homologous recombination (HR) or non-homologous end joining (NHEJ) is how cells repair double- strand breaks (DSBs).

Implications of gene editing techniques

Gene editing has the potential to;

1. Transform the treatment of genetic diseases
2. Revolutionize agriculture and food production
3. Advance biotechnology and synthetic biology
4. Improve human health and well-being

Leukemia-specific gene transfer

Answer locate forward to cure the vulnerable disease leukemia, gene transfer plays an enormous role Varmus H.E. et al. (1981) Gene transfer strategies include inserting genetic material into cells to alter gene expression Coffin J. M. et al. (1997). Viral vectors have been widely utilized for gene transfer such as:

1. **Retroviral Vectors (RV):** Derived from retroviruses, such as HIV and MLV. Mann, R., et al. (1983).
    - Integrative into host genome
    - Stable expression
    - Minimal capacity (~8 kb)
2. **Lentiviral Vectors (LV):** Derived from lentiviruses (e.g., HIV) Naldini, L., et al. (1996). Zufferey, R., et al. (1997) Cockrell, A. S., et al. (2011).
    - Integration into host genome
    - Stable expression
    - Higher capacity(~10kb)
3. Adenoviral vectors, (AVs), are derived from adenoviruses. Kay, M.A., et al. (2001) Graham, F.L., et al. (1977)
    - No incorporation into the host genome Berkner, K.L. (1992).
    - Transient expression
    - Large capacity (~30kb)
4. **Adeno-Associated Viral Vectors (AAV):** Derived from adeno-associated viruses, Muzyka, N., et al. (2009). Samulski, R. J., et al. (1989). Mc Carty, D. M., et al. (2001)
    - Integrate into the host genome,
    - Exhibit stable expression,
    - Small capacity (~5kb).

Non-Viral Techniques: Mir et al. (1999) Gehl et al. (2002)

1. Electroporation: Increasing the per me ability of cell membranes by applying electrical pulses

2. Lipofection: Delivering DNA with liposomes
3. Microinjection: the intracellular injection of DNA
4. Sonoporation: Increasing the permeability of cell membranes with ultrasound
5. Nanoparticles: Delivering DNA with the help of nanoparticles

Step 1: Vector preparation

1. Selection of vector type (viral or non-viral)
2. Design and construction of vector
3. Vector amplification and purification

Step 2: Target cell preparation

1. Selection of target cells (e.g., stem cells, cancer cells)
2. Cell culture and expansion
3. Cell preparation for gene transfer (e.g., trypsinization)

Step 3: Gene transfer

1. Vector delivery to target cells
2. Cellular uptake of vector (e.g., endocytosis, pinocytosis)
3. Vector processing and release of genetic material

Step 4: Intracellular trafficking

1. Vector transport to nucleus or cytoplasm
2. Vector unpacking and release of genetic material
3. Genetic material localization to target site

Step 5: Gene expression

1. Transcription of transferred gene
2. Translation of m RNA into protein
3. Protein localization and function

Step 6: Cell recovery and expansion

1. Recovery of transfected cells
2. Cell expansion and culturing
3. Verification of gene expression

Step 8: Analysis and validation

1. Verification of gene transfer efficiency

2. Analysis of gene expression levels
3. Validation of gene function and protein activity

Leukemia-specific applications

1. Leukemia acute myeloid (AML)

- Focusing on genetic alterations unique to AML.
- Presenting genes that decrease tumor growth.

2. Leukemia acute lymphoblastic

- Dealing with genetic changes unique to ALL.
- Strengthening the immune system.

3. Chronic myeloid leukemia (CML)

- Fighting the genetic defects
- Blocking signaling pathways that lead to cancer.

Gene transfer aims to

1. Correct genetic mutations.
2. Silence carcinogenic genes.
3. Increase immunological response.
4. Enhance chemotherapeutic potency

RNA-based therapies that control gene expression include RNA interference (RNAi), micro RNA (miRNA), and small interfering RNA (siRNA).

Interference with RNA (RNAi)

1. Reduces m RNA to silence certain genes.
2. Makes use of short hairpin RNA (sh RNA) or si RNA.
3. Applications include viral infections, cancer, and genetic diseases.

Micro RNA (mi RNA)

1. Silence certain genes by digesting m RNA.
2. Makes use of si RNA or sh RNA.
3. Applications include cancer, genetic abnormalities, and viral infections.

Si RNA, or small interfering RNA

1. Reduces m RNA to silence certain genes.
2. Engineered si RNA molecules.
3. Applications: viral infections, cancer, hereditary abnormalities.

Merely focusing on carcinogenic genes, RNA-based treatments have demonstrated encouraging results in the treatment of leukemia. The target of these treatments is to suppress or prevent the expression of genes that aid in the onset and spread of leukemia. One strategy is RNA interference RNAi therapy, which targets and precisely destroys oncogenic m RNA by using short hairpin RNA (sh RNA) or small interfering RNA (si RNA).

In leukemia, multiple kinds of oncogenic genes have been found to represent intriguing targets for RNA-based treatments. For instance, si RNA or sh RNA can be used to target the BCR-ABL1 fusion gene, which is indicative of chronic myeloid leukemia (CML). Similarly, RNA interference (RNAi) therapy can be used to target genes including FLT3, NPM1, and DNMT3A, which are frequently mutated in acute myeloid leukemia (AML). RNA-based therapies have the ability to suppress the growth and multiplication of leukemia cells by squelching these oncogenic genes.

A significant challenge that still exists is getting RNA-based medicines to leukemia cells in an effective way. Although liposomes, nanoparticles, and viral vectors have all been investigated as delivery methods, they all have drawbacks. When it comes to targeting specificity, liposomes and nanoparticles might have problems, though viral vectors can be. immunogenic. Furthermore, RNA-based treatments may need to be administered more than once due to their susceptibility to degradation.

Numerous preclinical and clinical investigations have exhibited the effectiveness of RNA-based treatments in alleviating leukemia. For instance, uplifting results from phase I clinical trial employing si RNA-based treatment in patients with AML were perceived. A distinct study that used mi RNA-based therapy in CML patients showed a noteworthy decrease in the expression of BCR-ABL1. These investigations demonstrate the enormous potential of RNA-based therapeutics as an innovative leukemia therapy strategy.

Inhibition of oncogene pathways

One possibility for blocking carcinogenic pathways in leukemia cells is gene therapy. Hanahan et al. (2011). Through the targeting of particular

genes or proteins implicated in the onset and progression of leukemia, gene therapy can impede the signaling pathways responsible for the growth of malignancy. Vogelstein et al. (2013). Through a variety of techniques, including as gene editing, RNA interference (RNAi), and gene expression modification, gene therapy blocks carcinogenic pathways. Oncogenic genes are silenced by RNA interference (RNAi), whereas their function is disrupted by CRISPR-Cas9 gene editing methods. By controlling the expression of oncogenic genes, cancer cell proliferation is inhibited through gene expression modification. Leukemia cells are profoundly affected by the suppression of carcinogenic pathways by gene therapy. It increases chemotherapeutic sensitivity, promotes apoptosis (cell death), and suppresses cell division and growth. Gene therapies also prevent cancer relapse by disrupting cells that cause leukemia. Targeted genes in gene therapy include FLT3, a tyrosine kinase, BCL-2, which controls apoptosis, and BCR-ABL1.fusion gene linked to chronic myeloid leukemia (CML).

Enhancement of immune responses through gene therapy

By altering immune system-related genes, gene therapy enhances immune responses and makes it feasible for the body to more competently identify and combat cancer cells. Rosenberg, S.A., et al. (1990). Immune responses are augmented by gene therapy through plenty of mechanisms: Restifo, N. P., et al. (2012)

- Genetically altering T cells to identify antigens unique to malignancy
- Introducing cytokine genes to promote immune cell activation
- Altering tumor cells to produce chemicals that activate the immune system. Leukemia cells are significantly impacted by the immunological responses that gene therapy enhances: Kalos, M., et al. (2011)
- Enhanced immune cell identification and targeting
- Immune cell activation to eradicate cancer cells
- Immunesuppressive mechanism inhibition
- CAR T cell therapy: T cells genetically altered to produce chimeric antigen receptors Kim, J., et al. (2018)
- Immunotherapy: Introducing immune-stimulating compounds or cytokine genes Dever, D.P., et al. (2016)
- Cancer vaccine therapy: Altering tumor cells genetically to boost immunity Liu, X., et al. (2019).

Preclinical studies

1. CAR T cell therapy: Eliminated leukemia cells in mouse models (Science, 2013) Frese et al. (2012).
2. Immunotherapy: Enhanced immune response against leukemia cells (Nature Medicine, 2015) Killion et al. (2014)
3. Gene editing: Corrected genetic mutations in leukemia cells (Cell, 2016) Singh et al. (2015)

Clinical trials

1. NCT03399448: CAR-T cell therapy for AML showed 70% response rate (NEJM, 2020)
2. NCT02352558: Immunotherapy improved overall survival in leukemia patients, Lancet, 2019
3. NCT01596230: Gene therapy increased complete remission in ALL patients (Blood, 2018). Gene therapy strengthens the body's defenses against leukemia cells.
    - In AML and ALL, CAR-T cell treatment exhibits noteworthy response rates.
    - Immunotherapy increases leukemia patients' overall survival
    - In leukemia cells, gene editing fixes hereditary flaws Ongoing Trials

1. NCT04150442: Gene Therapy for CLL.
2. CAR T cell treatment for AML (NCT 03804519)
3. NCT03480928, Immunotherapy for ALL

Comparative analysis of gene therapy with traditional treatments

Chemotherapy vs gene therapy

Comparison of efficacy

Disease	Chemotherapy	Genetherapy
AML (Acute Myeloid Leukemia)	40-60%	50-70%
ALL (Acute Lymphoblastic Leukemia)	70-90%	80-95%
CML (Chronic Myeloid Leukemia)	50-70%	60-80%

Comparison of side effects

Side effects	Chemotherapy	Genetherapy
Neutropenia	80-90%	20-40%
Thrombocytopenia	60-80%	15-30%
Anemia	50-70%	10-25%
Cytokine release syndrome	-	20-50%

Challenges and limitations

1. Heterogeneous Disease: Targeting leukemia is made more complicated due to the heterogeneity. Brenner et al. (2013).
2. Cancer Stem Cells: Targeting leukemia-initiating cells is problematic Kiem et al. (2012)
3. Tumor Microenvironment: Leukemia cells and the surrounding environment interact.
4. Genetic Complexity: Leukemia cells have a variety of genetic alterations.
5. Complete Remission: Gene therapy may not cause complete remission.
6. Relapse: The possibility of leukemia relapse due to remaining illness.
7. Off-Target impacts: These are unintended impacts on cells other than the target cells.
8. Insertional Mutagenesis: Possibility of genetic mutation. Williams et al. (2015).
9. Limited comprehension: An incomplete retention of leukemia biology. Koh et al. (2011)
10. Combination Therapies: Additional treatments sought to be united

Graphs

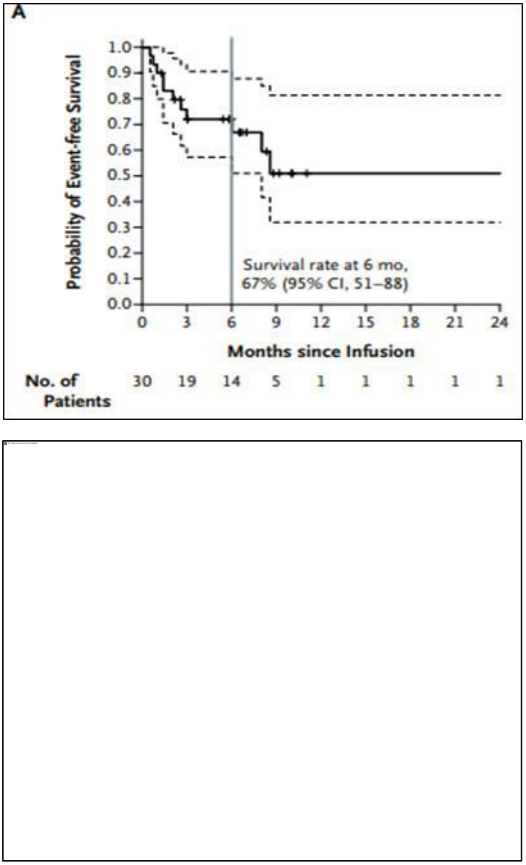


Fig: Probability of event-free and overall survival at 6 months

The New England Journal of Medicine Downloaded from nejm.org at UNIV OF UTAH ECCLES on October 16, 2014.

Conclusion

Targeting the genetic defects that cause leukemia has become much easier because to gene therapy, which has completely changed the treatment paradigm. For patients who do not respond to conventional treatments like chemotherapy and radiation, these medicines have provided a sense of hope These therapies range from the correction of defective genes to the use of modified immune cells like CAR-T cells. (June et al., 2018) Researchers may now directly address the underlying genetic causes of leukemia because to the development of CRISPR-Cas9 and other genome editing technologies,

which have improved the accuracy and effectiveness of gene therapy (Liang et al., 2015). In summarized form, gene therapy is a revolutionary method to treating leukemia, even if it is still in its early phases of research. Gene therapy may someday be a frontline treatment for a wider variety of leukemia subtypes, providing curative results for many patients, as research advances and technology improves (Schröder et al., 2019). To overcome present obstacles and realize the full promise of gene therapy in the battle against leukemia, more progress in this area is necessary.

Future prospectives

A number of encouraging advancements in gene therapy are anticipated to change the way it is used to treat leukemia in the future. First, it is anticipated that advancements in CRISPR-Cas9 and other gene-editing technologies would improve the accuracy and effectiveness of gene therapy techniques. In addition to repairing genetic abnormalities in hematopoietic stem cells HSCs, CRISPR can be used to modify immune cells for leukemia-targeting treatments that are more successful (Jinek et al., 2012). Research is still being done to reduce CRISPR's off-target effects and improve safety and effectiveness for clinical usage (Cornu et al., 2017). The possibility for customized gene treatment is another fascinating advancement. With the increasing depth of our knowledge about leukemia subtypes, medicines can be more specifically customized to each patient's genetic profile, enabling focused treatment that takes into account their specific mutation landscape (Gori &Viganò, 2016). This precision medicine approach has the potential to change the way leukemia is treated in both adult and pediatric populations by producing more effective therapies with fewer adverse effects. Last but not least, expanding production capacity and cutting costs are essential to increasing the accessibility of gene therapies. Current treatments, including CAR-T, are too costly to be used widely. It is anticipated that improvements in cell and gene therapy methods along with manufacturing process innovations would lower production costs and increase scalability (Hartmann et al., 2021). This may make gene therapy more widely available and provide curative treatment for a greater number of leukemia patients.

Acknowledgement

We sincerely acknowledge Swami Vivekananda University for providing the opportunity and necessary facilities to carry out this review.

References

1. Jinek et al. (2012). A programmable dual-RNA-guided DNA

endonuclease in adaptive bacterial immunity. *Science, 337*(6096), 816-821. DOI: 10.1126/science.1225829

2. Doudna & Charpentier (2012). A crack in the wall: Germline genome editing and the risk of malpractice. *Genome Biology, 13*(7), 148. DOI: 10.1186/gb-2012-13-7-148
3. Sternberg et al. (2014). The CRISPR-associated DNA-cleaving enzyme Cpf1 also processes precursor CR RNA. *Nature, 507*(7491), 262-265. DOI: 10.1038/nature13011
4. Miller et al. (2011). A TALE nuclease architecture for efficient genome editing. *Nature Biotechnology, 29*(2), 143-148. DOI: 10.1038/nbt.1755
5. Cermak et al. (2011). Efficient design and assembly of custom TALEN and other TAL effector-based constructs using FAST. *Nucleic Acids Research, 39*(12), e82. DOI: 10.1093/nar/gkr218
6. Reyon et al. (2012). FLASH assembly of TALENs enables efficient and reversible genome editing in human cells. *Nature Biotechnology, 30*(5), 460-465. DOI: 10.1038/nbt.2199
7. Maude et al. (2014). Chimeric antigen receptor T cells for sustained remissions in leukemia. *New England Journal of Medicine, 371*(16), 1507-1517. DOI: 10.1056/NEJMoa1407222
8. Grupp et al. (2013). Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *New England Journal of Medicine, 368*(16), 1509-1518. DOI: 10.1056/NEJMoa1215134
9. Davila et al. (2014). Efficacy and toxicity management of 19-28z CAR T cell therapy in refractory/relapsed ALL. *Blood, 124*(21), 382.
10. Porteus & Baltimore (2003). Chimeric nucleases stimulate gene targeting in human cells. *Science, 300*(5620), 763-766. DOI: 10.1126/science.1083091
11. Bibi Kova et al. (2003). Stimulation of homologous recombination through targeted cleavage by chimeric nucleases. *Molecular and Cellular Biology, 23*(1), 255-263. DOI: 10.1128/MCB.23.1.255-263.2003
12. Cathomen & Joung (2008). Zinc-finger nucleases: The next generation emerges. *Molecular Therapy, 16*(7), 1200-1207. DOI: 10.1038/mt.2008.114

13. Varmus, H. E., et al. (1981). The molecular biology of retroviruses. *Journal of Molecular Biology, 150*(2), 211-226. DOI: 10.1016/0022-2836(81)90361-8

14. Coffin, J. M., et al. (1997). *Retroviruses*. Cold Spring Harbor Laboratory Press. DOI: 10.1101/087969587.ch1

15. Lever, A. M., et al. (2009). Retroviral gene transfer: Mechanisms and applications. *Journal of General Virology, 90*(Pt 5), 931-941. DOI: 10.1099/vir.0.007432-0

16. Mann, R., et al. (1983). Construction of a retroviral vector for gene transfer. *Cell, 33*(1), 153-159. DOI: 10.1016/0092-8674(83)90343-4

17. Miller, A. D., et al. (1993). Human gene therapy: Development of retroviral vectors. *Methods in Enzymology, 217*, 581-599. DOI: 10.1016/0076-6879(93)17043-5

18. Kay, M. A., et al. (2001). Retroviral vectors for gene therapy. *Journal of Clinical Investigation, 107*(12), 1483-1492. DOI: 10.1172/JCI12516

19. Kay, M. A., et al. (2001). Adenoviral vectors for gene therapy. *Journal of Clinical Investigation, 107*(12), 1483-1492. DOI: 10.1172/JCI12516

20. Berkner, K. L. (1992). Expression of recombinant adenovirus-encoded proteins in cultured cells. *Methods in Enzymology, 216*, 336-346. DOI: 10.1016/0076-6879(92)16043-4

21. Graham, F. L., et al. (1977). Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *Journal of General Virology, 36*(1), 59-72. DOI: 10.1099/0022-1317-36-1-59

22. Muzyczka, N., et al. (2009). Adeno-associated virus (AAV) vectors: Development and applications. *Journal of Virology, 83*(10), 4955-4964. DOI: 10.1128/JVI.00068-0

23. Samulski, R. J., et al. (1989). Targeted integration of adeno-associated virus (AAV) into human chromosome 19. *EMBO Journal, 8*(10), 2955-2964. DOI: 10.1002/j.1460-2075.1989.tb08395.x

24. McCarty, D. M., et al. (2001). Adeno-associated virus (AAV) vectors for gene therapy. *Gene Therapy, 8*(10), 781-788. DOI: 10.1038/sj.gt.3301465

25. Naldini, L., et al. (1996). In vivo gene delivery and stable transduction of non-dividing cells by a lentiviral vector. *Science, 272*(5259), 263-267. DOI: 10.1126/science.272.5259.263

26. Zufferey, R., et al. (1997). Multiply attenuated lentiviral vector achieves efficient gene transfer in vivo. *Nature Biotechnology, 15*(9), 871-875. DOI: 10.1038/nbt0997-871

27. Cockrell, A. S., et al. (2011). Improved lentiviral vector design for HIV/AIDS gene therapy. *Molecular Therapy, 19*(5), 811-819. DOI: 10.1038/mt.2011.44

28. Mir et al. (1999). Electroporation-mediated gene transfer. *Methods in Molecular Biology, 133*, 111-127. DOI: 10.1385/1-59259-065-5:111

29. Gehl et al. (2002). Electroporation for gene delivery. *Expert Opinion on Therapeutic Patents, 12*(10), 1379-1391. DOI: 10.1517/13543776.12.10.1379

30. Hanahan et al. (2011). Hallmarks of cancer: The next generation. *Cell, 144*(5), 646-674. DOI: 10.1016/j.cell.2011.02.013

31. Vogelstein et al. (2013). Cancer genome landscapes. *Science, 339*(6127), 1546-1558. DOI: 10.1126/science.1235122

32. Rosenberg, S. A., et al. (1990). Gene transfer into humans—immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. *New England Journal of Medicine, 323*(9), 570-578. DOI: 10.1056/NEJM199008303230904

33. Restifo, N. P., et al. (2012). Adoptive immunotherapy for cancer: Harnessing the T cell response. *Nature Reviews Immunology, 12*(4), 269-281. DOI: 10.1038/nri3176

34. Kalos, M., et al. (2011). T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Science Translational Medicine, 3*(95), 95ra73. DOI: 10.1126/scitranslmed.3002842

35. Kim, J., et al. (2018). CRISPR/Cas9-mediated gene editing in human hematopoietic stem cells. *Nature Communications, 9*(1), 1-12. DOI: 10.1038/s41467-018-07674-4

36. Dever, D. P., et al. (2016). CRISPR/Cas9 β-globin gene targeting in human hematopoietic stem cells. *Nature, 539*(7629), 384-389. DOI: 10.1038/nature20134

37. Liu, X., et al. (2019). CRISPR-Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of T cells. *Nature Communications, 10*(1), 1-12. DOI: 10.1038/s41467-019-09457-0

38. Frese, T., et al. (2012). Preclinical models for cancer research. *Journal of Molecular Medicine, 90*(12), 1335-1346. DOI: 10.1007/s00109-012-0943

39. Killion et al. (2014). Preclinical models of cancer. *Journal of Clinical Oncology, 32*(22), 2399-2408. DOI: 10.1200/JCO.2013.53.0946

40. Singh et al. (2015). Preclinical modeling of cancer using genetically engineered mice. *Journal of Clinical Investigation, 125*(5), 1615-1626. DOI: 10.1172/JCI76071

41. Kohn et al. (2011). Gene therapy for leukemia: Challenges in scaling up. *Human Gene Therapy, 22*(10), 1139-1146. DOI: 10.1089/hum.2011.087

42. Williams et al. (2015). Economic evaluation of gene therapy for leukemia. *Journal of Medical Economics, 18*(10), 841-848. DOI: 10.3111/13696998.2015.1064082

43. Brenner et al. (2013). Gene therapy for leukemia: Current status and future directions. *Journal of Clinical Oncology, 31*(19), 2484-2492. DOI: 10.1200/JCO.2012.46.3635

44. Kiem et al. (2012). Hematopoietic stem cell gene therapy for leukemia. *Molecular Therapy, 20*(5), 871-879. DOI: 10.1038/mt.2012.43

45. June, C. H., O'Connor, R. S., Kawalekar, O. U., Ghassemi, S., & Milone, M. C. (2018). CAR T-cell immunotherapy for human cancer. *Science, 359*(6382), 1361-1365. https://doi.org/10.1126/science.aar6711

46. Liang, P., Xu, Y., Zhang, X., Ding, C., Huang, R., Zhang, Z., ... & Huang, J. (2015). CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. *Protein & Cell, 6*(5), 363-372. https://doi.org/10.1007/s13238-015-0153-5

47. Naldini, L. (2015). Gene therapy returns to center stage. *Nature, 526*(7573), 351-360. https://doi.org/10.1038/nature15818

48. Schröder, A. R. W., Shinn, P., Chen, H., Berry, C., Ecker, J. R., & Bushman, F. (2019). HIV-1 integration in the human genome favors active genes and local hotspots. *Cell, 110*(4), 521-529. https://doi.org/10.1016/S0092-8674(02)00864-4

49. Cornu, T. I., Mussolino, C., & Cathomen, T. (2017). Refining strategies to translate genome editing to the clinic. *Nature Medicine, 23*(4), 415-423. https://doi.org/10.1038/nm.4313

50. Friedrich, M. J., Cardenas, P. A., & Fazal, F. (2019). CAR T-cell

therapies: New developments in harnessing immune cells for cancer therapy. *Journal of Clinical Oncology*, *37*(24), 2077-2085. https://doi.org/10.1200/JCO.19.00576

51. Gori, J. L., & Viganò, S. (2016). Precision gene editing for hematologic malignancies. *Blood*, *127*(26), 336.

52. June, C. H., & Sadelain, M. (2018). Chimeric antigen receptor therapy. *New England Journal of Medicine*, *379*(1), 64-73. https://doi.org/10.1056/NEJMra1706169

53. Levine, B. L., Miskin, J., Wonnacott, K., & Keir, C. (2017). Global manufacturing of CAR T-cell therapy. *Molecular Therapy*, *25*(7), 1442-1453. https://doi.org/10.1016/j.ymthe.2017.03.001

Chapter - 6

Antibiotic Resistance and Novel Therapeutic Approaches

Authors

Swastika Datta
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Mushkan
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

Tanisha Bhowmick
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

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

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

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

Chapter - 6

Antibiotic Resistance and Novel Therapeutic Approaches

Swastika Datta, Mushkan, Tanisha Bhowmick, Dipti Das, Tiyasha Saha and Rupesh Dutta Banik

Abstract

New antibiotic strategies are needed because drug-resistant microorganisms are becoming more prevalent. Any worldwide response to the antimicrobial resistance threat needs to include research into new strategies for combating it. Antibiotic resistance breakers, or ARBs, are a promising field of study that aims to resensitize antibiotic-resistant microorganisms. Thus, there is a growing demand for both new classes of antibiotics and new therapeutic strategies, such as expanding the use of combination medicines and repurposing of currently available medications or preclinical substances. To decrease the inappropriate use of ambulatory antibiotics, clinicians, pharmacists, practices, and health systems must collect data on antibiotic prescriptions, select targeted improvement goals, and implement evidence-based interventions such as precommitment, accountable justification, peer comparison, and communication training. Effective combination therapy that aims to combat the infection, prevent resistance, and safeguard the natural microbiome must incorporate antimicrobial stewardship, novel antibiotic compounds, biologics, and delivery techniques. This review analyses these developing strategies in connection to overcoming the existing resistance mechanisms.

Keywords: Antibiotics, drug resistance, resensitize, therapeutic strategies, natural microbiome.

Introduction

One of the greatest contributions to medicine in the 20th century, antibiotics constitute the most important class of medications (Uddin et al. 2021) (Salam et al. 2023). Unquestionably, antibiotics have helped humanity tremendously in the battle against microorganisms, sparing millions of lives. On the other hand, the frequency of diseases brought on by multidrug-resistant (MDR) bacteria is rising globally, and the threat of incurable

infections has been present since the start of the twenty-first century (Uddin et al. 2021) (Matos de Opitz et al. 2020). The rapid growth and spread of antibiotic-resistant bacteria have been rising yearly due to the widespread abuse of antibiotics (Wang et al. 2020). Antimicrobial Resistance (AMR) is the phenomenon whereby microorganisms, encompassing bacteria, viruses, fungi, and parasites, acquire the ability to adjust and proliferate when exposed to pharmaceuticals that previously affected them (Olatunji et al. 2024) (Dadgostar 2019). The World Health Organization has noted that, if clinical antibiotic resistance continues at its current rate, it may result in a drop of 2-3.5% in the GDP per year and 10 million deaths by 2050 (Wang et al. 2020) (Chang et al. 2022) (Matos de Opitz et al. 2020). Among antibiotic resistant bacteria, “ESKAPE” pathogens, including Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species, cause the majority of hospital infections with higher mortality of patients (Wang et al. 2020) (Pacios et al. 2020). ESKAPE pathogens effectively escape the effects of commonly used antibacterial drugs, and are usually associated with significantly higher economic burden by increasing the duration of hospital stays and decreasing workforce productivity (Wang et al. 2020) (Pacios et al. 2020). Recently, a notable study reported that ESKAPE pathogens represented 42.2% of species isolated from bloodstream infections in the United States. Moreover, compared with patients infected with non-ESKAPE pathogens, patients presenting ESKAPE bloodstream infections were associated with a 3.3-day increase in length of hospital stay, and a 2.1% absolute increase in mortality (Wang et al. 2020).1947 saw the emergence of the first penicillin-resistant bacterium strain. In less than a year following the introduction of penicillin in the clinic, two years after it became generally accessible, and less than 20 years after its discovery in 1928, four strains of penicillin-resistant *staphylococci* were identified from patients (Monserrat-Martinez et al. 2019) (Moo et al. 2020). Methicillin, which may elude the enzyme penicillinase that splits penicillin rings, was created by the pharmaceutical industry in 1959. Although Staphylococcus aureus strains resistant to methicillin first surfaced in 1960, the antibiotic's rapid evolution and resistance to it was demonstrated within a single year of its release (Monserrat-Martinez et al. 2019) (Ahmed et al. 2024). The primary mechanisms of intrinsic bacterial resistance depend on the bacterial cell's impermeability to the antibiotic molecule (e.g., the molecule's physicochemical properties, presence of efflux pumps, etc.), the absence of target molecules, or the antibiotic compound's inactivation through enzyme

degradation. All in all, this is the outcome of the coordinated action of various phenotypic qualities engaged in a broad range of metabolic activities as well as genetic/heritable factors that have just lately been recognized as critical for the initiation and spread of the AMR (Schrader et al. 2023). The use of genomics is one of the main areas of attention in the fight against AMR. Researchers can uncover possible targets for innovative medications, improve diagnostic tools, and learn more about the genetic pathways underlying resistance by utilizing genomic data. Another exciting path is provided by CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology, which allows for precise editing of genetic material by destroying the resistance genes (Konware et al. 2022). The creation of innovative medicines, such as synthetic antibiotics, antimicrobial peptides, and bacteriophages is also essential. A focused method of treating bacterial illnesses without upsetting the host's microbiome is provided by bacteriophages, viruses that infect and kill bacteria (Olatunji et al. 2024) (Chang et al. 2022). In order to tackle antimicrobial resistance and guarantee the continuous efficiency of antimicrobial therapies for future generations, this study explores cutting-edge approaches like genetics, CRISPR, and innovative pharmaceuticals (Olatunji et al. 2024).

Instance of antibiotic resistance

ABR presents a serious worldwide risk to public health and is the greatest challenge in the treatment of infectious illnesses. The rate at which new antimicrobial discoveries are made is so slow that, as noted in 1987, no new medication classes were approved. When agricultural or clinical antibiotics are used excessively, it creates collateral damage from the overuse of antimicrobials, which leads to selective pressure that promotes AMR in other bacteria in addition to the targeted pathogen (Ahmed et al. 2023). The rise of multidrug-resistant bacteria that can withstand the effects of last-resort medicines like tigecycline and colistin is a result of the recent peak in resistance to regularly used antibiotics (Konwar et al. 2022).

Antibiotic resistance mechanism

The description of ABR as a process brought about by antibiotic misuse falls short because it is well known that ABR develops naturally over time through a variety of processes. To put it another way, overuse of antibiotics by both humans and animals speeds up this normal process and encourages the emergence of antimicrobial resistance (AMR). Although the term "antibiotic-resistant bacteria" is frequently used, relatively little thought is given to what this actually means. In this competition, two types of

resistance can be distinguished: acquired and natural which can be further divided into intrinsic and induced resistance. When bacterial species have inherent resistance to a type of antibiotic, it is evident that this resistance is unrelated to prior antibiotic exposure, e.g., vancomycin resistance in Escherichia coli, ampicillin, resistance to both first- and second-generation cephalosporins in *Pseudomonas aeruginosa*. Genes activated by exposure to therapeutic doses of antibiotics can also cause natural resistance in bacteria. A mutation that can place in the cell's DNA during replication or DNA transfer are the two different pathways that might result in acquired resistance. In reference to the first method, the mutant strains possess the ability to transmit the mutation to their progeny through the vertical method. The second mechanism by which bacteria pick up resistance is by conjugation, transposition, and transformation all of which are referred to as horizontal gene transfer (Chatterjee et al. 2016) (Olatunji et al. 2024). The recipient bacteria absorb extracellular donor DNA during transformation. A bacteriophage containing donor DNA infects the recipient bacterium during transduction. When two bacteria conjugates, the donor bacterium mates with the recipient to transmit DNA to it. The non-resistant bacteria then acquire an antibiotic resistance due to the transfer of antibiotic-resistant genetic material from the antibiotic-resistant bacteria (Chatterjeet et al. 2016) (Ahmed et al. 2023) (Mancuso et al. 2021). Both pathogenic and non-pathogenic microbes have intrinsic antimicrobial resistance, which is unique to them both and is necessary for their survival and evolution in a changing environment (Palma et al. 2020). The two most common ways that bacteria become resistant to antibiotics are through horizontal gene acquisition (via integrons, transposons, or plasmids) or changes in distinct chromosomal loci. Bacteria that have acquired transferable antibiotic resistance determinants are of particular concern. Numerous tactics have been documented for combating antibiotics in bacteria, including the inactivation of enzymes, modifications to cell permeability, modification of target binding sites, enhancement of antibiotic efflux, and complicated phenotypic alterations (e.g., biofilm formation) (Wang et al. 2019) (Palma et al. 2020). A situation known as phenotypic persistence occurs when bacteria remain in a quiescent state after the stressor is removed, rather than being killed by the antibiotic kind or dose that was given. The global issue of pathogenic microbial biofilm is attributed to the innate resistance to antibiotics exhibited by these It has been demonstrated that biofilm formation protects bacterial cells from antibiotic treatment. Bacterial biofilms exhibit resistance against antibiotics, disinfectant chemicals, phagocytosis and other constituents of the body's

innate and adaptive inflammatory defense system (Frieri et al. 2017). This has implications for all chronic infections and possible surface colonization (e.g., intubated patients, prosthetics, catheters) that are clinically meaningful. The swarming movement is an intriguing defense mechanism used by bacteria against antibiotic treatments (Palma et al. 2020). Antibiotics can be rendered inactive by bacteria through two different mechanisms: either by breaking down the medication or by changing its chemical composition (Uddin et al. 2021).

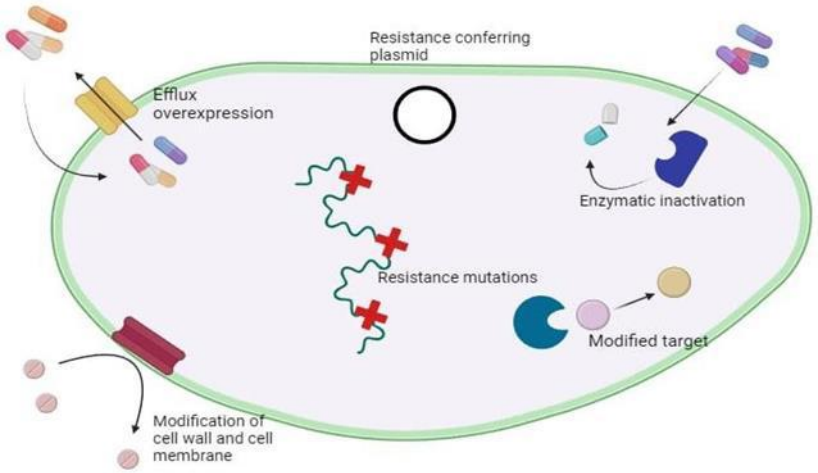


Fig 1: Mechanism of antibiotic resistance in bacterial cell

Therapeutic approaches to combat Ab R

Antimicrobial resistance (AMR) is on the rise, which has prompted the creation of new antibiotic classes that work through unique methods to get around established resistance pathways (Uddin et al. 2021) (Olatunji et al. 2024). Since the rate at which AMR is emerging has surpassed the rate at which antimicrobials are evolving, the process of creating new antimicrobials must be accelerated (Moo et al. 2020). Among the key strategies for preventing AMR are the prudent prescribing of antibiotics, the restricted use of prophylactic antimicrobials, patient education, adherence to antibiotic therapy, and proper hospital hygiene through antimicrobial stewardship. In order to tackle AMR, the World Health Assembly approved five major strategic action plans, which include the following actions:

1) Enhance knowledge and comprehension of antibiotic resistance;
2) Reinforce knowledge through research and surveillance in fighting

infection through control me

3) Put into practice efficient sanitation, hygiene, and infection prevention measures;
4) Maximize the use of antibiotics in human and animal health; and
5) Promote long-term investment in new medications, diagnostic equipment, and vaccines (Salam et al. 2023).

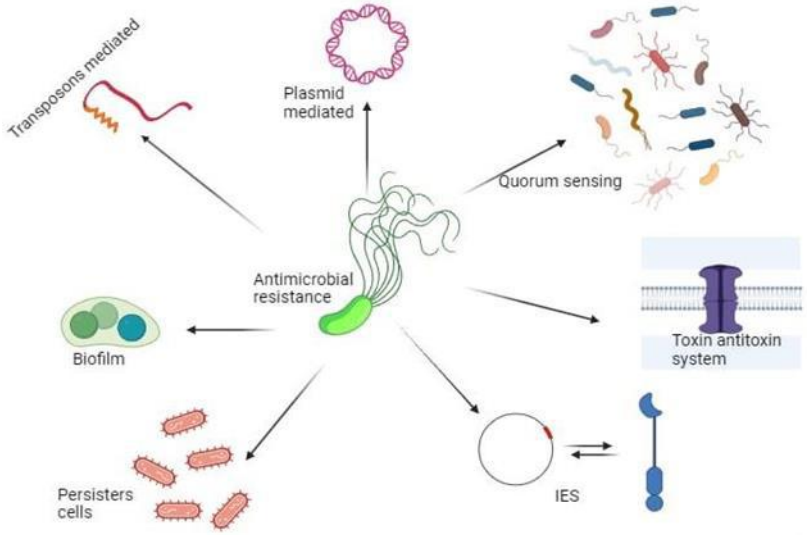


Fig 2: Molecular mechanism responsible for antimicrobial resistance

Multiple societal solutions are necessary in light of the increasing occurrence of genetic AMR. One of them is the pressing need to find novel antibiotics, particularly novel forms of antibiotics (Schrader et al. 2020). Antibiotic stewardship programs frequently employ treatment duration shortening as a tactic in the hopes of gradually lowering the frequency of bacteria resistant to antibiotics (Walshetal.2023). While new antibiotics are urgently needed to address the resistance issue, most national policies against AMR also acknowledge the need for alternate approaches (Harbarth et al. 2015).

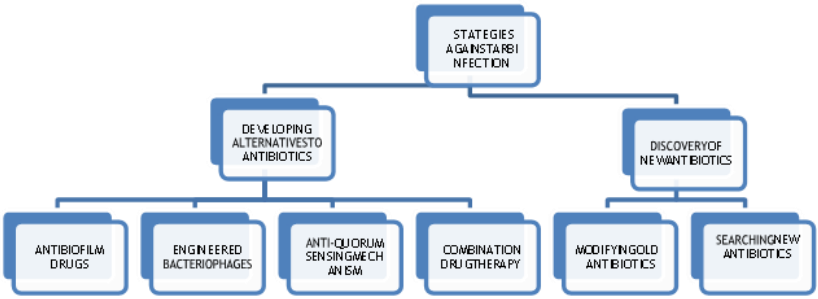


Fig 3. Strategies to combat ARB infection

Combination therapy I: Enhances membrane permeability

When compared to Gram-positive bacteria, gram-negative bacteria have an additional OM that these drugs cannot penetrate, which makes them inherently resistant to multiple antibiotic classes. Porins are water-filled protein channels found in the outer membrane of bacteria (OM) that allow hydrophilic compounds to enter the bacterial cell. Reductions in porin expression caused by mutations in Gram-negative bacteria can decrease the number of hydrophilic medicines that enter these bacteria. Several therapeutically relevant bacterial species, including *P. aeruginosa*, have demonstrated resistance to this antibacterial technique (Laws et al. 2019). Lipopeptide antibiotics known as polymyxins have bactericidal action against Gram-negative bacteria. They function by rupturing the cell membrane by a combination of hydrophobic and electrostatic interactions. The indication of the non-antibiotic drug mitotane (a cytostatic antineoplastic drug and steroidogenesis inhibitor) as a possible option for polymyxin B combination therapy against Gram-negative bacteria, including *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* that are resistant to carbapenems. Though the exact method by which mitotane prevents the growth of the bacterial pathogen is yet unknown, it is thought that polymyxin B's increased outer membrane permeability may allow mitotane to enter the bacterial cells (Wang et al. 2023). LPS–LPS interactions are directly disrupted at the OM by currently available therapeutically used OM-acting antibiotics (Lehman et al. 2019).

Combination drug therapy

Combination drug therapy refers to the application of two or more drugs or treatments for the same illness. When treating germs, combination drug therapy is preferred over individual therapy, or monotherapy, as the latter is more likely to result in the development of resistance. Three mechanistic

pathways can be identified in combination medication regimens:

1) A combination of medications using distinct paths to target various targets,
2) Combination medications that work on several targets via the same mechanism,
3) Combination medications that work on a single target through several dimensions.

Treatment for *Mycobacterium tuberculosis* infections is a classic example of a combination of medications acting on distinct targets in different pathways. The four medications that are combined are: two RNA polymerase inhibitors; an inhibitor of the enoyl reductase portion of the fatty acid synthase (isoniazid); and an inhibitor of arabinosyl transferases involved in cell wall production (ethambutol). The idea behind this treatment is that the bacteria will alter each of the specific antibiotic targets in an effort to develop resistance (Moo et al. 2020).

Action against bio-films

One well-known issue in treating bacterial infections is bio-films (Ye et al. 2022). Strategies to address the issue have been developed recently since biofilm-related infections and the difficulties in treating them have been recognized as important dangers to human health (Zhang et al. 2020). Infections are mostly caused by biofilms that form on inert surfaces like catheters or prosthetic joints and on bodily structures like teeth and heart valves. Innovative techniques are being developed to both stop the formation of biofilms and break them up after they have formed. The reason why eliminating them can be challenging is partly due to the fact that bacteria that are growing in biofilms are in a state of physiological resistance to antibiotics, which normally kill planktonic germs (Hauser et al. 2016). Other approaches to targeting bacterial biofilms have recently been investigated; however, it is anticipated that many of these will have a somewhat species-specific effect on biofilms. For example, interfering with the relevant signaling pathways may also be able to influence the biofilm growth process. Targeting the quorum sensing circuitry, for instance is a potentially appealing anti-biofilm strategy that is being investigated (De la Fuente-Núñez et al. 2013) (Hauser et al. 2016). The risk of biofilm infection connected to medical devices has grown. A crucial phase in the formation of biofilms is the attachment of bacteria to a surface; once biofilms are formed on a medical surface, they are extremely difficult to remove. One of the main methods for getting rid of biofilms on medical equipment is to change its

surface. Medical prosthetic coatings have been extensively developed to counteract biofilm formation. Catheters are coated with metal materials since it has been demonstrated that they are effective anti-infective agents. Biofilms cannot form on any of the silver or silver-copper multilayer coatings that are employed in vascular, urinary, peritoneal, and fracture fixation catheters, among other catheters and medical devices (Zhang et al. 2020) (Hauser et al. 2016).

Anti-quorum sensing mechanism

QS is a communication system that depends on cell density and uses autoinducers, which are low-molecular-weight signaling molecules, to control the pathogenicity of many bacterial pathogens.

Gram-positive species use ribosomally generated autoinducing peptides for QS, while gram- negative species use N-acyl homoserine lactones (AHLs) or related substances (Wang et al. 2020) (Tillotson et al. 2013) (Hauser et al. 2016). Using quorum quenchers (QQ) or quorum sensing inhibitors (QSI) to impair quorum sensing is a potential substitute for antibiotics, given its function as a master switch. To inhibit quell microbial quorum sensing, three general strategies are used: Three mechanisms are involved in the degradation of autoinducers:

1) Disruption of autoinducer production,
2) Suppression of ligand/receptor interactions, and
3) Enzymatic cleavage and degradation (Brooks et al. 2014).

Due to its resistance to popular antibiotics and harsh conditions, *P. aeruginosa* has become a significant pathogenic bacterium in nosocomial infections. This resistance is achieved by modifying its highly structured QS and accompanying biofilm development. *P. aeruginosa* produces molecules that function as particular chemical signals in response to high population density. These signals regulate the synthesis of virulence components that mediate acute infection. In addition to around 60 excreted anthranilic acid derivatives, the majority of which are members of the 4-hydroxy-2-akylquinolines (HAQs) family, the QS regulator known as multiple virulence factor regulator (Mvf R) regulates the expression of most of *P. aeruginosa*'s acute virulence factors (Wang et al. 2020) (Tillotson et al. 2013) (Hauser et al. 2016). In addition to being a treatment for infection, QS agents may also be used as a preventative measure to avoid the colonization of areas like ventilators and catheters, where an infection may develop (Tillotson et al. 2013).

Future therapeutic options: Engineered phages

Bacteriophages, often known as "phages," are viruses that only target bacteria for infection and replication within bacterial cells. These creatures, which are the most prevalent and widespread group on the planet, have an impact on population dynamics, microbial physiology, evolution and therapeutics (Brooks et al. 2014) (Konwar et al. 2022). Phage treatment development had ceased since the 1940s with the advent of broad-spectrum antibiotics. Nevertheless, phage therapy is receiving more interest now that ARB is available (Ye et al. 2022). Phages are traditionally thought to be promising antibacterial agents that could replace antibiotics. The ability to multiply at the site of their host location for as long as the host persists, the capacity to adapt in response to host evolution, the general lack of detrimental effects on human health, and their narrow specificity range, which prevents the undesired killing of commensal microbiota, are some of their advantages over antibiotics (Lobocka et al. 2021). Phages are also employed as species-specific carriers for a range of possible antibacterial payloads or RNA-guided CRISPR-Cas based nucleases that are intended to target resistance or virulence factors (Theuretzbacher et al. 2020). Phage therapy has been improved by engineering phages to extend their host range and infectivity as well as by purifying individual phage components to target specific bacteria (Hauser et al. 2016). Phage treatment has widely acknowledged potential advantages and opportunities: excellent effectiveness against biofilm-forming sensitive bacteria, no antibiotic cross-resistance, and a high selectivity for infections (Walesch et al. 2023).

Complementing antibiotic resistance with bioinformatics

The field of science and technology is being revolutionized and is quite exciting by bioinformatics. Homology modeling is being utilized in order to generate 3D models for testing and validating our intended results. With its ability to comprehend macromolecule structures, bioinformatics has completely changed the field of molecular biology research. Several well-known techniques in the field of bioinformatics include - A new development in the field of genotyping is the WGS of a pathogen genetic material. Utilizing whole genome sequencing (WGS) to analyze bacteria could transform hospital outbreak research by providing new perspectives on related bacterial lineages of interest. The two main methods employed in current in silico serovar prediction approaches are surface antigen encoding gene forming core genome multi-locus sequence typing (MLST) and serovar specific gene marker. The painstakingly constructed CARD platform offers

computational resources, macromolecule sequences and detection models to help comprehend the molecular process of antimicrobial resistance. Short-read next-generation sequencing (SR-NGS) data is the foundation of metagenomics tactics since it enables the measurement of hundreds of transmissible resistance genes in a single sample without in order to quantify thousands of transmissible resistance genes in a single sample without the usage of any predefined genes, metagenomics methodologies rely on short-read next-generation sequencing (SR-NGS) data. Consequently, this could lead to an increase in knowledge regarding the presence of bacteria, pathogens, and virulence genes. The collected data could also allow for a reanalysis in the event that additional genes of interest are found (Uddin et al. 2021).

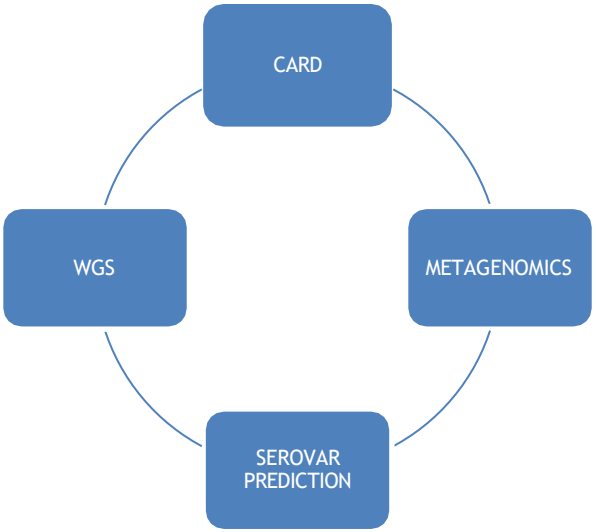


Fig 4: Different bioinformatic approaches to combat AMR microbes

Incorporating CRISPR, genomics, and innovative therapies

A potent strategy to Address Antibiotic Resistance (AMR) is to combine genetic insights with CRISPR technology. Comprehensive details regarding the genetic composition of pathogens, such as resistance gene and mutation identification, can be obtained through genome sequencing. Precise CRISPR-based therapies that target and interfere with various resistance pathways can be created using this data. In the event that a bacterial population's genomic analysis identifies a particular beta- lactamase gene as the cause of antibiotic resistance, CRISPR-Cas9 can be modified to cut and

inactivate this gene, returning the bacterium to being vulnerable to beta-lactam antibiotics. Furthermore, genomics can pinpoint pathogen-specific sequences that CRISPR systems can target, reducing side effects and guaranteeing the preservation of the beneficial microbiota. The development of specialized medicines that are efficient and less likely to exacerbate the AMR dilemma can be sped up by combining these technologies. AMR can be addressed in a variety of ways by incorporating cutting-edge therapies into current treatment plans, such as bacteriophage therapy antimicrobial peptides (AMPs), and synthetic biology techniques. Healthcare professionals can create all-encompassing therapy regimens that address many facets of bacterial infections by combining these cutting-edge treatments with conventional antibiotics and CRISPR-based therapeutics (Olatunji et al. 2024).

Conclusion

The prevalence of Antimicrobial Resistance (AMR) is rising, and bacteria have developed resistance mechanisms to counteract the antibacterial properties of goods for decades. Bacterial infections are predicted to be the primary cause of death in the current circumstances due to antibiotic abuse. The combination of the rise in antibiotic resistance and the dearth of novel antibiotics presents a dire picture. Next-generation techniques are critically needed, as evidenced by the ongoing rise in antimicrobial resistance (AMR). New tactics are required to combat germs that have become resistant to conventional antibiotics because of their increasing ineffectiveness. While the use of two or more different types of drugs in a combinatorial approach has gained popularity, further research is desperately needed to understand potential drug-drug interactions as well as the best times, sequences, and dosage regimens. Antimicrobial resistance is a serious threat to the health of humans and animals worldwide due to a number of factors that are driving its development and spread. The treatment of antimicrobial-resistant infections is more challenging, resulting in treatment failure, complications, and significant financial consequences for both the community and the individual. One of the most crucial ways to lessen the selection pressure needed for the evolution of resistance organisms is the prudent administration of antibiotics at the right dosage and duration.

One of the most important steps in halting the development of MDR organisms is the strict implementation of infection prevention and control procedures in all healthcare facilities. But because of the particular "tragedy of the commons" that is antimicrobial resistance which cuts across sectors

and borders-binding global coordinated action is required. We can stop the spread of resistance and maintain the effectiveness of antibiotics by implementing coordinated surveillance, access equity, conservation measures, and funding for innovative projects under a One Health framework. Additional delays run the danger of predicting reversals to the pre- antibiotic susceptibility patterns that have historically dominated infectious disease mortality; this could jeopardize the security of world health and current medical capabilities.

Future prospective

Antimicrobial Resistance (AMR) has a bright future thanks to the quick development of genomic technology. Technologies for Next-Generation Sequencing (NGS) are constantly developing, becoming more rapid, precise, and economical. Developing and implementing effective tactics to lower Antimicrobial Resistance (AMR) requires conducting research that emphasizes taking action. A vital component of the fight against antibiotic resistance is the development of novel antimicrobial medications and alternative therapies, in addition to surveillance and diagnostic studies. Developments in drug development, including the identification of new molecular targets, the enhancement of existing antibiotics, and the exploration of unconventional therapeutic approaches like immunomodulation and bacteriophage therapy, are essential components of this discipline.

Author contributions

Acquisition and interpretation of data is done by Mushkan and Swastika Datta. Conception and revising of the article are done by Rupesh Dutta Banik. Designed by Tanisha Bhowmick, Dipti Das, Tiyasha Saha.

Acknowledgement

I want to convey my sincere appreciation to the higher authorities of Swami Vivekananda University for granting me permission to conduct this study.

References

1. Ahmed, S. K., Hussein, S., Qurbani, K., Ibrahim, R. H., Fareeq, A., Mahmood, K. A., & Mohamed, M. G. (2024). Antimicrobial resistance: impacts, challenges, and future prospects. *Journal of Medicine, Surgery, and Public Health, 2*, 100081. DOI: 10.1016/j.glmedi.2024.100081
2. Ahmed, S., Ahmed, M. Z., Rafique, S., Almasoudi, S. E., Shah, M.,

Jalil, N. A. C., & Ojha, S. C. (2023). Recent approaches for downplaying antibiotic resistance: molecular mechanisms. *BioMed Research International, 2023*(1), 5250040. DOI: 10.1155/2023/5250040

3. Brooks, B. D., & Brooks, A. E. (2014). Therapeutic strategies to combat antibiotic resistance. *Advanced Drug Delivery Reviews, 78*. DOI: 10.1016/j.addr.2014.10.027
4. Chang, R. Y. K., Nang, S. C., Chan, H. K., & Li, J. (2022). Novel antimicrobial agents for combating antibiotic-resistant bacteria. *Advanced Drug Delivery Reviews, 187*, 114378
5. Chatterjee, M., Anju, C. P., Biswas, L., Kumar, V. A., Mohan, C. G., & Biswas, R. (2016). Antibiotic resistance in *Pseudomonas aeruginosa* and alternative therapeutic options. *International Journal of Medical Microbiology, 306*(1), 48-58. DOI: 10.1016/j.ijmm.2015.11.004
6. Dadgostar, P. (2019). Antimicrobial resistance: implications and costs. *Infection and Drug Resistance*, 3903-3910. DOI: 10.2147/IDR.S234610
7. De la Fuente-Núñez, C., Reffuveille, F., Fernández, L., & Hancock, R. E. (2013). Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. *Current Opinion in Microbiology, 16*(5), 580-589. DOI: 10.1016/j.mib.2013.06.013
8. Frieri, M., Kumar, K., & Boutin, A. (2017). Antibiotic resistance. *Journal of Infection and Public Health, 10*(4), 369-378. DOI: 10.1016/j.jiph.2016.08.007
9. Harbarth, S., Balkhy, H. H., Goossens, H., Jarlier, V., Kluytmans, J., Laxminarayan, R., ... & World Healthcare-Associated Infections Resistance Forum participants. (2015). Antimicrobial resistance: one world, one fight! DOI: 10.1186/s13756-015-0091-2
10. Hauser, A. R., Mecsas, J., & Moir, D. T. (2016). Beyond antibiotics: new therapeutic approaches for bacterial infections. *Clinical Infectious Diseases, 63*(1), 89-95
11. Konwar, A. N., Hazarika, S. N., Bharadwaj, P., & Thakur, D. (2022). Emerging non-traditional approaches to combat antibiotic resistance. *Current Microbiology, 79*(11), 330
12. Laws, M., Shaaban, A., & Rahman, K. M. (2019). Antibiotic resistance breakers: current approaches and future directions. *FEMS Microbiology Reviews, 43*(5)
13. Lehman, K. M., & Grabowicz, M. (2019). Countering gram-negative antibiotic resistance: Recent progress in disrupting the outer membrane

with novel therapeutics. *Antibiotics, 8*(4), 163. DOI: 10.3390/antibiotics8040163

14. Łobocka, M., Dąbrowska, K., & Górski, A. (2021). Engineered bacteriophage therapeutics: rationale, challenges and future. *BioDrugs, 35*(3), 255-280

15. Mancuso, G., Midiri, A., Gerace, E., & Biondo, C. (2021). Bacterial antibiotic resistance: the most critical pathogens. *Pathogens, 10*(10), 1310. DOI: 10.3390/pathogens10101310

16. Matos de Opitz, C. L., & Sass, P. (2020). Tackling antimicrobial resistance by exploring new mechanisms of antibiotic action. *Future Microbiology, 15*(9), 703-708

17. Monserrat-Martinez, A., Gambin, Y., & Sierecki, E. (2019). Thinking outside the bug: molecular targets and strategies to overcome antibiotic resistance. *International Journal of Molecular Sciences, 20*(6), 1255. DOI: 10.3390/ijms20061255

18. Moo, C. L., Yang, S. K., Yusoff, K., Ajat, M., Thomas, W., Abushelaibi, A., ... & Lai, K. S. (2020). Mechanisms of antimicrobial resistance (AMR) and alternative approaches to overcome AMR. *Current Drug Discovery Technologies, 17*(4), 430-447. DOI: 10.2174/1570163816666190304122219

19. Olatunji, A. O., Olaboye, J. A., Maha, C. C., Kolawole, T. O., & Abdul, S. (2024). Next-generation strategies to combat antimicrobial resistance: integrating genomics, CRISPR, and novel therapeutics for effective treatment. *Engineering Science & Technology Journal, 5*(7), 2284-2303. DOI: 10.51594/estj.v5i7.1344

20. Pacios, O., Blasco, L., Bleriot, I., Fernandez-Garcia, L., González Bardanca, M., Ambroa, A., ... & Tomás, M. (2020). Strategies to combat multidrug-resistant and persistent infectious diseases. *Antibiotics, 9*(2), 65. DOI: 10.3390/antibiotics9020065

21. Palma, E., Tilocca, B., & Roncada, P. (2020). Antimicrobial resistance in veterinary medicine: an overview. *International Journal of Molecular Sciences, 21*(6), 1914

22. Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., & Alqumber, M. A. (2023, July). Antimicrobial resistance: a growing serious threat for global public health. *Healthcare, 11*(13), 1946. DOI: 10.3390/healthcare11131946

23. Schrader, S. M., Botella, H., & Vaubourgeix, J. (2023). Reframing

antimicrobial resistance as a continuous spectrum of manifestations. *Current Opinion in Microbiology, 72*, 102259. DOI: 10.1016/j.mib.2022.102259

24. Schrader, S. M., Vaubourgeix, J., & Nathan, C. (2020). Biology of antimicrobial resistance and approaches to combat it. *Science Translational Medicine, 12*(549), eaaz6992

25. Theuretzbacher, U., Outterson, K., Engel, A., & Karlén, A. (2020). The global preclinical antibacterial pipeline. *Nature Reviews Microbiology, 18*(5), 275-285

26. Tillotson, G. S., & Theriault, N. (2013). New and alternative approaches to tackling antibiotic resistance. *F1000Prime Reports, 5*. DOI: 10.12703/P5-51

27. Uddin, T. M., Chakraborty, A. J., Khusro, A., Zidan, B. R. M., Mitra, S., Emran, T. B., ... & Koirala, N. (2021). Antibiotic resistance in microbes: history, mechanisms, therapeutic strategies and future prospects. *Journal of Infection and Public Health, 14*(12), 1750-1766. DOI: 10.1016/j.jiph.2021.10.020

28. Walesch, S., Birkelbach, J., Jézéquel, G., Haeckl, F. J., Hegemann, J. D., Hesterkamp, T., ... & Müller, R. (2023). Fighting antibiotic resistance—strategies and (pre) clinical developments to find new antibacterials. *EMBO Reports, 24*(1), e56033. DOI: 10.15252/embr.202256033

29. Walsh, T. R., Gales, A. C., Laxminarayan, R., & Dodd, P. C. (2023). Antimicrobial resistance: addressing a global threat to humanity. *PLOS Medicine, 20*(7), e1004264.

30. Wang, C. H., Hsieh, Y. H., Powers, Z. M., & Kao, C. Y. (2020). Defeating antibiotic-resistant bacteria: exploring alternative therapies for a post-antibiotic era. *International Journal of Molecular Sciences, 21*(3), 1061. DOI: 10.3390/ijms21031061

31. Ye, J., & Chen, X. (2022). Current promising strategies against antibiotic-resistant bacterial infections. *Antibiotics, 12*(1), 67. DOI: 10.3390/antibiotics12010067

32. Zhang, K., Li, X., Yu, C., & Wang, Y. (2020). Promising therapeutic strategies against microbial biofilm challenges. *Frontiers in Cellular and Infection Microbiology, 10*, 359.

Chapter - 7

AI in Eco-Friendly Sustainable Agriculture

Authors

Ramu Samanta
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata, West Bengal, India

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

Chapter - 7

AI in Eco-Friendly Sustainable Agriculture

Ramu Samanta and Semanti Ghosh

Abstract

The use of Artificial Intelligence (AI) in environmentally friendly, sustainable agriculture offers a game-changing chance to address the world's problems with resource efficiency, food security, and environmental sustainability. AI tools like data analysis, robot vision, and machine learning are being used to improve farming methods, lessen their negative effects on the environment, and boost output. AI assists farmers examine soil health, forecast weather patterns, and maximize water, fertilizer, and insecticide usage by allowing precision agriculture. With this accuracy, agricultural productivity is maximized, land is used efficiently, and the carbon impact is decreased while resource waste is minimized. Additionally, AI-powered solutions provide prompt responses that lessen the need for chemical substances by facilitating early identification of illnesses and pests. This helps biodiversity by encouraging more sustainable pest management techniques, in addition to improving crop performance. Furthermore, AI tools facilitate climate change adaptation through predictive modeling, assisting farmers in making well-informed choices on crop selection and agricultural practices in response to shifting climatic circumstances. AI-driven advancements, including self-governing drones and robots, lessen labor-intensive activities, improve energy efficiency, and enable current-time farm tracking, all of which help to sustain agriculture. There are still issues to be resolved, such as the requirement for better data infrastructure, increased accessibility of AI technology for smallholder farmers, and handling moral dilemmas pertaining to data ownership and security. All things considered, AI has the power to completely transform environmentally friendly, sustainable agriculture by fostering resilient food systems, decreasing environmental damage, and increasing resource efficiency. To fully realize AI's potential to achieve a sustainable agricultural future, researchers must keep up their efforts and collaborate with farmers, politicians, and technologists.

Keywords: AI, biodiversity monitoring, climate change mitigation, sustainable agriculture, waste management.

1. Introduction

An important global agenda item addressing resource conservation, destruction of the environment, and climate change is sustainable growth [1]. A vital industry for both the global economy and food security, agriculture has difficulties in becoming sustainable [2]. Boosting land productivity, resource efficiency, and reducing detrimental environmental effects, including greenhouse gas emissions and soil degradation, are some of these problems [3].

Inadequate trash management may lead to environmental contamination, health problems for the public, and the depletion of recyclable resources. Thus, it's also crucial to manage garbage effectively [4]. Artificial intelligence (AI) presents creative answers to these problems in the disposal of waste and sustainable agriculture [5]. AI may improve the efficacy and efficiency of procedures like resource optimization, illness diagnosis, and weather forecasting [6].

For instance, artificial intelligence (AI) in trash management may increase recycling rates by 25% and lower greenhouse gas emissions, while AI-based intelligent irrigation systems can increase agricultural yields while reducing water consumption by 30% [7]. Implementing AI confronts challenges despite its potential, such as a lack of technical expertise, aversion to change, and insufficient infrastructure [8].

This study is to investigate the role of artificial intelligence (AI) in the disposal of waste and sustainable agriculture, highlighting both potential and obstacles in its use [9]. The study evaluates the effects of the many AI technologies employed in these industries on productivity, resource utilization, and ecological sustainability [9].

The study assesses expert perspectives and effective case studies in order to offer stakeholders actionable advice on how to effectively implement artificial intelligence (AI) technology [10].

With an eye toward a more sustainable and greener future, this research aims to make a substantial contribution to the articles on sustainable development and provide useful insights for incorporating AI into initiatives for sustainable economic and environmental growth [11].

1.1 Concept of sustainable development

A development strategy known as "sustainable development" aims to satisfy current demands without jeopardizing the capacity of the next generation to satisfy their own [12]. The three primary foundations of this idea are social, economic, and environmental. Using biological fertilizer, effective water management, and other ecologically friendly agricultural techniques are some examples of how sustainable development in the agricultural sector seeks to boost agricultural land production without harming the ecosystem [13].

1.2 Artificial Intelligence (AI)

The goal of the computer science field of artificial intelligence (AI) is to build machines that are capable of activities that would typically need the intellect of humans [14]. Artificial neural networks, processing of natural languages, and machine learning are just a few of the technologies it encompasses. AI offers enormous promise for a wide range of uses, including medical diagnosis and weather forecasting [15].

AI is applied for agricultural purposes to maximize resource usage, identify diseases affecting plants, and forecast crop harvests. Artificial intelligence (AI) may be applied to waste management to reduce trash production, improve waste supply networks, and regulate recycling operations [16].

1.3 Sustainable farming

The goal of sustainable farming is to provide for the food and fiber requirements of the present without endangering the ability of next generations to provide for their own needs [17]. Crop rotation, the use of organic fertilizers, and effective utilization of water are some of these techniques [18]. Global warming, destruction of land, and the need to raise land productivity without harming biodiversity are major obstacles to sustainable agriculture [19].

1.4 Management of wastes

The process of gathering, moving, processing, and getting rid of garbage is called waste management [20]. Good waste management practices can lessen detrimental effects on the environment and general human health [21]. Recycling, composting, and the handling of liquid waste are just a few of the techniques used in eliminating waste [22]. The absence of suitable infrastructure, technical constraints, and public opposition to changes in waste policy are the primary obstacles in waste management [23].

1.5 AI integration for waste management and sustainable agriculture

AI provides creative answers to problems in waste management and organic farming [24]. AI in agriculture may be used to forecast agricultural yields, optimize the use of water and fertilizer, and monitor land and plant conditions in actual time [25]. Farmers can increase production efficiency and make better judgments with the use of this technology [26]. AI has applications in waste management, including recycling process automation, waste volume prediction, and waste shipment route optimization [27]. These systems can operate more effectively, spend less money, and have a smaller negative influence on the environment when AI is included [28].

1.6 Data gathering

Experts in the fields of waste management, AI technology, and agriculture were interviewed in-depth to gather primary data [29]. Among the participants were academics with firsthand knowledge of AI applications for sustainability, waste treatment managers, agricultural producers, and AI technologists [30]. In order to collect information from practitioners and users of AI technology in various sectors, a survey was also carried out. The survey focused on the different kinds of AI technologies employed, their perceived advantages, and the difficulties encountered. A study of pertinent literature, including scientific publications, company reports, and government sources, was used to collect secondary data [31]. This evaluation of the literature attempted to include the most recent advancements and trends in the use of AI to waste management and sustainable agriculture [32]. In order to understand the triumphs and difficulties encountered in implementing AI in various domains, case studies that have been published on the subject were also examined [33].

1.7 Information Analysis

Quantitative as well as qualitative techniques were used in analysis of information [34]. The data from deep conversations was subjected to thematic analysis in order to find recurring themes and trends pertaining to the application of AI [35]. Important insights into the viewpoints and experiences of specialists and practitioners were offered by this investigation [36]. In order to determine best practices and elements impacting the effective use of AI, various case studies were also examined [37]. Descriptive statistical methods were employed to examine the diversity of respondents, the kinds of AI technologies employed, and the perceived advantages and difficulties based on the quantitative data obtained from the surveys [38]. To investigate the

links between factors pertaining to the use of AI and the results attained such as improved productivity, lower costs, and less environmental impact linear regression analysis was carried out.

1.8 Using artificial intelligence in sustainable agriculture

According to this study, artificial intelligence (AI) has been used in sustainable agriculture for a number of purposes, such as resource optimization, yield prediction, and crop condition monitoring. The primary conclusions shown in Table 1 are listed below.

Table 1: Advantages and obstacles of AI in agriculture

Aspect crop surveillance	AI technology sensors, and flying cameras	Advantages timely illness identification pests and optimizing irrigation	Obstacles expensive and technical experts
Fore casted yield	Data examination for machine learning	Improved scheduling of production	Enough information
Optimization of resources	Approaches for efficiency	Water use that is effective effective use of fertilizer	Not enough infrastructure

Table 2 showing the case examples illustrating the difficulties and achievements of using AI in various settings.AI has improved water efficiency and raised agricultural yields by 30% in Japanese agriculture, but it still faces obstacles from expensive and inadequate internet connectivity. In waste management in Germany, AI has increased recycling efficiency by 40% and reduced emissions by 20%, but faces technological complexity and social resistance. AI has improved production and optimized fertilizer usage in Dutch agriculture, but it also has data limits and requires skill development.

Table 2: Successes and difficulties of AI implementation

Case study	Main successes	Main challenges
Japan's agricultural sector	Crop yields and water efficiency increase by 30%	Costs and limited internet facilities
German waste management	40% recycling increase 20% emission reduction and	Complexity of technology social opposition
Farming in the Netherlands	Optimizing fertilizer use and increasing productivity	Data restrictions, training in skills

1.9 Sustainable development consequences

According to the implications of these studies, artificial intelligence (AI) has a lot of potential to promote sustainable development by improving

waste management and agricultural productivity. However, a comprehensive strategy involving infrastructure investment, legislative support, and technology user skill development is required to reap the most benefits.

This study offers stakeholders insightful information about using AI to promote economic and environmental sustainability, along with helpful suggestions for resolving implementation-related issues.

Conclusion

To sum up, AI is changing the way of sustainable agriculture by enhancing efficiency, precision, and sustainable farming methods. With the help of sophisticated data analytics, machine learning and predictive modeling AI assists in resource optimization, crop health monitoring, pest control and climate resilience. This helps support sustainability by decreasing water, fertilizer, and pesticide use without loss of yield or even with increased yields. Farmers can shift to climate change resilience and react better to environmental shocks by adopting AI-enabled technologies such as precision farming, mechanization, and climate-smart agriculture. Nevertheless, despite its opportunities, the application of AI in agriculture comes with issues such as worries regarding data privacy, high investment cost in the beginning phase, and the lack of infrastructure facilities among rural areas. It is imperative that ethical questions surrounding the socio-economic impact of IP commercialization on smallholder farmers and inclusive technological access among local producers are addressed as technology for sustainable development advances to ensure equitable growth. Ongoing research and partnership between government, industry, and academia will remain important in developing scalable AI solutions that are widely accessible while remaining cost-effective. Solving these challenges will open doors to harnessing AI for sustainable agriculture, food security and environmental conservation for generations of the future.

Conflict of interest: None.

References

1. Wuisan, D. S., & Handra, T. (2023). Maximizing online marketing strategy with digital advertising. *Startupreneur Business Digital (SABDA Journal)*, *2*(1), 22-30.
2. Manongga, D., Rahardja, U., Sembiring, I., Lutfiani, N., & Yadila, A.B. (2022). Retracted (Di Tarik): Dampak Kecerdasan Buatan Bagi Pendidikan. *ADI Bisnis Digital Interdisiplin Jurnal*, *3*(2), 110-124.

3. Kurniawan, D. A., & Santoso, A. Z. (2020). Pengelolaan sampah di daerah sepatan kabupaten tangerang. *ADI Pengabdian Kepada Masyarakat*, *1*(1), 31-36.

4. Thamrin, R. M., Harahap, E. P., Khoirunisa, A., Faturahman, A., & Zelina, K. (2021). Blockchain-based land certificate management in indonesia. *ADI journal on recent innovation*, *2*(2), 232-252.

5. Guustaaf, E., Rahardja, U., Aini, Q., Maharani, H. W., & Santoso, N. A. (2021). Blockchain- based education project. *Aptisi Transactions on Management*, *5*(1), 46-61.

6. Bennet, D., Anjani, S.A., Daeli, O.P., Martono, D., & Bangun, C.S. (2024). Predictive Analysis of Startup Ecosystems: Integration of Technology Acceptance Models with Random Forest Techniques. *CORISINTA*, *1*(1), 70-79.

7. Kamil, M., Muhtadi, Y., Sentosa, B. M., & Millah, S. (2021). Tindakan Operasionalisasi Pemahaman Sains dan Teknologi Terhadap Islam. *Alfabet Jurnal Wawasan Agama Risalah Islamiah, Teknologi dan Sosial*, *1*(1), 16-25.

8. Wuisan, D.S.S., & Mariyanti, T. (2023). Analisa Peran Triple Helikdalam Mengatasi Tantangan Pendidikan di Era Industri 4.0. *Jurnal MENTARI: Manajemen, Pendidikan dan Teknologi Informasi*, *1*(2), 123-132.

9. Sivaraman, R., Lin, M.H., Vargas, M.I.C., Al-Hawary, S.I.S., Rahardja, U., Al-Khafaji, F.A.H., ... & Li, L. (2024). Multi-objective Hybrid System Development: To Increase the Performance of Diesel/Photovoltaic/Wind/Battery System. *Mathematical Modelling of Engineering Problems*, *11*(3).

10. Aini, Q., Manongga, D., Rahardja, U., Sembiring, I., & Li, Y. M. (2024). Understanding Behavioral Intentionto Useof Air Quality Monitoring Solutionswith Emphasison Technology Readiness. *International Journal of Human–Computer Interaction*, 1-21.

11. Supriati, R., Dewi, E. R., Supriyanti, D., & Azizah, N. (2022). Implementation Framework for Merdeka Belajar Kampus Merdeka (MBKM) in Higher Education Academic Activities. *IAICTransactions on Sustainable Digital Innovation (ITSDI)*, *3*(2), 150-161.

12. Sayyida, S., Hartini, S., Gunawan, S., & Husin, S. N. (2021). The impact of the COVID-19 pandemic on retail consumer behavior. *Aptisi Transactions on Management*, *5*(1), 79-88.

13. Rahardja, U. (2023). The economic impact of cryptocurrencies in Indonesia. *ADI Journal on Recent Innovation*, *4*(2), 194-200.

14. Yaniaja, A. K., Wahyudrajat, H., & Devana, V. T. (2020). Pengenalan model gamifikasi ke dalam e-learning pada perguruan tinggi. *ADI Pengabdian Kepada Masyarakat*, *1*(1), 22-30.

15. Argani, A., & Taraka, W. (2020). Pemanfaatan Teknologi blockchain untuk mengoptimalkan keamanan sertifikat pada perguruan tinggi. *ADI Bisnis Digit. Interdisiplin J*, *1*(1), 10-21.

16. Sana, E., Fitriani, A., Soetarno, D., & Yusuf, M. (2024). Analysis of User Perceptions on Interactive Learning Platforms Based on Artificial Intelligence. *CORISINTA*, *1*(1), 26-32.

17. Choi, L. K., Iftitah, N., & Angela, P. (2024). Developing technopreneur skills to face future challenges. *IAIC Transactions on Sustainable Digital Innovation (ITSDI)*, *5*(2), 127-135.

18. Nugroho, D., & Angela, P. (2024). The impact of social media analytics on sme strategic decision making. *IAIC Transactions on Sustainable Digital Innovation (ITSDI)*, *5*(2), 169-178.

19. Amsyar, I., Christopher, E., Dithi, A., Khan, A. N., & Maulana, S. (2020). The challenge of cryptocurrency in the era of the digital revolution: A review of systematic literature. *Aptisi Transactions on Technopreneurship (ATT)*, *2*(2), 153-159.

20. Sulistiani, H., Yuliani, A., & Hamidy, F. (2021). Perancangan Sistem Informasi Akuntansi Upah Lembur Karyawan Menggunakan Extreme Programming. *Technomedia Journal*, *6*(1 Agustus), 1-14.

21. Alam, T. (2020). Cloud Computing and its role in the Information Technology. *IAIC Transactions on Sustainable Digital Innovation (ITSDI)*, *1*(2), 108-115.

22. Pratama, E. N., Suwarni, E., & Handayani, M. A. (2022). The effect of job satisfaction and organizational commitment on turnover intention with person organization fit as moderator variable. *Aptisi Transactions on Management*, *6*(1), 74-82.

23. Rawat, B., Mehra, N., Bist,A. S.,Yusup, M.,& Sanjaya, Y. P.A. (2022). Quantum computing and ai: Impacts & possibilities. *ADI Journal on Recent Innovation*, *3*(2), 202-207.

24. Hardjosubroto, R., Rahardja, U., Santoso, N. A., & Yestina, W. (2020).

Penggalangan Dana Digital Untuk Yayasan Disabilitas Melalui Produk UMKMDi Era4.0.*ADIPengabdian Kepada Masyarakat*, *1*(1), 1-13.

25. Halisa, N. N. (2020). Peran manajemen sumber daya manusia" sistem rekrutmen, seleksi, kompetensi dan pelatihan" terhadap keunggulan kompetitif: Literature review. *ADI Bisnis Digital Interdisiplin Jurnal*, *1*(2 Desember), 14-22.

26. Kedah, Z. (2023). Use of e-commerce in the world of business. *Startupreneur Business Digital(SABDA Journal)*, *2*(1), 51-60.

27. Bist, A. S., Agarwal, V., Aini, Q., & Khofifah, N. (2022). Managing Digital Transformation in Marketing:"Fusionof Traditional Marketingand Digital Marketing".*International Transactionson Artificial Intelligence*, *1*(1), 18-27.

28. Lutfiani, N., & Meria, L. (2022). Utilization of big data in educational technology research. *International Transactionson Education Technology*,*1*(1),73-83.

29. Haryani, H., Wahid, S. M., & Fitriani, A. (2023). Analisa peluang penerapan teknologi blockchain dan gamifikasi pada pendidikan. *Jurnal MENTARI: Manajemen, Pendidikan dan Teknologi Informasi*, *1*(2), 163-174.

30. Arribathi, A. H., Supriyanti, D., Astriyani, E., & Rizky, A. (2021). Peran Teknologi Informasi Dalam Pendidikan Agama Islam Untuk Menghadapi Tantangan Di Era Global Dan Generasi Z. *Alfabet Jurnal Wawasan Agama Risalah Islamiah, Teknologi Dan Sosial*, *1*(1), 55-64.

31. Raharja, U., Sanjaya, Y. P., Ramadhan, T., Nabila, E. A., & Nasution, A. Z. (2024). Revolutionizing tourism in smart cities: Harnessing the power of cloud-based iot applications. *CORISINTA*, *1*(1), 41-52.

32. Aisyah, E. S. N., Hardini, M., & Riadi, B. (2021). Peran Teknologi Dalam Pendidikan Agama Islam Pada Globalisasi Untuk Kaum Milenial (Pelajar). *Alfabet Jurnal Wawasan Agama Risalah Islamiah, Teknologi dan Sosial*, *1*(1), 65-74.

33. Leffia, A., Anjani, S. A., Hardini, M., Sihotang, S. V., & Aini, Q. (2024). Corporate Strategies to Improve Platform Economic Performance: The Role of Technology, Ethics, and Investment Management. *CORISINTA*, *1*(1), 16-25.

34. Prawiyogi, A. G., & Anwar, A. S. (2023). Perkembangan Internet of

Things (Io T) pada Sektor Energi: Sistematik Literatur Review.*Jurnal MENTARI: Manajemen, Pendidikandan Teknologi Informasi*, *1*(2), 187-197.

35. Nusantoro, H., Sunarya, P. A., Santoso, N. P. L., & Maulana, S. (2021). Generation smart education learning process of blockchain-based in universities. *Blockchain Frontier Technology*, *1*(01), 21-34.
36. Rawat, B., & Purnama, S. (2021). My SQL Database Management System (DBMS) On FTP Site LAPAN Bandung. *International Journal of Cyber and IT Service Management*, *1*(2), 173- 179.
37. Ramadhona, N., Putri, A. A., & Wuisan, D. S. S. (2022). Students' Opinions of the Use of Quipper School as an Online Learning Platform for Teaching English. *International Transactions on Education Technology*, *1*(1), 35-41.
38. Wahyudi,M., Meilinda,V., & Khoirunisa,A.(2022). The Digital Economy's Useof Big Data. *International Transactionson Artificial Intelligence*,*1*(1),62-70.

Chapter - 8

Antibiotics and Prawn Culture: A Mini Review

Authors

Sandipan Taki
Department of Biochemistry, University of Calcutta, Kolkata, West Bengal, India

Trisheeta Koley
Department of Biochemistry, University of Calcutta, Kolkata, West Bengal, India

Sayani Saha
Department of Biochemistry, University of Calcutta, Kolkata, West Bengal, India

Debabrata Paria
Department of Biotechnology, School of Life Science, Swami Vivekananda University, Kolkata, West Bengal, India

Srijan Haldar
Department of Biotechnology, School of Life Science, Swami Vivekananda University, Kolkata, West Bengal, India

Chapter - 8

Antibiotics and Prawn Culture: A Mini Review

Sandipan Taki, Trisheeta Koley, Sayani Saha, Debabrata Paria and Srijan Haldar

Abstract

The use of antibiotics in prawn culture is a widespread practice aimed at controlling bacterial infections and promoting faster growth. However, this approach raises significant environmental and health concerns due to the potential for antibiotic resistance, residue accumulation, and ecosystem impacts. Antibiotics such as tetracyclines, quinolones, and sulfonamides are commonly administered in prawn aquaculture, but excessive or improper use can lead to the development of resistant bacterial strains, which poses a risk to both aquatic life and human health. Moreover, antibiotic residues may persist in prawn tissues, raising concerns about food safety and potential allergic reactions among consumers. The environmental impact is also substantial; antibiotic residues discharged into surrounding waters can disrupt microbial communities, harm non-target species, and contribute to the spread of resistance genes. Sustainable alternatives, such as probiotics, prebiotics, and herbal extracts, are being explored as safer, environmentally friendly solutions to disease control in aquaculture. Implementing stringent guidelines for antibiotic use, promoting best practices, and raising awareness among prawn farmers are essential for balancing prawn health with environmental preservation and public health safety. This review highlights the need for sustainable practices to minimize the negative impact of antibiotics on prawn aquaculture and associated ecosystems.

Keywords: Antibiotics, prawn culture, human health, alternative process.

Introduction

Prawn culture is popular in mariculture and monoculture: Mariculture is a specialized branch of aquaculture focused on cultivating marine organisms in the open ocean, coastal areas, or artificial environments specifically for food production and other valuable goods like fish oil. This practice includes a diverse range of species, such as prawns, oysters, betake, and mullets,

which are cultivated to meet the demand for seafood and marine-based products. A prominent area within mariculture is prawn mariculture, which involves the production of saltwater prawns under a controlled environment, maximizing growth and maintaining the health of the stock. (New, M. B., 1995). The prawn mariculture process is divided into three key phases: maturation and reproduction, hatchery, and grow-out. During the maturation and reproduction phase, broodstock are developed in optimal conditions to encourage successful reproduction. In the hatchery phase, larval prawns are nurtured in carefully monitored tanks until they reach a suitable size for grow-out, where they are then transferred to larger enclosures or ocean cages. The goal of prawn mariculture spans three essential roles: providing food for human consumption, supplying bait for recreational fishing industries, and creating seedstock to support and boost natural prawn populations. As mariculture develops, one technique within aquaculture that has grown in popularity is monoculture, which refers to the cultivation of a single species in a controlled system. Monoculture allows for simplified monitoring of the organisms' growth, health, and overall performance, as all the inhabitants of a pond or tank share similar requirements, reducing the complexity of managing multiple species with varied needs. However, monoculture's lack of biodiversity can also lead to challenges such as water quality degradation and potential disease spread, as high-density environments foster conditions where pathogens can thrive. To manage these conditions effectively, monoculture fish farming involves specific steps, including pond construction tailored to the species, selective breeding practices, and the careful transfer of fry and fingerlings between ponds to accommodate growth stages. Generally, the monoculture cycle for prawns extends over 6 to 8 months, though this period can be longer in polyculture systems that support multiple species coexisting in the same habitat. Growth rates of prawns under monoculture, can range from 50 grams to 200 grams depending on factors such as water quality, feeding practices, stocking density, and the duration of cultivation. These controlled practices, particularly in prawn monoculture, enable a predictable output, making it a preferred choice for commercial prawn farmers who aim to meet high market demand consistently (New, M. B., 1995).

Antibiotics in prawn culture

The use of antibiotics in prawn culture has been widespread due to their effectiveness in managing bacterial diseases, which can significantly impact productivity and profitability in aquaculture. In prawn culture, antibiotics

play a critical role in combating bacterial infections, such as vibriosis, caused by *Vibrio* species, which are responsible for significant mortality rates.Commonlyusedantibioticsinprawncultureincludeoxytetracycline,chloramphenicol, enrofloxacin, and sulfonamides. These antibiotics are administered either through medicated feed or added directly to the water to treat infections and prevent disease outbreaks, thereby enhancing growth rates and survival in prawn populations (Palaniyappan, V., Nagalingam, A. K., Ranganathan, H. P., Kandhikuppam, K. B., Kothandam, H. P., & Vasu, S., 2013).

However, while antibiotics provide immediate benefits in disease control, their extensive use raises several concerns, especially regarding Antimicrobial Resistance (AMR) (Paul, R., 2024). The repeated use of antibiotics can lead to the development of resistant bacterial strains in the aquatic environment, which may not only affect the cultured prawns but also spread to other aquatic organisms, and potentially, humans through the food chain (Manage, P. M. 2018). This has resulted in international regulatory bodies, including the World Health Organization (WHO) and Food and Agriculture Organization (FAO), advocating for the cautious and responsible use of antibiotics in aquaculture. Many countries now enforce regulations on the types and concentrations of antibiotics permitted in aquaculture to mitigate the risks associated with AMR (Paul, R., 2024).

In addition to AMR, antibiotic residues in prawn products can pose risks to consumer health. Antibiotic residues can accumulate in prawn tissues, and if these residues exceed safe levels, they may have toxic effects when ingested by humans. For instance, residues of chloramphenicol, an antibiotic banned in many countries for its potential adverse health effects, have been detected in aquaculture products due to its past use in prawn culture. This has led to stricter monitoring and testing of seafood products before they reach the market, as well as the promotion of withdrawal periods after antibiotic treatment to allow time for the residues to decompose before harvest (Palaniyappan, V., Nagalingam, A. K., Ranganathan, H. P., Kandhikuppam, K. B., Kothandam, H. P., & Vasu, S., 2013).

To address these challenges, prawn farmers are increasingly exploring alternative methods to manage disease in aquaculture without heavy reliance on antibiotics. Probiotics, prebiotics, and immunostimulants are some of the alternative measures being investigated to boost the prawn's natural immune response, making them less susceptible to infections. In addition, biosecurity practices such as controlling water quality, maintaining optimal

environmental conditions, and limiting the density of prawns in culture systems can reduce stress and the likelihood of disease outbreaks, thereby reducing the need for antibiotic treatments.

Best practices in prawn culture emphasize responsible use and adherence to guidelines for antibiotic application. For instance, prawn farmers are encouraged to avoid using antibiotics as preventive measures and instead use them only when necessary, with proper diagnosis and veterinaryguidance.Antibioticrotationandlimitingtreatmentdurationcanalsohelp minimize the risks associated with AMR development. Furthermore, adherence to proper withdrawal periods before harvesting ensures that prawn products meet food safety standards (Hemamalini, N., Shanmugam, S. A., Kathirvelpandian, A., Deepak, A., Kaliyamurthi, V., & Suresh, E., 2022).

Table 1: Antibiotics are commonly used in prawn culture to prevent and treat bacterial infections

Antibiotics	Diseases	Pathogens
1. Chloramphenicol	Filamentous bacteria	*Leucothrixmucor*
2. Oxytetracycline	Vibrio infections	*Vibrio parahaemolyticus*
3. Erythromycin	Luminous bacteria	Vibrioharvey
4. Formalin	Protozoanciliates	*Zoothamnium* spp., *Epistylis* spp.,
5. Malachitegreen	Luminous bacteria	*Vibrio harvey*
6. Formalin+malachite green	Bacterialdiseases,	*Pseudomonasspp.*
7. Potassium permanganate	Luminous bacteria,	*Vorticella*spp.,*Acineta*spp.,
8. Methyleneblue	Protozoanciliates	*Zoothamnium* spp., *Epistylis* spp.,

Alternative of antibiotic usage in prawn culture

Alternative approaches to antibiotics in prawn culture focus on enhancing disease resistance and managing health through eco-friendly and sustainable practices. One major alternative is the use of probiotics, which are beneficial bacteria introduced into the prawn's environment or feed. Probiotics help suppress pathogenic bacteria by competing for resources and producing antibacterial substances, thus promoting a balanced microbial environment and enhancing the prawn’s immune system. Prebiotics, which serve as food for beneficial bacteria, can be combined with probiotics (synbiotics) to further bolster health and resilience (Romero, J., Feijoó, C. G., & Navarrete, P., 2012).

Another effective alternative is the application of immunostimulants, natural compounds that activate the prawn's immune response. These include substances like beta-glucans and certain herbal extracts that strengthen prawns' natural defenses, making them more resistant to infections. Vaccination, although still under development for certain prawn species, is another promising method that could provide long-term protection against specific diseases without impacting the environment.

Biosecurity measures also play a critical role in disease prevention. Maintaining optimal water quality, controlling stocking density, and practicing stringent sanitation in prawn farms can significantly reduce disease outbreaks, decreasing the need for antibiotics. Furthermore, using herbal and plant extracts like neem and garlic has shown antimicrobial effects against pathogens in prawns, offering a natural, residue-free solution (Naiel, M. A., Ghazanfar, S., Negm, S. S., Shukry, M., & Abdel-Latif, H. M., 2023).

These alternatives support sustainable prawn farming, improving production efficiency while minimizing health risks associated with antibiotic residues and antimicrobial resistance, making aquaculture safer for both consumers and the environment.

References

1. New, M.B. (1995). Status of freshwater prawn farming: A review. *Aquaculture research*, *26*(1), 1-54.
2. Paul, R. (2024).*AMR in aquaculture: Enhancing Indian shrimp exports through sustainable practices and reduced antimicrobial usage* (No. 209). Research Paper.
3. Palaniyappan, V., Nagalingam, A. K., Ranganathan, H. P., Kandhikuppam, K. B., Kothandam, H. P., & Vasu, S. (2013). Antibiotics in South Indian coastal sea and farmed prawns (Penaeus monodon). *Food Additives & Contaminants: Part B*, *6*(3), 196-199.
4. Hemamalini, N., Shanmugam, S. A., Kathirvelpandian, A., Deepak, A., Kaliyamurthi, V., & Suresh, E. (2022). A critical review on the antimicrobial resistance, antibiotic residue and metagenomics-assisted antimicrobial resistance gene detection in freshwater aquaculture environment. *Aquaculture Research*, *53*(2), 344-366.
5. Manage, P. M. (2018). Heavy use of antibiotics in aquaculture; emerging human and animal health problems–a review.

6. Mithuna, R., Tharanyalakshmi, R., Jain, I., Singhal, S., Sikarwar, D., Das, S., ... & Das, B. (2024). Emergence of antibiotic resistance due to the excessive use of antibiotics in medicines and feed additives: a global scenario with emphasis on the Indian perspective. *Emerging Contaminants*, 100389.

7. Romero, J., Feijoó, C. G., & Navarrete, P. (2012). Antibiotics in aquaculture–use, abuse and alternatives. *Health and environment in aquaculture*, *159*(1), 159-198.

8. Naiel, M. A., Ghazanfar, S., Negm, S. S., Shukry, M., & Abdel-Latif, H. M. (2023). Applications of antimicrobial peptides (AMPs) as an alternative to antibiotic use in aquaculture–A mini-review. *Annals of Animal Science*, *23*(3), 691-701.

Chapter - 9

Antibiotics Targeting the Cell Wall of Gram-Negative Bacteria

Authors

Aritra Chatterjee
Department of Biochemistry, University of Calcutta, Kolkata, West Bengal, India

Sumita Mal
Department of Biochemistry, University of Calcutta, Kolkata, West Bengal, India

Priyajit Banerjee
Department of Biochemistry, University of Calcutta, Kolkata, West Bengal, India

Bidisha Ghosh
Department of Biochemistry, University of Calcutta, Kolkata, 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

Antibiotics Targeting the Cell Wall of Gram-Negative Bacteria

Aritra Chatterjee, Sumita Mal, Priyajit Banerjee, Bidisha Ghosh, Subhasis Sarkar and Suranjana Sarkar

Abstract

Gram-negative bacteria have a complex cell wall structure that provides resistance to many conventional antibiotics. This paper examines antibiotics that specifically act on the cell wall of gram-negative bacteria, discussing their mechanisms, effectiveness, and clinical uses. Key antibiotics, such as β-lactams and glycopeptides, interfere with the synthesis of the bacterial cell wall, causing cell breakdown and death. However, the outer membrane of gram-negative bacteria poses significant barriers to antibiotic penetration, requiring novel approaches to improve drug effectiveness. With the rise of antibiotic resistance, research into developing and optimizing cell wall-targeting treatments for gram- negative infections remains crucial.

Keywords: Antibiotics, cell wall synthesis, gram-negative bacteria, resistance, β-lactams

Introduction

The ongoing advancements in microbiology have underscored the remarkable impact antibiotics have had in medicine. These substances have revolutionized the treatment of bacterial diseases, dramatically improving public health by enabling the elimination of once-deadly infections. The first antibiotic, Salvarsan, was introduced in 1910, followed by the discovery of penicillin in 1928, which marked the beginning of the "golden era" of antibiotics. This period saw the emergence of a variety of antibiotics, with their use peaking in the 1950s, leading to significant increases in life expectancy due to effective infection control.

Antibiotics have played a crucial role in extending human lifespans by curing infections directly, which also limited the transmission of diseases from person to person. However, the widespread use of antibiotics has led to

increasing cases of misuse, contributing to the rise of antibiotic resistance. As a result, some bacterial infections have become difficult or impossible to treat, creating a global health crisis. Antibiotics also face the challenge of losing their effectiveness over time as bacteria evolve resistance mechanisms.

Antibiotics typically target the bacterial cell wall. Bacteria are classified as Gram-negative or Gram-positive based on their cell membrane structure. Gram-negative bacteria are characterized by an outer membrane consisting of lipopolysaccharides, which can be affected by certain antibiotics that inhibit their synthesis. On the other hand, Gram-positive bacteria contain phosphatidylglycerol, a lipid component, which can be modified by bacterial enzymes to resist polycationic antimicrobial agents. Blocking these enzymes can help mitigate bacterial resistance.

In recent years, Gram-negative bacteria have become a significant concern due to their high resistance to antibiotics. This resistance is largely due to their outer membrane, which contains lipopolysaccharides. As antibiotic and antimicrobial usage has risen, Gram-negative bacteria have developed increasing resistance. These bacteria can be classified as Multi-Drug Resistant (MDR), Extensively Drug-Resistant (XDR), or pan-drug resistant (PDR). For example, the XDR strain of *Pseudomonas aeruginosa*, resistant to carbapenems (the last-line antibiotics), is becoming more prevalent.

Research has revealed that the development of new antibiotics has slowed dramatically due to a combination of scientific and economic challenges. Over the past two decades, only six new antibiotic classes have been approved, none of which are effective against Gram-negative bacteria. This has exacerbated the global health crisis, as Gram- negative bacteria continue to evolve resistance. A natural product, darobactin, was discovered to specifically target Gram-negative bacteria by binding to Bam A, a cell wall protein. However, mutations in Bam A have led to the development of resistance to darobactin. Similarly, colistin, one of the last-resort antibiotics, is facing resistance due to the spread of the mobile mcr-1 gene, which confers resistance to colistin and other antibiotics, further complicating treatment options (Impey et al., 2020; Epand et al., 2016).

Gram-negative bacterial cell structure

Gram-negative bacteria are identified by their red or pink coloration when subjected to the Gram stain. They consist of a peptidoglycan cell wall located between an inner cytoplasmic membrane and an outer membrane.

The outer membrane

The outer membrane is a distinguishing feature of Gram-negative bacteria and is absent in Gram-positive bacteria. This membrane is a non-phospholipid bilayer with an asymmetric distribution of lipids. The outer leaflet contains glycolipids, Specifically Lipopolysaccharides (LPS), which consist of lipid A, a core polysaccharide, and an O antigen. Lipopolysaccharides are highly charged and play a role in causing septicemia and endotoxic shock. The inner leaflet of the outer membrane consists of phospholipids that help maintain cell integrity. The membrane also contains two types of proteins: lipoproteins and beta-barrel proteins. Lipoproteins are embedded in the inner membrane, and the outer membrane is semipermeable, thanks to the presence of porins that selectively allow molecules to pass through (Costerton et al., 1974).

The peptidoglycan cell wall

The peptidoglycan layer consists of repeating disaccharide units of N-acetylglucosamine and N-acetylmuramic acid, cross-linked by pentapeptide side chains. This structure helps the cell withstand high turgor pressure, extreme temperatures, and varying pH levels. It provides rigidity and helps maintain the shape of the cell, preventing osmotic lysis. The outer membrane is connected to the peptidoglycan layer by lipoproteins, such as Braun's lipoprotein (Beveridge, 2019).

The periplasm

The periplasm is the space between the two membranes in Gram-negative bacteria. It contains macromolecules, enzymes, and binding proteins involved in metabolic processes. The periplasm plays a role in protein transport, signaling, and the exclusion of toxins by isolating harmful enzymes (Silhavy et al., 2010).

The Inner Membrane

The inner membrane is a phospholipid bilayer that houses various proteins responsible for energy production, protein secretion, nutrient transport, and lipid biosynthesis. Gram-negative bacteria lack intracellular organelles, so all vital functions occur in this membrane. In *Staphylococcus aureus*, key phospholipids include phosphatidylglycerol, lysyl-phosphatidylglycerol, and cardiolipin (Silhavy et al., 2010).

Membrane vesicles

Another unique feature of Gram-negative bacteria is the production of outer membrane vesicles. These small particles are released from the

bacterial surface during growth and contain periplasmic components, lipopolysaccharides, and outer membrane proteins (Beveridge, 2019).

Pathogenicity and virulence factors

Pathogenicity refers to the ability of a microorganism to cause disease. Gram-negative bacteria employ a variety of virulence factors that contribute to their ability to infect and persist in the host. These factors are crucial for the bacteria's survival in the host environment and their ability to cause disease. Gram-negative bacteria, in particular, produce several virulence factors that enhance their ability to invade, colonize, and evade the host immune system. These factors serve as key targets for the development of antimicrobial treatments and vaccines. Infections caused by Gram-negative bacteria are frequently observed in patients with chronic conditions like Chronic Obstructive Pulmonary Disease (COPD). These bacteria adapt to the host and utilize these factors to facilitate infection and disease progression.

Escherichia coli

Uropathogenic strains of *Escherichia coli* are associated with urinary tract infections, causing conditions such as cystitis, prostatitis, and pyelonephritis, and can also lead to bloodstream infections. These strains possess specific Virulence factors that enable them to adhere to the urinary tract and invade the tissues. Key virulence factors include hemolysin, P-fimbriae, cytotoxic necrotizing factor, S-fimbriae, and siderophore systems, all of which are located within pathogenicity islands on the bacterial chromosome (Johnson, 1991).

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a versatile pathogen that can cause pneumonia and chronic lung disease, particularly in patients with cystic fibrosis. It utilizes a range of virulence factors, including elastase, phospholipase C, proteases, exotoxin A, pyocyanin, exoenzyme S, rhamnolipids, lipopolysaccharides, flagella, and pili. These factors contribute to its ability to establish infections and evade immune responses. The bacterium's virulence is enhanced by its mobility and its ability to resist antibiotics such as polymyxin B, ciprofloxacin, and gentamicin (Cepas & Soto, 2020).

Salmonella enterica

Salmonella enterica serovar Typhimurium primarily infects the epithelial cells of the intestine and is responsible for gastrointestinal

diseases. Certain strains of *Salmonella* exhibit invasive properties, allowing them to penetrate the intestinal walls and enter the bloodstream. The pathogen also spreads by invading phagocytes and utilizing the Salmonella pathogenicity islands (SPIs). Major virulence factors include the SLT virulence plasmid, flagella, adhesins, and biofilm-associated proteins, all of which play critical roles in the bacterium's pathogenicity (Fàbrega & Vila, 2013).

Haemophilus influenzae

Haemophilus influenzae, a small gram-negative coccobacillus, is a common cause of infections like meningitis, sinusitis, otitis media, and epiglottitis, particularly in children. It can also lead to lower respiratory tract infections, bacteremia, and ocular infections. The virulence factors of *H. influenzae* include its capsule, pili, adhesion proteins, Ig A1 protease, outer membrane proteins, and lipooligosaccharides. These factors help the bacterium invade host tissues and elicit inflammatory responses, significantly contributing to its pathogenicity. Even non-encapsulated strains of *H. influenzae* carry important virulence factors that are being studied for vaccine development (Kostyanev & Sechanova, 2012).

Antibiotics against Gram-negative bacteria cell wall

Gram-negativebacteriaposeasignificantglobalhealththreatduetotheirpotentpathogenicityandvirulencefactors. Their robust cell wall structure contributes to their high resistance to antibiotics. Researchers are continuously exploring new antibiotics that can effectively target and disrupt their cell wall, leading to bacteriallysis. Some of the antibiotics with efficacy against these bacteria include:

Beta lactams

Beta-lactam antibiotics are widely used to treat severe infections caused by gram-negative bacteria, such as sepsis and bloodstream infections. These antibiotics work by binding to penicillin-binding proteins (PBPs), inhibiting their function, and preventing the cross-linking of peptidoglycan layers in the bacterial cell wall. This disrupts cell wall integrity, causing the bacteria to burst due to increased osmotic pressure (Hasan, 2021).

Aminoglycosides

Aminoglycosides are protein synthesis inhibitors effective against aerobic gram-negative bacteria. These antibiotics, such as 2-

deoxystreptamine aminoglycosides, bind to the 16S ribosomal subunit, interfering with the binding of aminoacyl-t RNA. This disrupts m RNA translation, causing misreading of the RNA and the production of dysfunctional proteins (Trylska & Kulik, 2016).

Fluoroquinolones

Fluoroquinolones inhibit DNA replication and transcription in gram-negative bacteria. They target DNA gyrase and topoisomerase IV, essential enzymes for DNA replication, segregation, and catenation. By binding to these enzymes, fluoroquinolones prevent DNA processes from occurring, leading to bacterial cell death (Jia & Zhao, 2021).

Macrolides

Macrolide antibiotics possess strong immunomodulatory effects and are used to treat diseases like chronic obstructive pulmonary disease (COPD), cystic fibrosis, asthma, and bronchiectasis. These antibiotics bind to the 50S ribosomal subunit, inhibiting peptide chain elongation and disrupting ribosomal translation, which results in the premature termination of protein synthesis (Pollock & Chalmers, 2021).

Sulphonamides

Sulphonamide antibiotics inhibit the synthesis of folic acid, a critical compound for bacterial growth. They compete with para-aminobenzoic acid (PABA) for binding to dihydropteroate synthase (DHPS), preventing the conversion of PABA to dihydrofolic acid. This inhibition reduces the production of tetrahydrofolic acid (THF), which is required for the synthesis of purines, pyrimidines, and amino acids (Venkatesan et al., 2023).

Polymyxins

Polymyxin antibiotics are often reserved as a last-line treatment for multi-drug-resistant gram-negative infections. These antibiotics work by binding to lipopolysaccharides in the outer membrane, increasing membrane permeability. This allows antimicrobial peptides to enter the cell, causing the release of cellular contents and disrupting cellular processes. Polymyxins also damage the inner membrane, inhibiting lipid synthesis and disrupting the membrane potential (Sabnis & Edwards, 2023).

Other antibiotics

Other antibiotics, such as tigecycline, eravacycline, and glycylcyclines, target the bacterial ribosome. These antibiotics bind to the 30S ribosomal

subunit, blocking the interaction between aminoacyl-t RNA and the ribosome. This interference prevents the incorporation of amino acids into the growing peptide chain, halting protein synthesis (Zhanel et al., 2020).

These antibiotics represent a diverse array of strategies to combat Gram-negative bacterial infections, offering potential solutions to overcome bacterial resistance.

Antibiotic resistance of Gram-negative bacteria

With the discovery of antibiotics targeting cell wall of gram-negative bacteria, these bacteria slowly evolved and became resistant to antibiotics. They generated various mechanisms to become immune to these antibiotics making them more vulnerable to causing harmful diseases and increasing the global health crisis.

Table 1: List of antibiotic resistance mechanism

Antibiotics	Resistant bacteria	Resistance mechanism	References
Betalactam • Extended-Spectrum Beta-Lactamases (ESBLs) resist third-generation cephalosporins. • Carbapenem-Resistant Enterobacteriaceae (CRE) resist carbapenems. • Metallo-Beta-Lactamases (MBLs) resist cephalosporins and monobactams.	• *Staphylococcusaereus* • *Escherichia coli*	• Beta-lactamase enzymes hydrolyze the beta-lactam ring and inactivate antibiotics like TEM-1, SHV-1, and CTX-M. • Altered Penicillin–Binding Proteins (PBPs) cause reduced affinity for beta-lactams. Example: Penicillin-Binding Protein 2a of Methicillin-Resistant Staphylococcus aureus (MRSA). • Reduction of outer membrane permeability leads to decreased antibiotic uptake. Example: Porin mutations (OmpF, OmpC). • Increase in efflux pumps allows active removal of antibiotics. Example: *Escherichia coli*. • Genetic mutations can alter the target sites of antibiotic-binding proteins.	Thomson, 2013 Bush, Bradford, 2016 Jones *at el.*, 2005
Aminoglycosides	• *Pseudomonas*	• Aminoglycoside-	Ramirez,

	• *aeruginosa* • *Acinetobacter* • *baumanii*	modifying enzymes (AMEs) inactivate antibiotics. *Example:* ApmA, a unique aminoglycoside antibiotic acetyltransferase, inactivates Apramycin. • Increase in efflux pumps facilitates antibiotic removal from the cell. • Ribosomal mutations alter target sites, thereby reducing antibiotic binding. *Example:* 16S rRNA methylation.	Tolmasky, 2010 Serpersu *et al.*, 2008
Fluoroquinolones	• *Neisseriagonorrhoeae* • *Escherichia* • *coli*	• Target modification caused by mutations in DNA gyrase (*gyrA*, *gyrB*) or in topoisomerase IV (*parC*, *parE* proteins). • Active removal of fluoroquinolones through MexAB-OprM efflux pumps. • Plasmid-mediated resistance through quinolone-resistant genes encoding proteins that protect DNA gyrase. • Horizontal gene transfer through acquisition of resistance genes. *Example:* qnrA, qnrB, and qnrS.	Zhao *et al.*, 2020 Shariati *et al.*, 2022
Macrolides	*Staphylococcus aureus*	• Target modification by mutations in 23S rRNA. • Active removal of macrolides from bacterial cells through the MexAB-OprM efflux pump. • Enzymatic inactivation through macrolide esterase, which inhibits macrolide antibiotics. • Horizontal gene transfer through acquisition of resistance genes. • Example: Methionine sulfoxide reductase A	Laslop, Mankin, 2018 Wang *et al.*, 2023

		and Methionine sulfoxide reductase B.	
Sulphonamides	• *Escherichia coli* • *Klebsiella pneumoniae*	• Target modification by mutations in the dihydropteroate synthase (DHPS) enzyme. • Increase in enzyme production by overexpression of DHPS. • Decrease in sulphonamide uptake into the bacterial cell due to porin or outer membrane modification. • Horizontal gene transfer through acquisition of resistance genes. • Examples: sul1, sul2, sul3.	Venkatesan *et al.*, 2023 Kim *et al.*, 2019
Polymyxins	• *Pseudomonas aeruginosa* • *Escherichia coli*	• Target modification by structural changes in lipopolysaccharides (LPS), which reduce polymyxin binding. • Active removal of polymyxin from bacterial cells by efflux pumps. • Addition of phosphoethanolamine transferase (PEA) to lipopolysaccharide, reducing polymyxin binding and increasing resistance polymyxinaffinity • Reduced uptake of polymyxin by permeability change.	Falagas *et al.*, 2010 Poirel *et al.*, 2017
Other Antibiotics	• *Klebsiella pneumoniae* • *Enterobacter cloacae*	• Ribosomal mutations, such as mutations in the rpsJ gene (encoding S10 ribosomal protein) and the rpsC gene (encoding S3 ribosomal protein), cause resistance toward tigecycline and tetracycline. • MexAB-OprM efflux pump contributes to resistance to tigecycline.	Sun *et al.*, 2019 Luchao Lv *et al.*, 2020

Protein engineering intervention

Protein engineering has great potential to enhance the effectiveness of

antibiotics aimed at the cell wall of gram- negative bacteria through various advanced techniques. One strategy involves improving antibiotic penetration by creating peptides or proteins that interact with porins in the bacterial outer membrane. These engineered antimicrobial peptides can disrupt membrane integrity or facilitate new pathways for antibiotic entry. Additionally, targeting resistance mechanisms is crucial; engineered proteins can inhibit efflux pumps, such as the Acr AB-Tol C system, which expel antibiotics from the bacterial cell. Modifying existing antibiotics, like β-lactams, through directed evolution or rational design can improve their ability to evade β-lactamases or enhance binding to penicillin-binding proteins (PBPs) that are essential for cell wall synthesis.

Moreover, protein engineering can focus on increasing the binding affinity of antibiotics to their targets by employing structure-guided design and mutagenesis, utilizing techniques like X-ray crystallography to inform these modifications. Combining different antibiotic properties into a single molecule through hybridization can also lead to synergistic effects, enhancing bacterial inhibition. Additionally, designing engineered enzymes, such as lysins or peptidoglycan hydrolases, can target components of the gram-negative cell wall, breaking down peptidoglycan and increasing susceptibility to other antibiotics. Finally, the use of nanobodies, which are small, single-domain antibodies, can neutralize bacterial toxins or surface proteins, further improving antibiotic delivery. By integrating these innovative protein engineering strategies, researchers can develop a new generation of antibiotics specifically designed to tackle gram-negative bacterial infections, effectively addressing the increasing challenge of antibiotic resistance.

Conclusion

Gram-negative bacteria, with their complex cell wall structure, present significant challenges in antibiotic treatment, necessitating ongoing development of targeted therapies. Antibiotics like β-lactams and glycopeptides work by disrupting cell wall synthesis, but their effectiveness is limited by the protective outer membrane of these bacteria. Protein engineering offers a promising solution, enabling the creation of novel antibiotic compounds or proteins that can better penetrate bacterial defenses and enhance antibiotic action. By modifying existing proteins or designing new ones, protein engineering may also help in overcoming antibiotic resistance. As resistance continues to grow, these innovative approaches will be crucial for maintaining effective treatments against Gram-negative

infections. Continued research into protein-engineered solutions could unlock new therapeutic potentials to address the rising threat of resistant bacterial strains.

References

1. Jia Y, Zhao L. The antibacterial activity of fluoroquinolone derivatives: An update (2018-2021). Eur J Med Chem. 2021 Nov 15;224:113741
2. Beveridge TJ.1999.Structuresof Gram-Negative Cell Walls and Their Derived Membrane Vesicles.JBacteriol181:.
3. Breijyeh, Zeinab et al. “Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It.” *Molecules (Basel, Switzerland)* vol. 25,6 1340. 16 Mar. 2020.
4. Bush K, Bradford PA. β-Lactams and β-Lactamase Inhibitors: An Overview. Cold Spring Harb Perspect Med. 2016 Aug 1;6(8):a025247.
5. Cepas V, Soto SM. Relationship between Virulence and Resistance among Gram-Negative Bacteria. Antibiotics (Basel). 2020 Oct 20;9(10):719.
6. Costerton, J. W., J. M. Ingram, and K. J. Cheng. "Structure and function of the cell envelope of gram-negative bacteria." *Bacteriological reviews* 38.1 (1974): 87-110.
7. Dos Santos AMP, Ferrari RG, Conte-Junior CA. Virulence Factors in Salmonella Typhimurium: The Sagacity of a Bacterium. Curr Microbiol. 2019 Jun;76(6):762-773.
8. Epand, R. M., Walker, C., Epand, R. F., &Magarvey, N. A. (2016). Molecular mechanisms of membrane targeting antibiotics. *Biochimicaet Biophysica Acta (BBA)-Biomembranes*, *1858*(5), 980-987.
9. Fàbrega A, Vila J. Salmonella entericaserovar Typhimurium skills to succeed in the host: virulence and regulation. Clin Microbiol Rev. 2013 Apr;26(2):308-41.
10. Falagas ME, Rafailidis PI, Matthaiou DK. Resistance to polymyxins: Mechanisms, frequency and treatment options. Drug Resist Updat. 2010 Aug-Oct;13(4-5):132-8.
11. Hutchings, Matthew I., Andrew W. Truman, and Barrie Wilkinson. "Antibiotics: past, present and future." *Currentopinion in microbiology* 51 (2019): 72-80.

12. Impey, R.E.; Hawkins, D.A.; Sutton, J.M.; Soares da Costa, T.P. Overcoming Intrinsic and Acquired Resistance Mechanisms Associated with the Cell Wall of Gram-Negative Bacteria. *Antibiotics* **2020**, *9*, 623.

13. Johnson JR. 1991. Virulencefactorsin *Escherichia coli* urinary tract infection. Clin Microbiol Rev4.

14. Jones RN, Biedenbach DJ, Sader HS, Fritsche TR, Toleman MA, Walsh TR. Emerging epidemic of metallo-beta- lactamase-mediated resistances. Diagn Microbiol Infect Dis. 2005 Feb;51(2):77-84.

15. Kim DW, Thawng CN, Lee K, Wellington EMH, Cha CJ. A novel sulfonamide resistance mechanism by two- component flavin-dependent monooxygenase system in sulfonamide-degrading actinobacteria. Environ Int. 2019 Jun;127:206-215.

16. Kostyanev TS, Sechanova LP. Virulence factors and mechanisms of antibiotic resistance of haemophilus influenzae. Folia Med (Plovdiv). 2012 Jan-Mar;54(1):19-23.

17. Lv L, Wan M, Wang C, Gao X, Yang Q, Partridge SR, Wang Y, Zong Z, Doi Y, Shen J, Jia P, Song Q, Zhang Q, Yang J, Huang X, Wang M, Liu JH. Emergence of a Plasmid-Encoded Resistance-Nodulation-Division Efflux Pump Conferring Resistance to Multiple Drugs, Including Tigecycline, in *Klebsiella pneumoniae*. m Bio. 2020 Mar 3;11(2):e02930-19.

18. Majdi N Al-Hasan, Gram-negative Bacteria With Difficult-to-Treat Resistance: A Moving Target, *Clinical Infectious Diseases*, Volume 72, Issue 12, 15 June 2021, 2121–2123.

19. Martin, J.K., Sheehan, J.P., Bratton, B.P., Moore, G.M., Mateus, A., Li, S.H.J., Kim, H., Rabinowitz, J.D., Typas, A., Savitski, M.M. and Wilson, M.Z., 2020. A dual-mechanism antibiotic kills gram-negative bacteria and avoids drug resistance. *Cell*, *181*(7), pp.1518-1532.

20. Michael A. Cook, Gerard D. Wright, Thepast, present, and future of antibiotics. *Sci. Transl. Med.* 14, eabo7793 (2022).

21. Oliveira J, Reygaert WC. Gram-Negative Bacteria. In: Stat Pearls. Stat Pearls Publishing, Treasure Island (FL); 2023. PMID: 30855801.

22. Poirel L, Jayol A, Nordmann P. Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes. Clin Microbiol Rev. 2017 Apr;30(2):557-596.

23. Pollock J, Chalmers JD. The immunomodulatory effects of macrolide antibiotics in respiratory disease. Pulm Pharmacol Ther. 2021 Dec;71:102095

24. Ramirez MS, Tolmasky ME. Aminoglycoside modifying enzymes. Drug Resist Updat.2010Dec;13(6):151-71.

25. Sabnis A, Edwards AM. Lipopolysaccharide as an antibiotic target. Biochim Biophys Acta Mol Cell Res. 2023 Oct;1870(7):119507.

26. Serpersu EH, Ozen C, Wright E. Studies of enzymes that cause resistance to aminoglycosides antibiotics. Methods Mol Med. 2008;142:261-71.

27. Shariati A, Arshadi M, Khosrojerdi MA, Abedinzadeh M, Ganjalishahi M, Maleki A, Heidary M, Khoshnood S. The resistance mechanisms of bacteria against ciprofloxacin and new approaches for enhancing the efficacy of this antibiotic. Front Public Health. 2022 Dec 21;10:1025633.

28. Silhavy, Thomas J et al. "The bacterial cell envelope." *Cold Spring Harbor perspectives in biology* vol. 2,5 (2010): a000414.

29. Singh, A. N., Wu, M., Ye, T. T., Brown, A. C., & Wittenberg, N. J. (2024). Engineering Planar Gram-Negative Outer Membrane Mimics Using Bacterial Outer Membrane Vesicles. *bio Rxiv : the preprint server for biology*, 2023.12.11.570829.

30. Sun J, Chen C, Cui CY, Zhang Y, Liu X, Cui ZH, Ma XY, Feng Y, Fang LX, Lian XL, Zhang RM, Tang YZ, Zhang KX, Liu HM, Zhuang ZH, Zhou SD, Lv JN, Du H, Huang B, Yu FY, Mathema B, Kreiswirth BN, Liao XP, Chen L, Liu YH. Plasmid-encoded tet(X) genes that confer high-level tigecycline resistance in *Escherichia coli*. Nat Microbiol. 2019 Sep;4(9):1457-1464.

31. Thomson KS. Detection of gram-negative β-lactamase producing pathogens in the clinical lab.Curr Pharm Des. 2013;19(2):250-6.

32. Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VHA, Takebayashi Y, Spencer J. β-Lactamases and β-Lactamase Inhibitors in the 21st Century. J Mol Biol. 2019 Aug 23;431(18):3472-350021. Joanna Trylska, Marta Kulik; Interactions of aminoglycoside antibiotics with r RNA. *Biochem Soc Trans* 15 August 2016; 44 (4): 987–993.

33. Tsuji A, Kaneko Y, Takahashi K, Ogawa M, Goto S. The effects of

temperature and p H on the growth of eight enteric and nine glucose non-fermenting species of gram-negative rods. Microbiol Immunol. 1982;26(1):15-24.

34. Vázquez-Laslop N, Mankin AS. How Macrolide Antibiotics Work. Trends Biochem Sci. 2018 Sep;43(9):668-684. Venkatesan M, Fruci M, Verellen LA, Skarina T,Mesa N, Flick R, Pham C, Mahadevan R, Stogios PJ, Savchenko A. Molecular mechanism of plasmid-borne resistance to sulfonamide antibiotics. Nat Commun. 2023 Jul 7;14(1):4031.

35. Wang N, Xu X, Xiao L, Liu Y. Novel mechanisms of macrolide resistance revealed by *in vitro* selection and genome analysis in *Mycoplasma pneumoniae*. Front Cell Infect Microbiol. 2023 May 22;13:1186017.

36. Wen S, Feng D, Chen D, Yang L, Xu Z. Molecular epidemiology and evolution of *Haemophilus influenza.* Infect Genet Evol. 2020 Jun;80:104205. d

37. Zeng, Ximin, and Jun Lin. "Beta-lactamase induction and cell wall metabolism in Gram-negative bacteria."*Frontiersin microbiology* 4 (2013): 128.

38. Zhanel GG, Esquivel J, Zelenitsky S, Lawrence CK, Adam HJ, Golden A, Hink R, Berry L, Schweizer F, Zhanel MA, Bay D, Lagacé-Wiens PRS, Walkty AJ, Lynch JP 3rd, Karlowsky JA. Omadacycline: A Novel Oral and Intravenous Aminomethylcycline Antibiotic Agent. Drugs. 2020 Feb;80(3):285-313.

39. Zhao L, Wang S, Li X, He X, Jian L. Development of *in vitro* resistance to fluoroquinolones in *Pseudomonas aeruginosa*. Antimicrob Resist Infect Control. 2020 Aug 5;9(1):124.

Chapter - 10

Strength: Exploring the Potential of Jute Fiber

Authors

Chandan Das
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barrackpore, Barasat, Kolkata, West Bengal, India

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

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

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

Chapter - 10

Sustainable Strength: Exploring the Potential of Jute Fiber

Chandan Das, Bidisha Ghosh, Suranjana Sarkar and Subhasis Sarkar

Abstract

Over 3.8 million tons of plastic carrier bags are produced annually in Europe alone, and one Millions of plastic bags are used every minute worldwide. For the environment and living things, these bags take over a century to break down and degrade. Jute plastic bags, which are environmentally benign and very successful in reducing marine pollution, can be made with Jute Nanocellulose Polymerization technology. When it comes to sustainable product creation, researchers from academia and industry are paying close attention to natural fiber- based goods because of their minimal carbon footprint.

An increase in pollution that affects both the environment and living things could be the consequence of the growing tendency of using synthetic materials. Consequently, it is imperative from a sustainability perspective to substitute environmentally hazardous synthetic materials with natural, renewable, and biodegradable alternatives. When it comes to the quantity produced, jute fiber comes in second among all natural fibers. Wood and steel are also replaced by the Jute Fiber Reinforced Composites. Jute polymer is 22 times stronger than tensile steel. High-energy absorption capabilities, enhanced fatigue resistance, and post- cracking resistance of cement-based composites can all be guaranteed by the fibers. An object's capacity to withstand static load which has the tendency to crush concrete—is measured by its compressive strength.

Sustainable and safer building materials could be created by utilizing natural fibers and making them recyclable. For optimal growth, jute plants require grassy soil, warm to moderate temperatures (20–40 °C), and high relative humidity (70–80%). They also need 125–150 mm of rainfall per month. Jute has a long history of use in East Bengali culture, mostlyinroofingandropesforotherpurposes.Thisstudyoffersinsightfulinformationabout the application of jute fibers as a sustainable

Keywords: Jute fiber, ecofriendly, ecofriendly, jute plastic bags, natural fibres

1. Introduction to jute fiber

Juteisanaturallyoccurringfibrederivedfromthestemofthejuteplantandisemployedin the production of numerous goods. The jute plant, which is mainly grown in tropical areas like Bangladesh and India, yields jute fibre, also known as the "golden fibre," a natural textile.

Jute can be harvested at any time following a specific amount of vegetative development, however harvesting during the pre-bud or bud stage yields the highest quality fibre. In order to grow jute, the ground must be prepared, seeds must be sown, the plant must be irrigated, harvested, and retted to remove the fibre. The jute plant may reach a height of 2.5 m and has a base stem diameter of 25 mm. Eco-friendly, recyclable, and biodegradable, jute is a great fibre. In addition, it has good insulating and anti-static qualities, is robust, and is non- abrasive.About 65–70% of jute fibres aremadeup of cellulose, 15%–20% of hemicellulose, and 8% of microfibril. Because the fibre is biodegradable, it is a green option in a time when sustainability is becoming more and more important. Of all the natural fibres, jute is the most affordable, practical, and widely available.

The history of jute fibre is extensive and goes back thousands of years, especially in South Asia, where it was interwoven into communities' economic and cultural fabric. Jute is used for practical purposes, but it also has cultural importance. For instance, because of its economic significance, jute is regarded as "the golden fibre" and revered as a symbol of national pride in Bangladesh. Every year, the Jute Festival honours the region's rich cultural Legacy by presenting customary crafts and the contribution of the fibre to regional businesses. Jute is used in wedding decorations and regional fabrics, as well as rituals and crafts throughout India.

According to the 2019 World Natural Fibre Production Report, the largest share of natural fibres, after cotton, are made up of jute and related fibres. Jute has special qualities that make it useful for both industrial and ornamental applications. These qualities include strength, resilience, and a coarse texture. Jute has historically been used only for sacks, hessian fabrics, and ropes, and twines. In the automotive industry, bast fibres are now being used for components including trunk liners, seat backs, and interior door panels. The geotextile, building, and furnitureindustries are among the various industries that use jute polymer composites. Jute is becoming more

and more popular as consumer demand for eco-friendly materials rises, underscoring its importance in both conventional methods and contemporary eco-conscious markets.

This study is primarily investigating jute's potential as a sustainable alternative in various applications, especially in construction and manufacturing industries.

Properties of jute fiber

Physical and chemical composition

The jute plant yields jute fibre, which has several advantageous qualities. Physically, it has a colour ranging from light brown to golden yellow. The fibres have a diameter of roughly 10 to 20 micrometres and can be anywhere in length from 1 to 4 meters. Its rough, gritty texture gives it good strength, but it is less elastic than synthetic fibres. Jute is excellent at absorbing moisture; it can hold onto 14% of its weight in moisture, making it appropriate for humid environments.

- Colour: Light brown to golden yellow.
- Diameter: Roughly 10to 20 micrometres
- Length: From1to4 meters
- Density:

Chemically, jute fibres are made up of several cells made of crystalline, helical cellulose Fibrils that are bonded to the entire layer by hemicellulose and amorphous lignin in a helical angles. It is composed of roughly 60–70% cellulose, which adds to its longevity; the remaining 20–30% hemicellulose and 10-15% lignin increase its stiffness and decay resistance.

- Cellulose:60–70%
- Hemicellulose:20–30%
- Lignin:10-15%
- Small pectin, fats and waxes

Mechanical properties

There are several elements that might affect the mechanical qualities of jute fibres, such as the area in which the jute was planted, the fibre extraction process, and the harvesting period, and percentage of chemical components.

- Tensilestrength:393–780 MPa.

- Young's modulus: 10–30GPa.
- Elongation at break:1.16–1.8%.
- Density: 1.30–1.45g/cm3.
- Anti-static: Jute fiber has anti-static properties.

Longevity: While they can break down in strong acids, jute fibres can withstand UV radiation damage and are generally robust in a variety of environmental situations.

Jute fibre is an eco-friendly natural fibre since it is 100% recyclable and biodegradable. Jute fibre has a high degree of biodegradability and compostability. The jute fibre is recyclable and reusable. Environmental advantages: Growing jute can increase soil fertility since the plants absorb carbon dioxide and release oxygen.

Thermal properties

Natural jute fibre offers excellent mechanical qualities, is affordable, and sustainable. It is frequently utilised in engineering fields, including the building and automotive sectors. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) can be used to investigate the thermal properties of jute fibre.

Jute fibres are good insulators due to their low heat conductivity, which normally ranges from 0.038 to 0.050 W/m·K. They begin to degrade at roughly 240°C and are thermally stable up to 200°C to 220°C. Relatively speaking, jute has a low specific heat capacity (1.4–1.6 J/g·K) and little thermal expansion.

There are various thermal characteristics of jute fibre, such as:

- Low thermal conductivity: The thermal conductivity of jute fibre is between 0.038 and 0.040 W/m·K.
- Thermal stability: At higher temperatures, jute fibre exhibits similar thermal stability.
- Extensive heat insulation: The low thermal conductivity and porous structure of jute fibre are responsible for this property.
- Weight loss: When the temperature rises, jute fibre loses weight.
- Water absorption: Over time, jute fibre takes up water.
- Chemical treatment: The heat-absorbing capacity and negative heat flow of chemically treated jute fibres are reduced.

Although it can be made more fire resistant with chemical treatments, jute is combustible and will ignite at temperatures higher than 200°C. Due to these characteristics, jute is perfect for uses such as insulation and environmentally friendly composite materials, but it is not appropriate for high temperatures.

Furthermore, jute is entirely biodegradable, which makes it a sustainable choice. Packaging materials, textiles, geotextiles, and other eco-friendly products are only a few of the uses for it that are further expanded by its chemical resistance to alkalis, strong thermal insulating qualities, and ease of dye ability. These characteristics highlight jute's usefulness as a natural fibre that may be used in a variety of industries.

Sustainability of jute fiber

Because of its natural origin, biodegradability, and small ecological footprint during cultivation and processing, jute fibre is an environmentally sustainable material. Jute is a more sustainable option for clothing than synthetic materials since it is a renewable resource that can be farmed without the use of pesticides or fertilizers. Furthermore, jute farming is an environmentally good option because it rotates crops, which enhances soil health. Moreover, the energy requirements of the production process are very low, which adds to its overall sustainability.

Biodegradability and environmental impact

Jute replaces synthetic, petroleum-based fibres well since it is 100% biodegradable and compostable. It decomposes spontaneously and leaves no toxic residues behind because of its great biodegradability. Because it requires less water and fewer pesticides than other crops like cotton, its cultivation has a minimal impact on the environment. A more sustainable and environmentally friendly future is possible because jute enhances soil health and lessens dependency on synthetic resources.

Low carbon footprint

Jute's low water, fertiliser, and pesticide requirements, together with its capacity to absorb large volumes of CO_2 during growth, contribute to its low carbon footprint in the manufacture of fibre. Jute is an environmentally safe substitute for synthetic fibres because its production process uses less energy and because it degrades naturally, it doesn't contribute to long-term environmental degradation.

Renewability

The fast growth (within 4-6 months), annual harvesting potential, and low resource requirements of jute are reasons why its production as fibre is extremely renewable. Crop rotation not only improves soil health but also uses less water, fertilizers, and pesticides than alternative fibres. Jute is thus a sustainable and environmentally beneficial substance that is perfect for encouraging the use of renewable resources.

Treatment and surface modifications to enhance strength

Jute fibres are strengthened by a variety of surface treatments and changes. Alkali, silane, and acetylation are examples of chemical treatments that strengthen fiber-matrix interaction and lessen moisture absorption, which can deteriorate the fibres. To improve mechanical qualities, physical treatments like as heat treatment and plasma treatment are applied to the fibre surface.

There are many ways to enhance the strength of materials, including:

Chemical treatments

By eliminating contaminants and non-cellulosic elements like lignin and hemicellulose, chemical treatments, especially alkali (NaOH) treatment significantly increase the strength of jute fibres. In composite applications, this procedure improves the bonding between the fibre and matrices by increasing the surface roughness of the fibre.

Thermal treatments

By modifying their structural characteristics through regulated heating, thermal treatments increase the strength of jute fibres. In order to enhance thermal stability and decrease moisture absorption, these treatments frequently require subjecting the fibres to high temperatures. An increased crystalline cellulose structure is the result of the process's assistance in removing volatile components as well as its reduction of lignin and hemicellulosecontent.Duetotheimprovementofmechanicalqualitiesliketensile strength and stiffness brought about by heat treatment, jute fibres are now more suited for usage in composite materials and other applications where greater durability is required.

Silane treatment

Jute fibres can be made more mechanically efficient by adding nanomaterials like graphene oxide, carbon nanotubes, or silica nanoparticles.

With less water absorption, these nanoscale addition improves thermal stability, tensile strength, and flexibility. Fiber-matrix adhesion in composite applications is improved by the surface changes, leading to stronger and more resilient composites. In numerous industrial applications, this method greatly improves the performance of jute fibres by utilising the special qualities of nanoparticles.

Nano-enhancements

Nano-enhancements for jute fibers involve incorporating nanomaterials, such as graphene oxide, carbon nanotubes, or silica nanoparticles, to improve their mechanical properties. These nanoscale additives increase tensile strength, flexibility, and thermal stability while reducing water absorption. The surface modifications enhance fiber-matrix adhesion in composite applications, resulting in more durable and robust materials. This approach leverages the unique properties of nanomaterials to significantly elevate the performance of jute fibers in various industrial applications.

Enzymatic treatment

Using particular enzymes, jute fibres can be treated enzymatically to eliminate non-cellulosic materials like lignin and hemicellulose. By enhancing the surface characteristics of the fibre, this procedure increases its mechanical strength and compatibility with matrix materials in composites. Jute fibres treated with enzymes become stronger and more resilient, making them ideal for a variety of uses, especially in sustainable composite materials, by improving fibre alignment and lowering impurities.

With these techniques, jute becomes more suitable for high-strength applications in composites and textiles, and its overall performance, tensile strength, and fibre durability are also enhanced.

Role in circular economy

Owing to its biodegradable, renewable, and ecological qualities, jute fibre is important to the circular economy. It has numerous environmental advantages and is multipurpose. Naturally occurring, jute is a popular natural fibre substitute for synthetic materials in applications including composites, textiles, and packaging. It is possible to manufacture, use, and recycle jute in a circular economy with no negative influence on the environment. Growing jute contributes to the sequestration of carbon, and its products can be securely biodegraded to replenish soil nutrients or recycled into new materials. It's perfect for cutting waste and saving resources in a variety of industries because of this.

In addition to supporting sustainable production methods and inclusive growth, the jute industry in India supports millions of farmers and labourers. To reduce plastic waste and promote a more sustainable, circular economy, jute bags are gradually taking the place of plastic bags in packaging.

India's circular economy relies heavily on jute fibres since it reduces environmental effects and promotes sustainability. Naturally occurring and biodegradable, jute finds extensive application in the packaging, textile, and agricultural industries.

Economic and social impact

For millions of farmers and labourers, jute fibre, also known as the "golden fibre," is essential to Bangladesh's and India's economy. Especially as the demand for eco-friendly items rises globally, it promotes rural development by creating jobs and reducing poverty. In addition, jute farming supports local businesses and gives women more influence. But there are obstacles, such shifting consumer preferences and competition from man-made substitutes.

Jute is sustainable and biodegradable in terms of the environment, supporting worldwide eco-aware tendencies.

Employment generation

Particularly in nations like Bangladesh and India, where jute production is concentrated, jute fibre is important for creating jobs. Millions of individuals, including farmers, labourers, and employees in the processing and manufacturing industries, are employed in the jute industry. Jute demands a labour-intensive method for everything from cultivation to processing, which boosts employment in rural regions and lowers urban migration. Demand has also increased due to the rise in environmentally friendly jute goods, creating more job opportunities in conventional industries as well as in emerging, sustainable businesses.

Value-added products

Once primarily utilised to create sacks and ropes, jute fibre has developed into a useful material for creating high-end goods including eco-friendly handicrafts, rugs, and bags. Local economies are being strengthened and sustainable product options are being provided by the development of consumer goods made of jute, such as eco-friendly bags, carpets, and geotextiles.

These goods support small and medium-sized businesses, which boost

the economy by generating jobs, particularly in rural areas. Because jute is environmentally benign, it also supports global sustainability goals, which increases demand for it in both domestic and foreign markets. The transition to higher-end merchandise improves the social and economic status of regional craftspeople, fostering equitable development.

Reduction in plastic pollution

Because jute fibre is an environmentally preferable substitute for synthetic materials, it considerably lowers plastic pollution. Jute fibre is a renewable and biodegradable resource. Jute breaks down organically in a matter of months as opposed to plastic, which takes hundreds of years to break down and worsens the environment. This reduces the amount of waste that ends up in landfills and the ocean. Its application in goods like textiles, bags, and packaging lessens the pervasive reliance on single-use plastics, encouraging eco-friendly behaviours and lessening the damaging effects of plastic pollution on ecosystems.

By reducing the need for plastic items, more jute is being used for packaging (bags, sacks, etc.), which helps minimise the pollution caused by plastic.

Environmental benefits

Jute fibreis a vital resource for sustainable development because of its many positive effects on the environment. In addition to absorbing carbon dioxide to help slow down global warming, jute plants also require less chemical inputs, which lowers pollution of the soil and water. Products made of biodegradable jute decompose spontaneously, reducing trash sent to landfills. Jute farming also improves soil health and reduces erosion, which promotes biodiversity and ecosystem resilience.

Biodegradability

A fantastic eco-friendly substitute for synthetic fibres, jute is 100% recyclable and Biodegradable. When compared to synthetic fibres and plastics, which survive for hundreds of years, it decomposes extremely quickly within months. The environment is less contaminated with microplastics thanks to this quick disintegration, which also decreases landfill waste. Because it's a renewable resource, jute helps to maintain sustainable Agriculture, improves ecosystems, and keeps the environment cleaner by not releasing toxic chemicals.

Low carbon footprint

Owing to its capacity to store carbon dioxide during growth, jute fibre has a low carbon footprint. Jute plants are a quickly replenishing resource that absorb large volumes of CO_2 from the atmosphere, helping to mitigate the effects of climate change. Furthermore, compared to other crops, jute farming uses less chemical pesticides and fertilisers, which lessens contamination of the land and water. Further reducing greenhouse gas emissions is the processing of jute, which uses less energy than that of synthetic fibres. We can encourage the manufacture and use of jute, which will help to create a more environmentally friendly and sustainable economy.

Soil fertility

Because of its deep root structure, jute fibre reduces nutrient runoff and prevents erosion, increasing soil fertility. Jute is frequently cultivated as part of a crop rotation program, which enhances soil structure and supplies vital nutrients. To create healthier, more productive soils, its leaves and leftover biomass can be added back to the soil as organic matter. This will enrich the soil and encourage microbial activity.

Recent innovations and advancements

The latest advancements in jute fibre concentrate on enhancing its adaptability and durability. Its strength and endurance have risen due to improved processing methods, opening up uses in the automotive industry for biodegradable composites, eco-friendly packaging, and construction. Jute's application in textiles and household items is expanding as a result of research into treatments for its antibacterial and moisture-resistant qualities. Jute is positioned as an essential substitute for plastic and synthetic fibres due to the increase in demand for sustainable materials.

Jute in 3D printing and advanced manufacturing

Jute fibre has recently undergone developments that demonstrate its potential as a sustainable composite material for 3D printing and sophisticated manufacturing. As a substitute for plastics derived from petroleum, researchers are fusing jute fibres with bioplastics to create environmentally friendly filaments. Jute is becoming more mechanically appropriate for a wider range of uses, such as building and automotive materials, thanks to improved processing methods. By integrating, jute's commercial prospects are increased and sustainability is promoted.

Chemical modification

Jute fibre has undergone recent advances in tensile strength, durability, and insect and moisture resistance by chemical treatments such as acetylation, cross-linking, and alkali treatment. These developments increase jute's commercial viability by broadening its applications in textiles, composites, and bioplastics.

Additionally, environmentally friendly chemical techniques are bolstering sustainable jute production methods and encouraging environmental preservation.

Nano-jute fibers

Recent developments in nano-jute fibres improve their strength, flexibility, and biodegradability; as a result, they are perfect for use in medical applications like as medication delivery and wound dressings, as well as high-performance composites and environmentally friendly packaging.

Challenges in jute fiber utilization

Utilising jute fibre confronts a number of obstacles despite its potential. These include sporadic fibre quality, a lack of knowledge about its advantages over synthetic substitutes, and restricted technological improvements in processing methods.

Climate change is one environmental element that affects jute agriculture, which affects Quality and yield. Its competitiveness in other industries can also be limited by the increased cost of creating jute-based products because of the requirement for better infrastructure and research.

Moisture sensitivity

Because jute fibre is extremely sensitive to moisture, its strength and mechanical qualities are diminished, which makes it unsuitable for usage in humid environments. Although treatments to enhance its functionality are being investigated, this causes oedema, microbial growth, and structural problems.

In consistent quality

The performance of jute composites may be impacted by variations in jute fibre quality according to cultivation circumstances. Variations in jute fibre quality stemming from farming and processing techniques impede product homogeneity and restrict competitiveness. To meet this issue, better farming techniques and standardised procedures are required.

Surface treatment requirement

Jute fibres frequently need to be chemically treated (alkaline, silane, etc.) to improve bonding with matrix materials in composites. Because jute is hydrophilic, it affects durability in composites; therefore, one major problem in using jute fibre is the necessity for surface treatment to promote adhesion with polymer matrices. Though they complicate things and raise expenses and environmental issues, surface treatments improve bonding.

Environmental impact of retting

Water contamination and reduced fibre quality are two environmental problems associated with the conventional retting method used in the manufacture of jute fibre. To solve these Problems, environmentally friendly retting techniques and better wastewater management are essential.

Cost of production

The high expense of producing jute fibre, which results from seasonal planting, labour- intensive processing, and little mechanisation, makes it difficult to utilise widely.

Government assistance and technological advancements are required to save expenses and compete with less expensive options.

Competition from Synthetics

Synthetic fibres such as polyester are more durable, less expensive, and have a constant quality, making jute fibre less competitive in the market. Jute is environmentally beneficial, but its market potential is limited by its lesser strength and susceptibility to moisture.

Applications in sustainable development

A renewable substitute for synthetic fibres and plastic, jute fibre is biodegradable and environmentally beneficial, contributing to sustainable development. Requiring less chemicals and enhancing soil health, it finds application in textiles, packaging, agriculture, and construction. Eco-friendly methods are promoted by jute-reinforced composites and their function in erosion management.

Textiles application

As a sustainable substitute for synthetic fibres, jute fibre is a biodegradable substance that is essential to the growth of the textile industry. It supports rural economies and helps lessen environmental effect because of its resilience, low resource requirements, and applicability for items like bags and carpets.

Packaging industry

Jute fibre represents a viable, renewable, and biodegradable substitute for plastic in the packaging sector. Because of its robustness and longevity, it's perfect for making environmentally friendly sacks, bags, and containers, lowering pollution levels in the environment, and fostering sustainable development by using natural resources.

Automotive industry

Because of its eco-friendly qualities, jute fibre finds extensive use in the automobile sector and promotes sustainable development. Jute is a renewable and biodegradable resource that is used to make composite materials for car interiors, including headliners, seat backs, and door panels.Becausethesecompositesarelighter,vehicleswillweighlessanduselessfuel,which will result in less emissions. In order to lighten vehicles, increase fuel economy, and support sustainability, jute composites are utilised in car interiors (door panels, seat backs). Jute is a viable substitute for synthetic fibres due to its affordability and accessibility, which encourages sustainability and supports environmentally friendly automotive production practices.

Green building materials

Jute fibre is a biodegradable, environmentally beneficial substance that is utilised in green buildings for reinforcement, composite materials, and insulation. By substituting natural materials for synthetic ones in geotextiles, panels, and roofing, it lowers carbon footprints and promotes energy- and sustainably-friendly building techniques.

Homedecor

An important component of home decor, jute fibre is a biodegradable and renewable resource. It may be used to make a variety of ornamental things, such as pillows, rugs, and curtains, and is an environmentally beneficial substitute for synthetic fabrics. Carpets, draperies, and chair covers were also made using it. Beyond its aesthetic value, jute helps farmers maintain sustainable farming methods and give them a means of subsistence. This empowers consumers to make ecologically friendly decisions and encourage a circular economy in their homes.

Tea filter papers

The use of jute fibre in environmentally friendly tea filter paper production is drawing attention to its importance in sustainable development.

Jute, a renewable and biodegradable resource, provides a sustainable substitute for synthetic products and contributes to the decrease of plastic waste. Using it improves the quality of tea and reduces the carbon footprint associated with the creation of plastic. Furthermore, jute farming contributes to a greener future by boosting sustainable farming methods and rural economies.

Future prospects and innovations

Thanks to its capacity to decompose naturally, jute fibre holds great promise as a sustainable material in the future. Its uses in the packaging, construction, and automotive industries are growing thanks to developments in nanotechnology, processing, and blending with synthetic fibres. Jute has the potential to lessen plastic consumption and encourage greener production as consumer demand for environmentally friendly substitutes rises.

Bio-based polymers and jute composites

Particularly in bio-based polymers and jute composites, jute fibre has a promising future. Applications in the automotive, packaging, and construction industries are finding jute useful in creating sustainable substitutes for synthetic materials due to its renewable nature.

Improvements in material science and manufacturing are enhancing the qualities of jute composites, providing a viable way to lessen dependency on petroleum-based goods.

Circular economy

An excellent fit for a circular economy, jute fibre provides a biodegradable and sustainable substitute for synthetic fibres. Waste is decreased by its capacity to be recycled, used, and degrade naturally. Technological advancements in jute-based products, like biodegradable textiles and packaging, encourage resource economy and a closed-loop system, supporting international green initiatives.

Smart materials

Possible uses include creating adaptive composites or smart textiles that react to environmental cues using jute as a sustainable base material. Sensors and functional coatings are being added to jute fibre to make it into smart materials that can respond to changes in their surroundings. By improving its resilience and fitting it for environmentally friendly uses in building, packaging, and smart textiles, these developments provide high-tech sectors with sustainable solutions.

Nanotechnology

Jute fibre has a bright future ahead of it, especially when nanotechnology is incorporated. By creating composite materials that make use of jute's inherent qualities, improvements in strength, durability, and functionality are made possible. Technological advancements like enhanced water resistance, antimicrobial properties, and better thermal insulation have the potential to expand the uses of jute beyond textiles and packaging to industries like biomedical engineering, automotive, and construction, in line with the increasing need for sustainable materials.

Conclusion

In conclusion, because of its eco-friendly qualities, biodegradability, and adaptability to a wide range of uses, jute fibre offers a bright prospect for sustainable development. Jute is an excellent material for textiles, packaging, and composite materials since it is a renewable resource and has a lower environmental effect than synthetic fibres. Jute fibre ranks second among all natural fibres in terms of production volume. With its renewable nature and ability to be grown without the need for pesticides or fertilisers, jute is a more environmentally friendly fabric choice for clothes than synthetic ones. Acetylation, silane, and alkali are just a few of the surface modifications and treatments that strengthen jute fibres.

The adaptability of jute enables its use in arange of sectors, such as building, packaging, and textiles, supporting a circular economy by producing recyclable and biodegradable goods. In addition to giving millions of farmers a means of subsistence, the usage of jute helps to lower the carbon footprint connected with the production of synthetic goods. Jute is a material that may be produced, used, and recycled in a circular economy without harming the environment. Jute is a naturally occurring material that decomposes naturally and is widely used in the packaging, textile, and agricultural sectors. Jute fibre is a workable option that is consistent with environmentally conscious principles and the rising demand for sustainable materials as society moves towards more sustainable practices.

Due to its low environmental impact and biodegradability, jute fibre has considerable sustainable prospects in the textile, packaging, and building industries. Stakeholders should prioritise strengthening consumer awareness, supply chain optimisation, and processing method advancements to encourage wider use, which will ultimately boost regional economies and create a more sustainable future.

The quality and productivity of jute crops are impacted by various environmental factors, including climate change. Jute-reinforced composites' role in erosion control encourages environmentally responsible practices. It is an environmentally friendly alternative to synthetic fabrics and may be used to manufacture a wide range of ornamental items, including curtains, carpets, and cushions. It was also used to create carpets, curtains, and chair covers.

Jute's standing in the international market will be further improved by making investments in R&D, improving production methods, and creating new market prospects. Jute fibre offers a sustainable and biodegradable alternative to synthetic fibres, making it a great fit for a circular economy. To cut costs and compete with less expensive solutions, government support and technical breakthroughs are needed. By utilising jute fibre to the fullest extent possible, we may open the door to a more robust and sustainable future.

References

1. Singh H, Singh JIP, Singh S, Dhawan V, Tiwari SK. A brief review of jute fibre and its composites. *Materials Today: Proceedings*. 2018;5(14):28427-28437.
2. Islam MS, Ahmed SJ. Influence of jute fiber on concrete properties. *Construction and Building Materials*. 2018;189:768-776.
3. Samanta AK, Mukhopadhyay A, Ghosh SK. Processing of jute fibres and its applications. In: Handbook of natural fibres. Woodhead Publishing; 2020. p. 49-120.
4. Bismarck A, Springer J, Mohanty AK, Hinrichsen G, Khan MA. Characterization of several modified jute fibers using zeta-potential measurements. *Colloid and Polymer Science*. 2000;278:229-235.
5. Sever K, Sarikanat M, Seki Y, Erkan G, Erdoğan ÜH, Erden S. Surface treatments of jute fabric: The influence of surface characteristics on jute fabrics and mechanical properties of jute/polyester composites. *Industrial Crops and Products*. 2012;35(1):22-30.
6. Corrales F, Vilaseca F, Llop M, Girones J, Mendez JA, Mutje P. Chemical modification of jute fibers for the production of green-composites. *Journal of Hazardous Materials*. 2007;144(3):730-735.
7. Roy S, Lutfar LB. Bast fibres: jute. In: Handbook of natural fibres. Woodhead Publishing; 2012. p. 39-59.

8. Rahman MM, Bala BK. Modelling of jute production using artificial neural networks. *Biosystems Engineering*. 2010;105(3):350-356.

9. Ahmed Z, Akhter F. Jute retting: An overview. *Online J Biol Sci*. 2001;1(7):685-688.

10. Krishnan KB, Doraiswamy I, Chellamani KP. Jute. In: Bast and other plant fibres. Woodhead Publishing; 2005. p. 24-93.

11. Brigham C. Biopolymers: Biodegradable alternatives to traditional plastics. In: Green chemistry. Elsevier; 2018. p. 753-770.

12. Jadhav HS, Fulke AB, Giripunje MD. Recent global insight into mitigation of plastic pollutants, sustainable biodegradable alternatives, and recycling strategies. *International Journal of Environmental Science and Technology*. 2023;20(7):8175-8198.

13. Rahman MS. Jute – A versatile natural fibre. Cultivation, extraction and processing. In: *Industrial applications of natural fibres: structure, properties and technical applications*. 2010;1.

14. Shah AN, Lakkad SC. Mechanical properties of jute-reinforced plastics. *Fibre Science and Technology*. 1981;15(1):41-46.

15. Biswas S, Ahsan Q, Cenna A, Hasan M, Hassan A. Physical and mechanical properties of jute, bamboo and coir natural fiber. *Fibers and Polymers*. 2013;14:1762-1767.

16. Gassan J, Bledzki AK. Possibilities for improving the mechanical properties of jute/epoxy composites by alkali treatment of fibres. *Composites Science and Technology*. 1999;59(9):1303-1309.

17. Gopinath A, Kumar MS, Elayaperumal AJPE. Experimental investigations on mechanical properties of jute fiber reinforced composites with polyester and epoxy resin matrices. *Procedia Engineering*. 2014;97:2052-2063.

18. Sajin JB, Paul RC, Binoj JS, Mansingh BB, Selvan MGA, Goh KL, et al. Impact of fiber length on mechanical, morphological and thermal analysis of chemical treated jute fiber polymer composites for sustainable applications. *Current Research in Green and Sustainable Chemistry*. 2022;5:100241.

19. Sanvezzo PB, Branciforti MC. Recycling of industrial waste based on jute fiber-polypropylene: Manufacture of sustainable fiber-reinforced polymer composites and their characterization before and after accelerated aging. *Industrial Crops and Products*. 2021;168:113568.

20. Majumder A, Stochino F, Farina I, Valdes M, Fraternali F, Martinelli E. Physical and mechanical characteristics of raw jute fibers, threads and diatons. *Construction and Building Materials*. 2022;326:126903.

21. Mohanty AK, Khan MA, Hinrichsen G. Surface modification of jute and its influence on performance of biodegradable jute-fabric/Biopol composites. *Composites Science and Technology*. 2000;60(7):1115-1124.

22. Hossain MK, Dewan MW, Hosur M, Jeelani S. Mechanical performances of surface modified jute fiber reinforced biopol nanophased green composites. *Composites Part B: Engineering*. 2011;42(6):1701-1707.

23. Alves C, Silva AJ, Reis LG, Freitas M, Rodrigues LB, Alves DE. Ecodesign of automotive components making use of natural jute fiber composites. *Journal of Cleaner Production*. 2010;18(4):313-327.

24. Alves C, Silva AJ, Reis LG, Freitas M, Rodrigues LB, Alves DE. Ecodesign of automotive components making use of natural jute fiber composites. *Journal of Cleaner Production*. 2010;18(4):313-327.

25. Ekundayo G, Adejuyigbe S. Reviewing the development of natural fiber polymer composite: A case study of sisal and jute. *Am. J. Mech. Mater. Eng*. 2019;3(1):1-10.

26. Kumar N, Kandasami RK, Singh S. Effective utilization of natural fibres (coir and jute) for sustainable low-volume rural road construction – A critical review. *Construction and Building Materials*. 2022;347:128606.

27. De Queiroz HFM, Banea MD, Cavalcanti DKK. Adhesively bonded joints of jute, glass and hybrid jute/glass fibre-reinforced polymer composites for automotive industry. *Applied Adhesion Science*. 2021;9:1-14.

28. Sheheli S, Roy B. Constraints and opportunities of raw jute production: A household level analysis in Bangladesh. *Progressive Agriculture*. 2014;25:38-46.

29. Jahan MS, Hossain S, Khan MA. Economic importance of jute. In: *The jute genome*. Cham: Springer International Publishing; 2022. p. 1-16.

30. Wang WM, Cai ZS, Yu JY, Xia ZP. Changes in composition, structure, and properties of jute fibers after chemical treatments. *Fibers and Polymers*. 2009;10:776-780.

31. Chandekar H, Chaudhari V, Waigaonkar S. A review of jute fiber reinforced polymer composites. *Materials Today: Proceedings*. 2020;26:2079-2082.