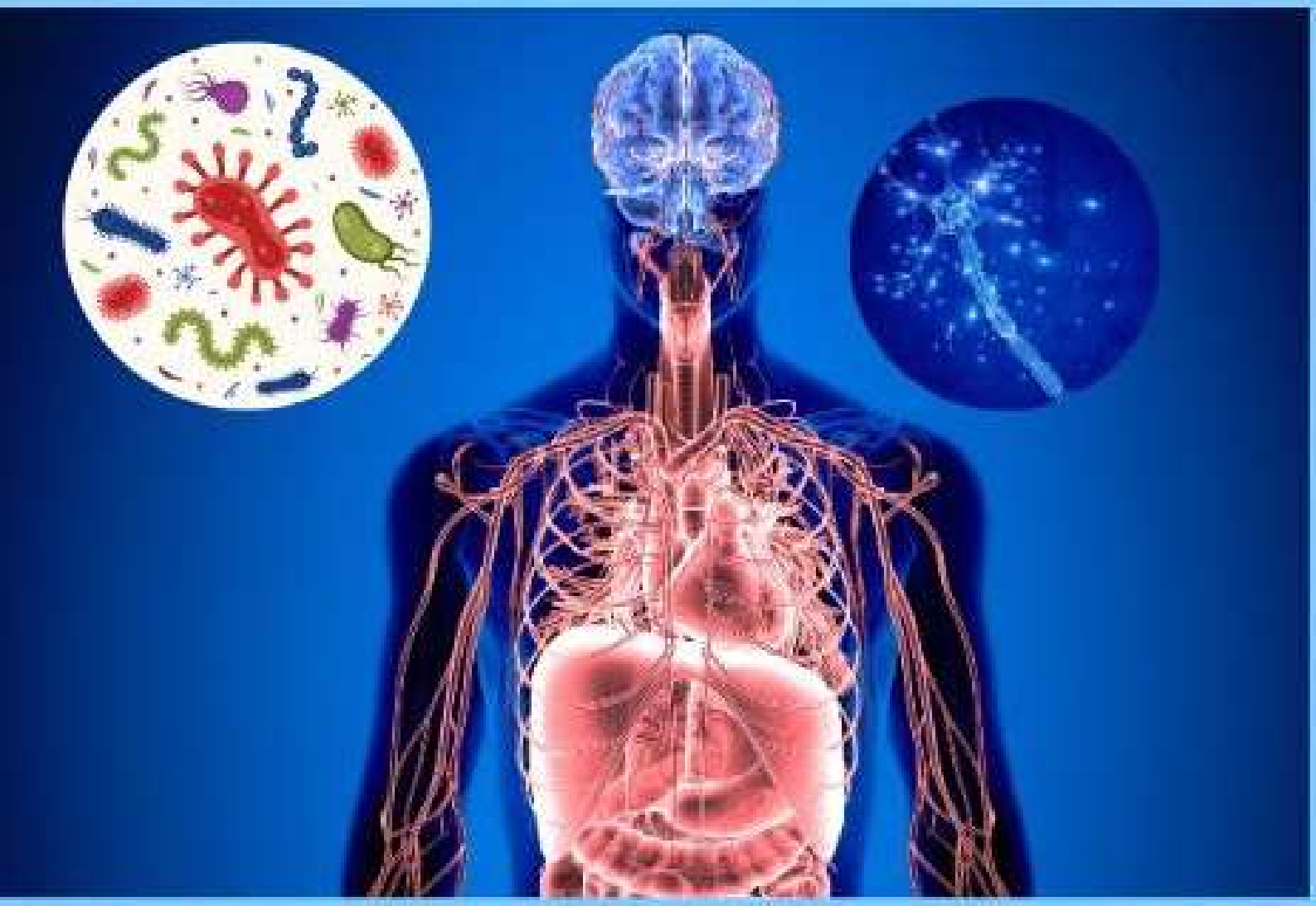
RECENT UPDATES IN MEDICINAL CHEMISTRY AND APPLIED MICROBIOLOGY



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Recent Updates in Medicinal Chemistry and Applied Microbiology

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PREFACE

We are glad to present the book entitled **Recent Updates in Medicinal Chemistry and Applied Microbiology (ISBN: 978-81-966183-3-9**)to the students, faculty members and researchers of Chemical sciences and Life Sciences. We have observed that eminent professors and active researchers from various technical institutions across the Nation contributed to the book chapters which are focused on state-of-the-art areas related to Medicinal Chemistry, Microbiology, and Biotechnology.

We hope that the research issues covered in the book will be helpful to the readers. We are grateful to the publisher and all the authors who contributed to the publication of **Recent Updates in Medicinal Chemistry and Applied Microbiology**: First Edition.

Editors

Dr. T. Gopalakrishnan

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Edited Book Entitled

"Recent Updates in Medicinal Chemistry and Applied Microbiology"

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COMPREHENSIVE INSIGHTS INTO MUCORMYCOSIS

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ABSTRACT

Mucormycosis, a rare but life-threatening fungal infection, has gained global attention due to its association with the COVID-19 pandemic. This study provides a comprehensive overview of mucormycosis, encompassing its epidemiology, risk factors, pathogenesis, diagnosis, and treatment. The objective is to enhance understanding and aid in the development of effective strategies for prevention and management. Epidemiological trends demonstrate an alarming rise in mucormycosis cases, particularly in individuals with COVID-19, diabetes, or immunocompromised conditions. The pathogenesis involves the invasion of tissues by Mucorales fungi, leading to devastating consequences. Early diagnosis is critical, involving a combination of clinical, radiological, and microbiological assessments. Timely intervention with antifungal therapy and surgical debridement is imperative for patient survival. The study concludes that increased awareness, risk factor mitigation, and healthcare system preparedness are essential in the battle against *Mucormycosis*.

***Key words**:* Mucormycosis; COVID-19; Epidemiology; Pathogenesis; Diagnosis and Treatment.

INTRODUCTION

American pathologist R.D. Baker coined the term *Mucormycosis,* also recognized as *Zygomycosis*. This refers to a subtle fungal infection caused by members of *Mucorales* and *zygomycotic* species. *Mucormycotina*, common saprobes originating from decaying matter or soils, are implicated. Infections involving *Mucorales* are characterized by swift progression (Kwon-Chung, 2012). In 1885, the German pathologist Paltauf documented the first case of Mucormycosis, terming it *Mycosis Mucorina* (Mohammadi *et al*., 2014). According to a study in France, there was a 7.4% annual increase in occurrence of *Mucormycosis* (Bitar *et al*., 2009). The universal incidence and the potential for seasonal variation in mucorales infection have been acknowledged (Petrikkos *et al*., 2012). These potent and extremely damaging infections primarily manifest in individuals with compromised immune systems, particularly in those undergoing hematopoietic stem cell transplantation or individuals with haematological malignancies. Distinct risk groups include diabetic patients with ketoacidosis and individuals with transfusional/dyserythropoetic iron overload. Challenges in diagnosis and subsequent antifungal treatment, compounded by significant intrinsic resistance to commonly employed antifungal drugs, contribute to elevated mortality rates in specific patient populations (Binder *et al*., 2014).

Mucormycosis, caused by fungi of the order *Mucorales*, is primarily acquired by humans through the inhalation of sporangiospores, ingestion of contaminated food, or inoculation (Ribes *et al*., 2000; Richardson, 2009). The fungi belonging to *Mucorales* are widespread, exhibiting a

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morphological appearance of broad, aseptate, or sparsely septate ribbon-like hyphae. Approximately 11 genera and ~27 species within *Mucorales* are associated with human infections. Globally, *Rhizopus arrhizus* stands as the most prevalent agent causing mucormycosis, followed by species such as *Lichtheimia*, *Apophysomyces, Rhizomucor*, *Mucor,* and Cunninghamella (Jeong *et al*., 2019; Prakash *et al*., 2018). The emergence of COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been linked to a diverse range of opportunistic bacterial and fungal infections. *Aspergillus* and *Candida* have been identified as the primary fungal pathogens for co-infections in individuals with COVID-19. Recently, there has been a notable increase in reported cases of *Mucormycosis* among individuals with COVID-19 worldwide (Singh *et al*., 2021).

EPIDEMIOLOGY AND CLINICAL PRESENTATION OF MUCORMYCOSIS

Rhizopus spp., *Lichtheimia*, *Mucor* spp., and spp. (formerly Absidia and Mycocladus) are the predominant causative agents of mucormycosis, with less common occurrences of genera like *Rhizomucor, Saksenaea, Cunninghamella*, and *Apophysomyces* (Petrikkos *et al*., 2012). The etiology of mucormycosis exhibits significant variations across different countries. In Europe, *Rhizopus* spp. (34%), *Lichtheimia* spp. (19%), and *Mucor* spp. (19%) are the most commonly identified agents in patients with mucormycosis (Skiada *et al*., 2011). In India, while *Rhizopus* species remain the leading cause, emerging species like *Apophysomyces elegans*, *A. variabilis*, and *Rhizopus homothallicus* are observed, along with rare agents like *Mucor irregularis* and *Thamnostylum lucknowense* (Chander *et al*., 2015). Additionally, a new species of *Apophysomyces, A. mexicanus*, has been reported in Mexico (Bonifaz *et al*., 2014).

Moreover, in Europe, *mucormycosis* ranks as the third most significant disease among hematological patients, following *Aspergillosis* and *Candidiasis* (Ibrahim *et al*., 2005). This infection poses a substantial threat primarily to immunocompromised individuals, including those with uncontrolled diabetes, undergoing corticosteroid treatment, recipients of bone marrow or solid organ transplantation, individuals with hematological malignancies, and those with traumatic injuries. Mucormycosis manifests in various forms: (1) Rhinocerebral mucormycosis, affecting the sinuses and brain, leading to symptoms such as fever, facial swelling, black lesions in the mouth or on the face, headaches, and sinus congestion; (2) Pulmonary mucormycosis, predominantly infecting the lungs, resulting in chest pain, difficulty breathing, cough, and fever; (3) Cutaneous mucormycosis, causing local skin infections characterized by ulcers or blisters, swelling, and redness; (4) Gastrointestinal mucormycosis, uncommon in adults but more frequent in premature neonates, leading to symptoms like nausea, gastrointestinal bleeding, abdominal pain, and vomiting; (5) Disseminated mucormycosis, occurring in patients with multiple medical complications, making it challenging to distinguish from other infectious diseases; (6) Uncommon presentations such as renal infection (Skiada *et al*., 2013; Lewis and Kontoyiannis, 2013; Ibrahim *et al*., 2005; Ribes *et al*., 2000).

The morbidity and mortality associated with mucormycosis vary depending on the affected organ, the causative fungal species, and the patient's medical status. For instance, a mortality

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rate of 46% was observed in patients with sinus infections, while pulmonary and disseminated mucormycosis infections reported mortality rates of 76% and 96%, respectively (Roden *et al*., 2005). Among patients undergoing stem cell and solid organ transplantation, the chances of survival are comparatively better, with reported mortality rates of 8% and 2%, respectively (Park *et al*., 2011). Diabetes stands out as the most common clinical risk factor distinguishing mucormycosis from Fusariosis or Pseudallescheriasis, as well as other uncommon Mold diseases (Quan, 2010). Diabetes is particularly prominent in rhino-orbital and rhinorbital-cerebral mucormycosis and is associated with mucormycosis infections affecting the sinuses and brain but does not play a role in pulmonary mucormycosis (Schachtschabel *et al*., 2008).

Globally, the occurrence of mucormycosis varied from 0.005 to 1.7 per million population, while its occurrence is nearly 80 times higher (0.14 per 1000) in India compared to industrialized countries, in a current estimate of year 2019–2020. In other words, India has highest cases of the mucormycosis in the world (Skiada *et al*., 2020). Highly, diabetes mellitus (DM) has been the most public risk factor linked with mucormycosis in India, although haematological malignancies and organ transplant takes the lead in Europe and the USA (Prakash and Chakrabarti, 2019).

RISK FACTORS

While there have been occasional reports of Mucormycosis in immunocompetent individuals, it is predominantly recognized as an opportunistic disease with identified risk factors across diverse patient populations. Particularly, the disease predominantly affects patients with haematological malignancies (HM) and persistent severe neutropenia. Notably higher in incidence compared to other fungal infections is the prevalence among patients with poorly controlled diabetes, especially complicated by ketoacidosis (DKA), individuals with iron overload, or those who have undergone major trauma (Roden *et al*., 2005; Schofield *et al*., 2013). Individuals most at risk for developing mucormycosis are those with reduced amounts of mononuclear and polymorphonuclear phagocytes, which would typically inhibit spore germination in healthy individuals, or those whose underlying conditions disrupt the function of their phagocytic cells. This includes patients with HM, individuals who have undergone hematopoietic stem cell transplantation, and those who have received high-dose corticosteroid treatment (Roilides *et al*., 2011; Dabritz *et al*., 2012).

Recently, a growing number of mucormycosis cases in individuals with COVID-19 have been reported worldwide, particularly in India. The primary factors contributing to the germination of Mucorales spores in people with COVID-19 seem to be the conducive environment of low oxygen (hypoxia), steroid-induced hyperglycemia, high glucose levels (diabetes, new-onset hyperglycemia, diabetic ketoacidosis (DKA)), acidic conditions (metabolic acidosis), elevated iron levels (increased ferritins), and reduced phagocytic activity of white blood cells (WBC) due to immunosuppression (SARS-CoV-2 mediated, steroid-mediated, or background comorbidities). These factors are compounded by several shared risk factors, including prolonged hospitalization with or without mechanical ventilators (Singh *et al*., 2021).

IRON UPTAKE AND MUCORMYCOSIS PATHOGENESIS

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In addition to host factors contributing to the predisposition of patients to mucormycosis, Mucorales exhibit virulence factors that enable the organism to cause disease. One significant trait is their capacity to acquire iron from the host, a crucial element for cell growth and development involved in various essential cellular processes (Howard, 1999). Successful pathogens employ multiple processes to procure iron from the host. Recent data indicate that the level of available, unbound iron in serum plays a critical role in uniquely predisposing patients with diabetic ketoacidosis (DKA) to mucormycosis.

In mammalian hosts, iron is typically bound to carrier proteins such as transferrin, lactoferrin, and ferritin, preventing the toxic effects of free iron. This sequestration of iron is a fundamental universal host defense mechanism against microbes, including Mucorales. Serum iron may also be increased in patients undergoing dialysis or multiple transfusions. In the past, the iron chelator deferoxamine was used in these cases. This is a bacterial siderophore and is actually utilized by Mucorales as a xenosiderophore for acquiring iron from the host. It soon became apparent that deferoxamine was a risk factor for mucormycosis, most often disseminated (Singh and Sun, 2008). Clinical observations supporting the role of iron uptake in mucormycosis pathogenesis include the unique susceptibility of patients with DKA. These individuals exhibit elevated levels of free iron in their serum, promoting the growth of *R. oryzae* under acidic conditions (pH 7.3–6.88) but not under alkaline conditions (pH 7.78–8.38) (Artis *et al*., 1982; Boelaert *et al*., 1993).

Moreover, experiments adding exogenous iron to serum demonstrated profuse growth of *R. oryzae* under acidic conditions, but not at pH 7.4. Simulated acidic conditions reduced the iron-binding capacity of serum samples from healthy volunteers, indicating that acidosis disrupts transferrin's ability to bind iron, potentially through proton-mediated displacement of ferric iron from transferrin. Animal data corroborated these findings, revealing that mice with DKA were protected from *R. oryzae* infection by the administration of iron chelators such as deferiprone and deferasirox, which *Mucorales* do not utilize as xenosiderophores. However, not all *Mucorales* have the same susceptibility to effective iron chelators; for instance, *Cunninghamella bertholletiae* and *Mucor* species display higher deferasirox minimal inhibitory and fungicidal concentrations than *Rhizopus* species (Ibrahim *et al*., 2008; Lewis *et al*., 2011).

DIAGNOSTIC METHOD

The diagnosis of mucormycosis involves a comprehensive assessment of clinical manifestations, including the meticulous use of magnetic resonance imaging (MRI) modalities and the early utilization of computed tomography (CT). Specialist evaluation of cytological and histological provisions, along with the optimal application of clinical microbiological techniques and molecular detection, is crucial in the diagnostic process. Detection of host factors plays a significant role in estimating a patient's likelihood of invasive mucormycosis.

Various laboratory techniques are employed for the detection of mucor, including PAS stains, direct examination, calcofluor, histopathological examination, Gomori methenamine silver stain, culture, molecular methods, and fluorescent in situ hybridization (Walsh *et al*., 2012). However, identifying mucormycosis is challenging due to its ambiguous clinical presentation and recurrent occult distribution, necessitating the development of sensitive nonculture-based

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investigative methods (Kontoyiannis *et al*., 2005). Tissue-based analysis remains the gold standard for confirmation.

In the realm of differential diagnosis, distinguishing mucormycosis from conditions such as maxillary sinus neoplasia, maxillary sinus aspergillosis, soft tissue infarction, soft tissue radionecrosis, and other deep fungal infections is crucial (Sciubba *et al*., 2002). Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry can enhance the identification of cultured specimens, but the development of comprehensive databases is essential for broader application (Cornely *et al*., 2019).

Molecular methods, including polymerase chain reaction (PCR)-based approaches, are increasingly employed for their ability to enhance detection in tissues and facilitate species-level identification using targets such as the internal transcribed spacer (ITS) or 18s ribosomal RNA. Non-invasive approaches to fungal identification, such as gene expression profiling, next-generation sequencing, and breath-based metabolomics, are also under exploration (Koshy *et al*., 2017). However, further development and validation of these methods are necessary for their widespread clinical utility.

MEDICAL MANAGEMENT OF MUCORMYCOSIS

The successful treatment of mucormycosis necessitates prompt and accurate diagnosis, surgical debridement, and the administration of appropriate drugs. Adjunctive measures such as hyperbaric oxygen, recombinant cytokines, or granulocyte transfusion, along with the use of a prosthetic obturator, have been proposed to enhance treatment outcomes (Sipsas *et al*., 2018). Recognizing the limitations of current monotherapies, especially in hematological patients, Spellberg *et al*. (2012) advocate for a "Combination therapy" approach to improve outcomes.

Management by Using Antifungal Drug Therapies

Antifungal therapies, including AmB Deoxycholate, Liposomal AmB (5-10mg/kg), AmB lipid complex, AmB colloidal dispersion, and Posaconazole (400mg bid), are commonly employed, accompanied by the management of underlying conditions. Second-line treatments involve combinations such as caspofungin with lipid AmB or a combination of lipid AmB and Posaconazole, with a recommendation against combining it with Deferasirox (Skiada *et al*., 2013). The antifungal drugs used for mucormycosis treatment are detailed in Table 1 (Alastruey-Izquierdo *et al*., 2009; Dannaoui *et al*., 2003; McCarthy *et al*., 2014; Vitale *et al*., 2012).

Chronic administration of corticosteroids and other immunosuppressive agents, commonly used in transplantation, malignancies, and autoimmune diseases, poses a significant risk factor for mucormycosis. Prolonged (>3 weeks) high-dose systemic corticosteroids are particularly associated with increased risk (Hoang *et al*., 2020). The concurrent challenges of diabetes and the widespread use of corticosteroids in COVID-19 patients create a concerning synergy that heightens susceptibility to mucormycosis. Effective management of hyperglycaemia and a judicious, evidence-based application of corticosteroids in COVID-19 patients are crucial to mitigate the devastating impact of fatal mucormycosis (Singh *et al*., 2021).

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Table 1. Antifungal drugs used for the treatment of Mucormycosis

Antifungal drug	Dose and Route	Common Side Effects
AmB - deoxycholate	1 to 1.5 mg/kg/day IV	Infusion reactions Phlebitis Acute kidney injury Hypokalemia and hypomagnesemia Anemia
Liposomal AmB	5 to 10 mg/kg/day IV	
ABLC	5 to 10 mg/kg/day IV	
Posaconazole	**V formulation**: 300 mg twice daily on day 1, followed by 300 mg daily **Oral suspension**: 200 mg four times daily, followed by 400 mg twice daily after stabilization of disease. **Delayed-release tablets**: 300 mg twice daily on day 1, followed by 300 mg daily	Nausea, vomiting, diarrhea, and headache QTc prolongation Hepatotoxicity
Isavuconazole	**IV formulation:** 372 mg every 8 h for 6 doses, followed by 372 mg once daily Oral tablets: 372 mg (2 capsules) every 8 h for 6 doses, followed by 372 mg (2 capsules) once daily	Nausea, vomiting, diarrhea, headache, and rash

SURGICAL MANAGEMENT

The exorbitant cost and limited availability of liposomal Am-B have led physicians to resort to surgical interventions to preserve the lives of patients. Surgical debridement, aimed at eliminating all necrotic lesions, remains the cornerstone of effective mucormycosis treatment in COVID-19 patients. Extensive surgery is advisable at the earliest opportunity, with preoperative utilization of MRI or CT scans to assess the extent of affected tissues and determine tissue margin involvement. Repetitive surgical excision of necrotic lesions has demonstrated enhanced outcomes. Following successful treatment, patients may undergo reconstructive surgery (Sipsas *et al*., 2018). Surgical recommendations vary based on the affected site and the severity of the condition.

PREVENTIVE MEASURES

The Indian Council of Medical Research (ICMR) has issued a set of general recommendations for preventing mucormycosis in COVID-19 patients (Chatterjee *et al*., 2020):

- Ensuring effective control of sugar levels during COVID-19, whether or not steroids are used.

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- Administering steroids in a judicious manner, with the correct dosage, appropriate timing, and for a suitable duration.
- Exercising caution in the use of antibiotics/antifungals.
- Employing sterile or clean water as humidifiers during oxygen therapy.
- Furthermore, for individuals in the post-COVID-19 recovery phase, adopting modest preventive measures is advised to mitigate the risk of mucormycosis:
- Maintaining personal hygiene through thorough bathing and body scrubbing.
- Utilizing face masks and face shields when in polluted or unsanitary environments.
- Wearing covered shoes, long trousers, long-sleeved shirts, and gloves when handling soil, manure, moss, etc., particularly during gardening activities.

CONCLUSION

Mucormycosis is a formidable fungal infection with a rising incidence, posing significant challenges to public health. Its association with COVID-19 and the surge in cases among diabetic and immunocompromised patients have sounded an alarm. This study has highlighted the critical aspects of mucormycosis, shedding light on its epidemiology, risk factors, pathogenesis, diagnosis, and treatment.

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REFERENCES

1. Gupta MP, Sharma K, Khan BU, Kapoor BC, Bajpai PK, Kumar A *et al*. Tissue specific esterase isozyme variation in *Clarias batrachus* and *C. gariepinus*. Global Journal of Pharmacology. 2009; 3(1):1-5

2. Alastruey-Izquierdo A, Castelli MV, Cuesta I, Zaragoza O, Monzón A, Mellado E, Rodríguez-Tudela JL. In vitro activity of antifungals against Zygomycetes. Clinical Microbiology and Infection. 2009; 15:71-6.

3. Artis WM, Fountain JA, Delcher HK, Jones HE. A mechanism of susceptibility to mucormycosis in diabetic ketoacidosis transferrin and iron availability. Diabetes. 1982;31(12):1109-14.

4. Binder U, Maurer E, Lass-Flörl C. Mucormycosis–from the pathogens to the disease. Clinical Microbiology and Infection. 2014; 20:60-6.

5. Bitar D, Van Cauteren D, Lanternier F, Dannaoui E, Che D, Dromer F, Desenclos JC, Lortholary O. Increasing incidence of Zygomycosis (Mucormycosis), France, 1997–2006. Emerging infectious diseases. 2009;15(9):1395.

6. Boelaert JR, de Locht M, Van Cutsem J, Kerrels V, Cantinieaux B, Verdonck A, Van Landuyt HW, Schneider YJ. Mucormycosis during deferoxamine therapy is a siderophore-mediated infection. In vitro and in vivo animal studies. The Journal of clinical investigation. 1993;91(5):1979-86.

ISBN : 978-81-966183-3-9

7. Bonifaz A, Stchigel AM, Guarro J, Guevara E, Pintos L, Sanchis M, Cano-Lira JF. Primary cutaneous mucormycosis produced by the new species Apophysomyces mexicanus. Journal of Clinical Microbiology. 2014;52(12):4428-31.
8. Chander J, Stchigel AM, Alastruey-Izquierdo A, Jayant M, Bala K, Rani H, Handa U, Punia RS, Dalal U, Attri AK, Monzon A. Fungal necrotizing fasciitis, an emerging infectious disease caused by *Apophysomyces (*Mucorales). Revista iberoamericana de micologia. 2015;32(2):93-8.
9. Chatterjee P, Nagi N, Agarwal A, Das B, Banerjee S, Sarkar S, Gupta N, Gangakhedkar RR. The 2019 novel coronavirus disease (COVID-19) pandemic: A review of the current evidence. The Indian journal of medical research. 2020;151(2-3):147.
10. Cornely OA, Alastruey-Izquierdo A, Arenz D, Chen SC, Dannaoui E, Hochhegger B, Hoenigl M, Jensen HE, Lagrou K, Lewis RE, Mellinghoff SC. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. The Lancet infectious diseases. 2019;19(12):405-21.
11. Däbritz J, Attarbaschi A, Tintelnot K, Kollmar N, Kremens B, Loewenich FD, Schrod L, Schuster F, Wintergerst U, Weig M, Lehrnbecher T. Mucormycosis in paediatric patients: demographics, risk factors and outcome of 12 contemporary cases. Mycoses. 2011;54(6):785-8.
12. Dannaoui E, Meletiadis J, Mouton JW, Meis JF, Verweij PE. In vitro susceptibilities of zygomycetes to conventional and new antifungals. Journal of Antimicrobial Chemotherapy. 2003;51(1):45-52.
13. Hoang K, Abdo T, Reinersman JM, Lu R, Higuita NI. A case of invasive pulmonary mucormycosis resulting from short courses of corticosteroids in a well-controlled diabetic patient. Medical mycology case reports. 2020; 29:22-4.
14. Howard DH. Acquisition, transport, and storage of iron by pathogenic fungi. Clinical Microbiology Reviews. 1999;12(3):394-404.
15. Ibrahim AS, Spellberg BJ, Avenissian V, Fu Y, Filler SG, Edwards Jr JE. Vaccination with recombinant N-terminal domain of Als1p improves survival during murine disseminated candidiasis by enhancing cell-mediated, not humoral, immunity. Infection and immunity. 2005;73(2):999-1005.
16. Ibrahim AS, Spellberg B, Edwards Jr J. Iron acquisition: a novel perspective on mucormycosis pathogenesis and treatment. Current opinion in infectious diseases. 2008;21(6):620-5.

17. Jeong W, Keighley C, Wolfe R, Lee WL, Slavin MA, Kong DC, Chen SA. The epidemiology and clinical manifestations of mucormycosis: a systematic review and meta-analysis of case reports. Clinical microbiology and infection. 2019;25(1):26-34.
18. Kontoyiannis DP, Lionakis MS, Lewis RE, Chamilos G, Healy M, Perego C, Safdar A, Kantarjian H, Champlin R, Walsh TJ, Raad II. Zygomycosis in a tertiary-care cancer center in the era of Aspergillus-active antifungal therapy: a case-control observational study of 27 recent cases. The Journal of infectious diseases. 2005;191(8):1350-60.

ISBN : 978-81-966183-3-9

19. Koshy S, Ismail N, Astudillo CL, Haeger CM, Aloum O, Acharige MT, Farmakiotis D, Baden LR, Marty FM, Kontoyiannis DP, Fredenburgh L. Breath-based diagnosis of invasive mucormycosis (IM). InOpen Forum Infectious Diseases 2017 (Vol. 4, No. suppl_1, pp. S53-S54). US: Oxford University Press.
20. Kwon-Chung KJ. Taxonomy of fungi causing mucormycosis and entomophthoramycosis (zygomycosis) and nomenclature of the disease: molecular mycologic perspectives. Clinical Infectious Diseases. 2012; 54:8-15.
21. Lewis RE, Kontoyiannis DP. Epidemiology and treatment of mucormycosis. Future microbiology. 2013;8(9):1163-75.
22. Lewis RE, Pongas GN, Albert N, Ben-Ami R, Walsh TJ, Kontoyiannis DP. Activity of deferasirox in Mucorales: influences of species and exogenous iron. Antimicrobial agents and chemotherapy. 2011:411-3.
23. McCarthy M, Rosengart A, Schuetz AN, Kontoyiannis DP, Walsh TJ. Mold infections of the central nervous system. New England Journal of Medicine. 2014;371(2):150-60.
24. Mohammadi R, Nazeri M, Sayedayn SM, Ehteram H. A successful treatment of rhinocerebral mucormycosis due to Rhizopus oryzae. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 2014;19(1):72.
25. Park BJ, Pappas PG, Wannemuehler KA, Alexander BD, Anaissie EJ, Andes DR, Baddley JW, Brown JM, Brumble LM, Freifeld AG, Hadley S. Invasive non-Aspergillus mold infections in transplant recipients, United States, 2001–2006. Emerging infectious diseases. 2011;17(10):1855.
26. Petrikkos G, Skiada A, Lortholary O, Roilides E, Walsh TJ, Kontoyiannis DP. Epidemiology and clinical manifestations of mucormycosis. Clinical Infectious Diseases. 2012;(54)23-34.
27. Prakash H, Chakrabarti A. Global epidemiology of mucormycosis. Journal of Fungi. 2019;5(1):26.
28. Prakash H, Chakrabarti A. Global epidemiology of mucormycosis. Journal of Fungi. 2019;5(1):26.
29. Prakash H, Ghosh AK, Rudramurthy SM, Singh P, Xess I, Savio J, Pamidimukkala U, Jillwin J, Varma S, Das A, Panda NK. A prospective multicenter study on mucormycosis in India: Epidemiology, diagnosis, and treatment. Medical mycology. 2019;57(4):395-402.
30. Quan C, Spellberg B. Mucormycosis, pseudallescheriasis, and other uncommon mold infections. Proceedings of the American Thoracic Society. 2010 May 15;7(3):210-5.
31. Ribes JA, Vanover-Sams CL, Baker DJ. Zygomycetes in human disease. Clinical microbiology reviews. 2000;13(2):236-301.
32. Richardson M. The ecology of the Zygomycetes and its impact on environmental exposure. Clinical Microbiology and Infection. 2009;15:2-9.
33. Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, Schaufele RL, Sein M, Sein T, Chiou CC, Chu JH, Kontoyiannis DP. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. Clinical infectious diseases. 2005;41(5):634-53.

ISBN : 978-81-966183-3-9

34. Roilides E, Kontoyiannis DP, Walsh TJ. Host defenses against zygomycetes. Clinical infectious diseases. 2012;54:61-6.
35. Schachtschabel D, David A, Menzel KD, Schimek C, Wöstemeyer J, Boland W. Cooperative biosynthesis of trisporoids by the (+) and (−) mating types of the zygomycete Blakeslea trispora. ChemBioChem. 2008;9(18):3004-12.
36. Schofield C, Stern A, Jevtic A. Disseminated zygomycosis due to Mycocladus corymbifera with cutaneous and cerebral involvement. Australasian Journal of Dermatology. 2013;54(1): 8-11.
37. Sciubba JJ, Regezi JA, Rogers RS. PDQ oral disease: diagnosis and treatment. Pmph-usa; 2002.
38. Singh AK, Singh R, Joshi SR, Misra A. Mucormycosis in COVID-19: a systematic review of cases reported worldwide and in India. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2021;15(4):102146.
39. Singh N, Sun HY. Iron overload and unique susceptibility of liver transplant recipients to disseminated disease due to opportunistic pathogens. Liver Transplantation. 2008;14(9):1249-55.
40. Sipsas NV, Gamaletsou MN, Anastasopoulou A, Kontoyiannis DP. Therapy of mucormycosis. Journal of Fungi. 2018;4(3):90.
41. Skiada A, Lanternier F, Groll AH, Pagano L, Zimmerli S, Herbrecht R, Lortholary O, Petrikkos GL. Diagnosis and treatment of mucormycosis in patients with hematological malignancies: guidelines from the 3rd European Conference on Infections in Leukemia (ECIL 3). haematologica. 2013;98(4):492.
42. Skiada A, Pavleas I, Drogari-Apiranthitou M. Epidemiology and diagnosis of mucormycosis: an update. Journal of fungi. 2020;6(4):265.
43. Spellberg B, Ibrahim A, Roilides E, Lewis RE, Lortholary O, Petrikkos G, Kontoyiannis DP, Walsh TJ. Combination therapy for mucormycosis Clinical infectious diseases. 2012;54:73-8.
44. Steinbrink JM, Miceli MH. Mucormycosis. Infectious Disease Clinics. 2021;35(2):435-52.
45. Vitale RG, De Hoog GS, Schwarz P, Dannaoui E, Deng S, Machouart M, Voigt K, Van De Sande WW, Dolatabadi S, Meis JF, Walther G. Antifungal susceptibility and phylogeny of opportunistic members of the order mucorales. Journal of clinical microbiology. 2012;50(1):66-75.
46. Walsh TJ, Gamaletsou MN, McGinnis MR, Hayden RT, Kontoyiannis DP. Early clinical and laboratory diagnosis of invasive pulmonary, extrapulmonary, and disseminated Mucormycosis (Zygomycosis). Clinical Infectious Diseases. 2012;54:55-60.

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NANOPARTICLES AS THERAPEUTIC BIOMATERIALS

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ABSTRACT

Recent drug delivery systems are formulated with well-developed properties such as smaller particle size, increased permeability, increased solubility, efficacy, specific site targeting, stability, toxicity, and sustained delivery. Nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. The nanoparticles have been used *in vivo* to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. Various biomaterials have been used in the formulation of nanoparticles for drug delivery research to increases therapeutic benefit, while minimizing side effects. Here, we review various aspects of nanoparticle formulation, characterization, effects and their applications.

Key words*:* Nanoparticles, Drug delivery, Targeting, Drug release.

INTRODUCTION

Nanoparticles are well-defined as particulate dispersions with a dimension in the range of 10-1000nm. The drug is dissolved, deceived, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nano capsules can be obtained. Nano capsules are systems in which the drug is limited to a cavity surrounded by a unique polymer membrane, whereas nanospheres are matrix systems in which the drug is essentially and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly(ethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes (1-4). The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen.

Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation, targeting to site of action and reduction toxicity or side effects, their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability. On the other hand, polymeric nanoparticles offer some specific advantages

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over liposomes. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties (5, 6). The advantages of using nanoparticles as a drug delivery system include the following: 1. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration. 2. They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects. 3. Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity. 4. Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance. 5. The system can be used for numerous routes of administration including oral, nasal, parenteral, intra-ocular etc. In spite of these advantages, nanoparticles do have limitations. For example, their small size and large surface area can lead to particle aggregation, making physical handling of nanoparticles problematic in liquid and dry forms.

In addition, small particles size and large surface area readily result in limited drug loading and burst release. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available. The present review details the latest development of nanoparticulate drug delivery systems, surface modification issues, drug loading strategies, release control and potential applications of nanoparticles.

Preparation of Nanoparticles

Nanoparticles may be organized from a spread of materials including proteins, polysaccharides and synthetic polymers. the choice of matrix substances is depending on many factors including (7) : (a) size of nanoparticles required; (b) inherent houses of the drug, e.g., aqueous solubility and stability; (c) floor traits which include price and permeability; (d) degree of biodegradability, biocompatibility and toxicity; (e) Antigenicity of the final product. Nanoparticles have been organized most frequency by using 3 methods: (1) dispersion of preformed polymers; (2) polymerization of monomers; and (three) ionic gelation or coacervation of hydrophilic polymers. but other strategies which include supercritical fluid generation eight and particle replication in non-wetting templates (9) have also been defined inside the literature for production of nanoparticles. The latter turned into claimed to have absolute manipulate of particle size, shape and composition, which could set an instance for the destiny mass manufacturing of nanoparticles in enterprise. Dispersion of preformed polymers: Dispersion of preformed polymers is a commonplace method used to put together biodegradable nanoparticles from poly (lactic acid) (PLA); poly (D,L-glycolide), PLG; poly (D, L-lactide-co-glycolide) (PLGA) and poly (cyanoacrylate) (PCA), (10-12). This approach can be utilized in diverse methods as described under. Solvent evaporation approach: on this approach, the polymer is dissolved in a natural solvent which includes dichloromethane, chloroform or ethyl acetate which is likewise used because the solvent for dissolving the hydrophobic drug. The combination of polymer and drug answer is then emulsified in an

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aqueous answer containing a surfactant or emulsifying agent to shape an oil in water (o/w) emulsion. After the formation of stable emulsion, the natural solvent is evaporated both by way of lowering the stress or by continuous stirring. Particle size become determined to be inspired by means of the kind and concentrations of stabilizer, homogenizer speed and polymer awareness (13).

That allows to produce small particle length, often a high-velocity homogenization or ultrasonication (14). Spontaneous emulsification or solvent diffusion method: that is a modified version of solvent evaporation approach (15). On this technique, the water miscible solvent along with a small amount of the water immiscible organic solvent is used as an oil section. Because of the spontaneous diffusion of solvents an interfacial turbulence is created among the 2 phases leading to the formation of small particles. because the concentration of water miscible solvent increases, a decrease inside the size of particle can be accomplished. each solvent evaporation and solvent diffusion techniques can be used for hydrophobic or hydrophilic capsules, within the case of hydrophilic drug, a multiple w/o/w emulsion wishes to be formed with the drug dissolved inside the inner aqueous section. Polymerization method in this technique, monomers are polymerized to form nanoparticles in an aqueous manner. Drug is included either by way of being dissolved within the polymerization medium or via adsorption onto the nanoparticles after polymerization completed. The nanoparticle suspension is then purified to eliminate diverse stabilizers and surfactants hired for polymerization by means of ultracentrifugation and re-suspending the debris in an isotonic surfactant-free medium. This approach has been suggested for making polybutylcyanoacrylate or poly (alkyl cyanoacrylate) nanoparticles (16,17). Nano capsule formation and their particle length relies upon at the awareness of the surfactants and stabilizers (18). Coacervation or ionic gelation technique much studies has been focused at the guidance of nanoparticles the usage of biodegradable hydrophilic polymers along with chitosan, gelatin and sodium alginate. Calvo evolved a technique for making ready hydrophilic chitosan nanoparticles by means of ionic gelation (19, 20). The technique entails an aggregate of aqueous stages, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a poly anion sodium tripolyphosphate. In this approach, positively charged amino organization of chitosan interacts with bad charged tripolyphosphate to shape coacervates with a size inside the variety of nanometer. Coacervates are formed because of electrostatic interplay among two aqueous stages, while, ionic gelation involves the fabric undergoing transition from liquid to gel (1) because of ionic interaction conditions at room temperature.

Manufacturing of nanoparticles using supercritical fluid era conventional techniques such as solvent extraction-evaporation, solvent diffusion and organic segment separation methods require using natural solvents that are unsafe to the surroundings as well as to physiological structures. Consequently, the supercritical fluid generation has been investigated as an opportunity to put together biodegradable micro- and nanoparticles due to the fact supercritical fluids are environmentally safe (21).

A supercritical fluid may be commonly defined as a solvent at a temperature above its essential temperature, at which the fluid remains a single section no matter pressure (21). Supercritical

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CO2 (SC CO2) is the maximum extensively used supercritical fluid due to its slight vital situations nontoxicity, non-flammability, and coffee rate. The most not unusual processing strategies regarding supercritical fluids are supercritical anti-solvent (SAS) and fast growth. The system of SAS employs a liquid solvent, eg methanol, that's absolutely miscible with the supercritical fluid (SCCO2), to dissolve the solute to be micronized; on the system situations, because the solute is insoluble within the supercritical fluid, the extract of the liquid solvent by means of supercritical fluid ends in the instant precipitation of the solute, resulting the formation of nanoparticles eight. Thote and Gupta (2005) defined the usage of a changed SAS approach for formation of hydrophilic drug dexamethasone phosphate drug nanoparticles for microencapsulation purpose (21) .RESS differs from the SAS manner in that its solute is dissolved in a supercritical fluid (along with supercritical methanol) after which the solution is unexpectedly expanded via a small nozzle right into a location lower pressure(22), as a consequence the solvent power of supercritical fluids dramatically decreases and the solute sooner or later precipitates. This approach is clean because the precipitate is essentially solvent unfastened. RESS and its modified method were used for the fabricated from polymeric nanoparticles (23). Supercritical fluid generation method, even though environmentally friendly and appropriate for mass manufacturing, calls for specially designed system and is more steeply-priced.

Effect of Characteristics of Nanoparticles on medicine Delivery flyspeck size and size distribution are the most important characteristics of nanoparticle systems. They determine the in vivo distribution, natural fate, toxin and the targeting capability of nanoparticle systems. In addition, they can also impact the medicine lading, medicine release and stability of nanoparticles. numerous studies have verified that nanoparticles of sub-micron size have a number of advantages over microparticles as a medicine delivery system (24). Generally, nanoparticles have fairly advanced intracellular uptake compared to microparticles and available to a wider range of natural targets due to their small size and relative mobility. That 100 nm nanoparticles had a2.5-fold lesser uptake than 1 μm microparticles, and 6-fold lesser uptake than 10 μm microparticles in a Caco- 2 cell line (25). In a posterior study (26), the nanoparticles entered throughout the submucosal layers in a rat in situ intestinal circle model, while microparticles were generally localized in the epithelial filling. It was also reported that nanoparticles can across the blood- brain hedge following the opening of tight junctions by hyperactive bibulous mannitol, which may give sustained delivery of remedial agents for delicate- to- treat conditions like brain excrescences (27). Tween 80 carpeted nanoparticles have been shown to cross the blood- brain hedge (28).

In some cell lines, only submicron nanoparticles can be taken up efficiently but not the larger size microparticles (29). Medicine release is affected by flyspeck size. lower patches have larger face area, thus, utmost of the medicine associated would be at or near the flyspeck face, leading to fast medicine release. Whereas, larger patches have large cores which allow further medicine to be reprised and sluggishly verbose out (30). lower patches also have lesser threat of aggregation of patches during storehouse and transportation of nanoparticle dissipation. It's

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always a challenge to formulate nanoparticles with the lowest size possible but maximum stability. Polymer declination can also be affected by the flyspeck size.

Thus, it was hypothecated that larger patches will contribute to faster polymer declination as well as the medicine release. still, Panyam *et al* set PLGA patches with different size ranges and set up that the polymer declination rates in vitro weren't mainly different for different size patches (31,32). Presently, the fastest and most routine system of determining flyspeck size is by photon- correlation spectroscopy or dynamic light scattering. Photon- correlation spectroscopy requires the density of the medium to be known and determines the periphery of the flyspeck by Brownian stir and light scattering parcels (33). The results attained by photon-correlation spectroscopy is generally vindicated by surveying or transmission electron microscopy (SEM or TEM). When nanoparticles are administered intravenously, they're fluently honored by the body vulnerable systems, and are also cleared by phagocytes from the rotation (34). Piecemeal from the size of nanoparticles, their face hydrophobicity determines the quantum of adsorbed blood factors, substantially proteins(opsonin). This in turn influences the in vivo fate of nanoparticles (35). List of these opsonin onto the face of nanoparticles called opsonization acts as a ground between nanoparticles and phagocytes. Indeed, formerly in the blood sluice, face on-modified nanoparticles (conventional nanoparticles) are fleetly opsonized and largely cleared by the macrophages of MPS rich organs (36)

Generally, it is IgG, compliment C3 components that are used for recognition of foreign substances, especially foreign macromolecules. Hence, to increase the likelihood of the success in drug targeting by nanoparticles, it is necessary to minimize the opsonization and to prolong the circulation of nanoparticles in vivo. This can be achieved by (a) surface coating of nanoparticles with hydrophilic polymers/surfactants; (b) formulation of nanoparticles with biodegradable copolymers with hydrophilic segments such as polyethylene glycol (PEG), polyethylene oxide, polyoxamer, poloxamine and polysorbate 80 (Tween 80). PEG surfaces in brush-like and intermediate configurations reduced phagocytosis and complement activation whereas PEG surfaces in mushroom-like configuration were potent complement activators and favoured phagocytosis (37). The zeta potential of a nanoparticle is commonly used to indicate the surface charge property of nanoparticles (38). It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. Nanoparticles with a zeta potential above (+/-) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles.

The zeta potential can also be used to determine whether a charged active material is encapsulated within the center of the nano capsule or adsorbed onto the surface (1) Drug loading Ideally, a successful nanoparticulate system should have a high drug-loading capacity thereby reduce the quantity of matrix materials for administration. Drug loading can be done by two methods: • Incorporating at the time of nanoparticles production (incorporation method) • Absorbing the drug after formation of nanoparticles by incubating the carrier with a concentrated drug solution (adsorption /absorption technique). Drug loading and entrapment efficiency very much depend on the solid-state drug solubility in matrix material or polymer (solid dissolution or dispersion), which is related to the polymer composition, the molecular

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weight, the drug polymer interaction and the presence of end functional groups (ester or carboxyl) (38- 41). The PEG moiety has no or little effect on drug loading (42). The macromolecule or protein shows greatest loading efficiency when it is loaded at or near its isoelectric point when it has minimum solubility and maximum adsorption. For small molecules, studies show the use of ionic interaction between the drug and matrix materials can be a very effective way to increase the drug loading (43, 44). Drug release to develop a successful nanoparticulate system, both drug release and polymer biodegradation are important attention factors. In general, drug release rate depends on: (1) solubility of drug; (2) desorption of the surface bound/adsorbed drug; (3) drug diffusion through the nanoparticle matrix; (4) nanoparticle matrix erosion/degradation; and (5) combination of erosion/diffusion process. Thus solubility, diffusion and biodegradation of the matrix materials govern the release process. The rapid initial release or 'burst' is mainly attributed to weakly bound or adsorbed drug to the large surface of nanoparticles (45). It is obvious that the method of incorporation has an effect on release profile. If the drug is loaded by combination method, the system has a relatively small burst effect and better sustained release characteristics (46). If the nanoparticle is coated by polymer, the release is then controlled by diffusion of the drug from the core across the polymeric membrane. Furthermore, release rate can also be affected by ionic interaction between the drug and addition of auxillary ingredients. When the drug is elaborate in interaction with auxillary ingredients to form a less water soluble complex, then the drug release can be very slow with almost no burst release effect ; Various methods which can be used to study the in vitro release of the drug are: (1) side-by-side diffusion cells with artificial or biological membranes; (2) dialysis bag diffusion technique; (3) reverse dialysis bag technique; (4) agitation followed by ultracentrifugation/centrifugation; (5) Ultra-filtration or centrifugal ultra-filtration techniques. Usually, the release study is carried out by controlled agitation followed by centrifugation. Due to the time-consuming nature and technical difficulties encountered in the separation of nanoparticles from release media, the dialysis technique is generally preferred.

Applications of Nanoparticulate Delivery Systems Tumor targeting using nanoparticulate delivery systems The rationale of using nanoparticles for tumor targeting is based on 1) nanoparticles will be able to deliver a concentrate dose of drug in the (1) vicinity of the tumor targets via the enhanced permeability and retention effect or active targeting by ligands on the surface of nanoparticles; 2) nanoparticles will reduce the drug exposure of health tissues by limiting drug distribution to target organ. Verdun *et al* confirmed in mice treated with doxorubicin combined into poly (isohexylcyanoacrylate) nanospheres that higher concentrations of doxorubicin manifested in the liver, spleen and lungs than in mice treated with free doxorubicin (47). The exact underlying mechanism is not fully understood but the biodistribution of nanoparticles is rapid, within ½ hour to 3 hours, and it likely involves MPS and endocytosis/phagocytosis process (48). Recently Bibby *et al* reported the biodistribution and pharmacokinetics (PK) of a cyclic RGD doxorubicin-nanoparticle formulation in tumor bearing mice (49). Their biodistribution studies revealed decreasing drug concentrations over time in the heart, lung, kidney and plasma and accumulating drug concentrations in the liver,

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spleen and tumor. The majority injected dose appeared in the liver (56%) and only 1.6% in the tumour at 48 hrs. post injection, confirming that nanoparticles have a great tendency to be captured by liver. This indicates the greatest challenge of using nanoparticles for tumour targeting is to avoid particle uptake by mononuclear phagocytic system (MPS) in liver and spleen. Such propensity of MPS for endocytosis/phagocytosis of nanoparticles provides an opportunity to effectively deliver therapeutic agents to these cells. This biodistribution can be of benefit for the chemotherapeutic treatment of MPS- rich organs/tissues localized tumors like hepatocarcinoma, hepatic metastasis arising from digestive tract or gynecological cancers, bronchopulmonary tumors, primitive tumors and metastasis, small cell tumors, myeloma and leukemia. Histological examination showed a considerable accumulation of nanoparticles in the lysosomal vesicles of Kupffer cells, whereas nanoparticles could not be clearly identified in tumoral cells. Thus, Kupffer cells, after a massive uptake of nanoparticles by phagocytosis, were able to induce the release of doxorubicin, leading to a gradient of drug concentration, favorable for a prolonged diffusion of the free and still active drug towards the neighboring metastatic cells (50). When conventional nanoparticles are used as carriers in chemotherapy, some cytotoxicity against the Kupffer cells can be expected, which would result in deficiency of Kupffer cells and naturally lead to reduced liver uptake and decreased therapeutic effect with intervals of less than 2 weeks administration (51). Long circulating nanoparticles to be successful as a drug delivery system, nanoparticles must be able to target tumors which are localized outside MPS-rich organs. In the past decade, a great deal of work has been devoted to developing so-called "stealth (1) particles or PEGylated nanoparticles, which are invisible to macrophages or phagocytes (52).

A prime step forward inside the area got here whilst using hydrophilic polymers (together with polyethylene glycol, poloxamines, poloxamers, and polysaccharides) to efficaciously coat traditional nanoparticle surface produced an opposing impact to the uptake with the aid of the MPS (53). Those coatings provide a dynamic "cloud" of hydrophilic and neutral chains on the particle floor which repel plasma proteins (54,55). Coating traditional nanoparticles with surfactants or PEG to acquire an extended-circulating service has now been used as a popular approach for drug concentrated on in vivo. Thinking about that fact that folate receptors are over said at the surface of some human malignant cells and the cell adhesion molecules which includes selectins and integrins are concerned in metastatic events, nanoparticles bearing particular ligands such as folate can be used to target ovarian carcinoma while particular peptides or carbohydrates can be used to target integrins and selectins (56). Oyewumi *et al* confirmed that the benefits of folate ligand coating have been to facilitate tumor cell internalization and retention of Gd-nanoparticles within the tumor tissue (57). MDR takes place specially because of the over expression of the plasma membrane glycoprotein (Pgp), that is able to extruding diverse definitely charged xenobiotics, including a few anticancer tablets, out of cells (58). The cause in the back of the affiliation of medicine with colloidal carriers, including nanoparticles, towards drug resistance derives from the fact that Pgp possibly acknowledges the drug to be effluxed out of the tumoral cells most effective whilst this drug is present in the

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plasma membrane, and not when it's far placed in the cytoplasm or lysosomes after endocytosis (59, 60).

Nanoparticles for oral shipping of peptides and proteins enormous advances in biotechnology and biochemistry have led to the invention of a big wide variety of bioactive molecules and vaccines based on peptides and proteins. (1) reality that bioavailability of those molecules is confined by means of the epithelial limitations of the gastrointestinal tract and their susceptibility to gastrointestinal degradation via digestive enzymes. Polymeric nanoparticles permit encapsulation of bioactive molecules and protect them in opposition to enzymatic and hydrolytic degradation. for example, it has been located that insulin-loaded nanoparticles have preserved insulin pastime and produced blood glucose reduction in diabetic rats for up to 14 days following the oral management (61). The surface vicinity of human mucosa extends to two hundred instances that of pores and skin (62). The gastrointestinal tract offers a ramification of physiological and morphological boundaries against protein or peptide shipping, e.g., (a) proteolytic enzymes within the gut lumen like pepsin, trypsin and chymotrypsin; (b) proteolytic enzymes at the brush border membrane (endopeptidases); (c) bacterial intestine vegetation; and (d) mucus layer and epithelial mobile lining itself (63). The histological architecture of the mucosa is designed to efficiently prevent uptake of particulate depend from the surroundings. positive glycoproteins and lectins bind selectively to this form of floor shape by means of specific receptor-mediated mechanism. extraordinary lectins, along with bean lectin and tomato lectin, had been studied to beautify oral peptide adsorption sixty (64, 65). The capability to increase oral bioavailability of diverse peptides (e.g., granulocyte colony stimulating aspect, erythropoietin) and debris by covalent coupling to diet B-12 has been studied (66,67). For this intrinsic procedure, mucoprotein is required, which is ready through the mucus membrane inside the stomach and binds especially to cobalamin. The mucoprotein absolutely reaches the ileum wherein resorption is mediated by means of unique receptors. The paracellular course of absorption of nanoparticles utilizes less than 1% of mucosal surface place. the use of polymers which includes chitosan (68), starch (69) or poly(acrylate) (70) can increase the paracellular permeability of macromolecules. This technique is initiated by way of an unspecific bodily adsorption of cloth to the cellular floor by electrostatic forces which include hydrogen bonding or hydrophobic interactions (71). Adsorptive endocytosis depends basically on the size and floor homes of the fabric. If the floor rate of the nanoparticles is tremendous or uncharged, it will offer an affinity to adsorptive enterocytes although hydrophobic, while if it is negatively charged and hydrophilic, it shows greater affinity to adsorptive enterocytes and M cells.

This shows that an aggregate of size, surface fee and hydrophilicity play a primary position in affinity. that is demonstrated with poly (styrene) nanoparticles and whilst it's far carboxylated (72). Nanoparticles for gene transport Polynucleotide vaccines paintings via delivering genes encoding applicable antigens to host cells where they're expressed, generating the antigenic protein inside the area of expert antigen presenting cells to provoke immune response. (1) humoral and cellular-mediated immunity because intracellular production of protein, as opposed to extracellular deposition, stimulates both fingers of the immune machine (73). Nanoparticles loaded with plasmid DNA can also function a green sustained release gene

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shipping system due to their rapid break out from the degradative endo-lysosomal compartment to the cytoplasmic compartment (74). Hedley *et al*. (75) stated that following their intracellular uptake and endo lysosomal break out, nanoparticles ought to launch DNA at a sustained fee ensuing in sustained gene expression. It efficaciously prevents the passage of water-soluble molecules from the blood movement into the CNS, and can also reduce the brain awareness of lipid-soluble molecules with the aid of the function of enzymes or efflux pumps (76). as an instance, polysorbate eighty/LDL, transferrin receptor binding antibody (which include OX26), lactoferrin, cellular penetrating peptides and melanotransferrin had been proven able to delivery of a self non transportable drug into the brain through the chimeric construct that may undergo receptor-mediated transcytosis (77-81). it has been suggested poly (butyl cyanoacrylate) nanoparticles became capable of supply hexapeptide dalargin, doxorubicin and different sellers into the mind that's massive due to the exceptional issue for tablets to go the BBB 77. despite some said fulfillment with polysorbate 80 coated NPs, this device does have many shortcomings such as desorption of polysorbate coating, rapid NP degradation and toxicity because of presence of high attention of polysorbate 80.37. OX26 MAbs (anti-transferrin receptor MAbs), the most studied BBB targeting antibody, have been used to enhance the BBB penetration of liposomes (82). however, currently, Ji *et al*. demonstrated that mind uptake of lactoferrin, an iron-binding glycoprotein belonging to the transferrin (Tf) circle of relatives, is two times that of OX26 and transferrin in vivo 79. it's miles feasible quickly we can see these BBB particular molecules used for focused on nanoparticles to the brain.

CONCLUSION

The foregoing studies shows that nanoparticulate structures have notable potentials, being able to convert poorly soluble, poorly absorbed and labile biologically lively substance into promising deliverable capsules. The middle of this device can enclose a spread of medication, enzymes, genes and is characterized through a long move time because of the hydrophilic shell which prevents popularity with the aid of the reticular-endothelial gadget. To optimize this drug transport gadget, more understanding of the extraordinary mechanisms of organic interactions, and particle engineering, is still required. In addition, advances are wished a good way to turn the concept of nanoparticle technology into a sensible application as the subsequent generation of drug transport device.

REFERENCES

1. Langer R. Biomaterials in drug delivery and tissue engineering: one laboratory's experience. Acc Chem Res 2000; 33:94-101.

2. Bhadra D, Bhadra S, Jain P, Jain NK. Pegnology: a review of PEG-ylated systems. Pharmazie 2002; 57: 5-29.

3. Kommareddy S, Tiwari SB, Amiji MM. Long-circulating polymeric nano vectors for tumor-selective gene delivery. Technol Cancer Res Treat 2005; 4: 615- 25.

4. Lee M, Kim SW. Polyethylene glycol-conjugated copolymers for plasmid DNA delivery. Pharm Res 2005; 22: 1-10.

ISBN : 978-81-966183-3-9

5. Vila A, Sanchez A, Tobio M, Calvo P, Alonso MJ. Design of biodegradable particles for protein delivery. J Control Release 2002; 78: 15-24.
6. Mu L, Feng SS. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol(R)): PLGA nanoparticles containing vitamin E TPGS. J Control Release 2003; 86: 33-48. 7. Kreuter J. Nanoparticles. In Colloidal drug delivery systems, J, K., Ed. Marcel Dekker: New York, 1994; 219-342.
8. Reverchon E, Adami R. Nanomaterials and supercritical fluids. The Journal of Supercritical Fluids 2006; 37: 1-22.
9. Rolland JP, Maynor BW, Euliss LE, Exner AE, Denison GM, DeSimone JM. Direct fabrication and harvesting of monodisperse, shape-specific nano biomaterials. J. Am. Chem. Soc. 2005; 127: 10096-10100
10. Kompella UB, Bandi N, Ayalasomayajula SP. Poly (lactic acid) nanoparticles for sustained release of budesonide. Drug Deliv. Technol. 2001; 1: 1-7.
11. Ravi MN, Bakowsky U, Lehr CM. Preparation and characterization of cationic PLGA nanospheres as DNA carriers. Biomaterials 2004; (25)1771-1777.
12. Li YP, Pei YY, Zhou ZH, Zhang XY, Gu ZH, Ding J, Zhou JJ, Gao, XJ, PEGylated polycyanoacrylate nanoparticles as tumor necrosis factor-[alpha] carriers. J Control Release 2001; (71)287-296.
13. Kwon, HY, Lee JY, Choi SW, Jang Y, Kim JH. Preparation of PLGA nanoparticles containing estrogen by emulsification-diffusion method. Colloids Surf. A: Physicochem. Eng. Aspects 2001; (182) 123-130.
14. Zambaux M, Bonneaux F, Gref R, Maincent P, Dellacherie E, Alonso M, Labrude P, Vigneron C. Influence of experimental parameters on the characteristics of poly (lactic acid) nanoparticles prepared by double emulsion method. J. Control. Release 1998; (50) 31-40.
15. Niwa T, Takeuchi H, Hino T, Kunou N, Kawashima Y. Preparation of biodegradable nanoparticles of water-soluble and insoluble drugs with D, Llactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method, and the drug release behavior. J. Control. Release 1993; (25)89-98.
16. Zhang Q, Shen Z, Nagai T. Prolonged hypoglycemic effect of insulin-loaded polybutylcyanoacrylate nanoparticles after pulmonary administration to normal rats. Int. J. Pharm. 2001; (218)75-80.
17. Boudad H, Legrand P, Lebas G, Cheron M, Duchene D, Ponchel G. Combined hydroxypropyl-[beta]- cyclodextrin and poly(alkylcyanoacrylate) nanoparticles intended for oral administration of saquinavir. Int J. Pharm. 2001; (218)113-124.
18. Puglisi G, Fresta M, Giammona G, Ventura CA. Influence of the preparation conditions on poly(ethylcyanoacrylate) nanocapsule formation. Int. J. Pharm. 1995; (125) 283-287.
19. Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ. Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. J. Appl. Polymer Sci. 1997;(63) 125-132.
20. Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ. Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. Pharm Res. 1997; (14) 1431-1436.

ISBN : 978-81-966183-3-9

21. Jung J, Perrut M. Particle design using supercritical fluids: Literature and patent survey. J. Supercritical Fluids 2001; (20) 179-219.
22. Thote AJ, Gupta RB. Formation of nanoparticles of a hydrophilic drug using supercritical carbon dioxide and microencapsulation for sustained release. Nanomedicine: Nanotech. Biology Medicine 2005; (1)85-90.
23. Sun Y, Mezian M, Pathak P, Qu L. Polymeric nanoparticles from rapid expansion of supercritical fluid solution. Chemistry 2005; 11: 1366-73.
24. Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Adv Drug Deliv Rev 2003; 55: 329-47.
25. Desai MP, Labhasetwar V, Walter E, Levy RJ, Amidon G L, The mechanism of uptake of biodegradable microparticles in Caco-2 cells is size dependent. Pharm Res 1997; 14: 1568-73.
26. Desai MP, Labhasetwar V, Amidon GL, Levy RJ. Gastrointestinal uptake of biodegradable microparticles: effect of particle size. Pharm Res 1996; 13: 1838-45.
27. Kroll RA, Pagel MA, Muldoon LL, Roman-Goldstein S, Fiamengo SA, Neuwelt EA. Improving drug delivery to intracerebral tumor and surrounding brain in a rodent model: a comparison of osmotic versus bradykinin modification of the blood-brain and/or blood-tumor barriers. Neurosurgery 1998; 43: 879-86.
28. Kreuter J, Ramge P, Petrov V, Hamm S, Gelperina SE, Engelhardt B, Alyautdin R, von Briesen H, Begley DJ. Direct evidence that polysorbate-80-coated poly(butyl cyanoacrylate) nanoparticles deliver drugs to the CNS via specific mechanisms requiring prior binding of drug to the nanoparticles. Pharm Res 2003; 20: 409-16.
29. Zauner W, Farrow NA, Haines AM. In vitro uptake of polystyrene microspheres: effect of particle size, cell line and cell density. J Control Release 2001; 71: 39-51.
30. Redhead HM, Davis SS, Illum L. Drug delivery in poly(lactide-co-glycolide) nanoparticles surface modified with poloxamer 407 and poloxamine 908: Mohanraj & Chen 572 Trop J Pharm Res, June 2006; 5.
31. Dunne M, Corrigan OI, Ramtoola Z. Influence of particle size and dissolution conditions on the degradation properties of polylactide-co-glycolide particles. Biomaterials 2000; 21: 1659-1668.
32. Panyam J, Dali MM, Sahoo S K, Ma W, Chakravarthi SS, Amidon GL, Levy RJ, Labhasetwar V. Polymer degradation and in vitro release of a model protein from poly(,-lactide-co-glycolide) nano- and microparticles. J Control Release 2003; 92: 173- 187.
33. Swarbrick J, Boylan J. Encyclopedia of pharmaceutical technology. 2nd ed.; Marcel Dekker: New York, 2002.
34. Muller RH, Wallis KH. Surface modification of injectable biodegradable nanoparticles with poloxamer polymers and poloxamine 908. Int. J. Pharm. 1993; 89: 25-31.
35. Brigger I, Dubernet C, Couvreur P. Nanoparticles in cancer therapy and diagnosis. Adv. Drug Deliv. Rev. 2002; 54: 631-651.
36. Grislain L, Couvreur P, Lenaerts V, Roland M, Deprez Decampeneere D, Speiser P. Pharmacokinetics and distribution of a biodegradable drug-carrier. Int. J. Pharm. 1983; 15: 335-345.

ISBN : 978-81-966183-3-9

37. Olivier JC. Drug transport to brain with targeted nanoparticles. NeuroRx 2005; 2: 108-119.
38. Couvreur P, Barratt G, Fattal E, Legrand P, Vauthier C. Nanocapsule technology: a review. Crit Rev Ther Drug Carrier Syst 2002; 19: 99-134.
39. Govender T, Stolnik S, Garnett MC, Illum L, Davis SS. PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water-soluble drug. J. Control. Rel. 1999; 57: 171-185.
40. Govender T, Riley T, Ehtezazi T, Garnett MC, Stolnik S, Illum L, Davis SS. Defining the drug incorporation properties of PLA-PEG nanoparticles. Int J Pharm 2000; 199: 95-110.
41. Panyam J, Williams D, Dash A, Leslie-Pelecky D, Labhasetwar V. Solid-state solubility influences encapsulation and release of hydrophobic drugs from PLGA/PLA nanoparticles. J Pharm Sci 2004; 93: 1804-14.
42. Peracchia M, Gref R, Minamitake Y, Domb A, Lotan N, Langer R. PEG-coated nanospheres from amphiphilic diblock and multiblock copolymers: investigation of their drug encapsulation and release characteristics. J Control Release 1997; 46: 223–231
43. Chen Y, McCulloch, RK, Gray BN. Synthesis of albumin-dextran sulfate microspheres possessing favorable loading and release characteristics for the anti-cancer drug doxorubicin. J Control Release 1994; 31: 49-54.
44. Chen Y, Mohanraj VJ, Parkin JE. Chitosan-dextran sulfate nanoparticles for delivery of an antiangiogenesis peptide. Letters in Peptide Science 2003; 10: 621-627.
45. Magenheim B, Levy MY, Benita S. A new in vitro technique for the evaluation of drug release profile from colloidal carriers - ultrafiltration technique at low pressure. Int. J. Pharm. 1993; 94: 115-123.
46. Fresta M, Puglisi G, Giammona G, Cavallaro G, Micali N, Furneri PM. Pefloxacin mesilate-and ofloxacin loaded polyethylcyanoacrylate nanoparticles; characterization of the colloidal drug carrier formulation. J. Pharm. Sci. 1995; 84: 895-902.
47. Verdun C, Brasseur F, Vranckx H, Couvreur P, Roland M. Tissue distribution of doxorubicin associated with polyhexylcyanoacrylate nanoparticles. Cancer Chemother. Pharmacol 1990; 26: 13-18.
48. Couvreur P, Kante B, Lenaerts V, Scailteur V, Roland M, Speiser P. Tissue distribution of antitumor drugs associated with polyalkylcyanoacrylate nanoparticles. J Pharm Sci 1980; 69: 199-202.
49. Bibby DC, Talmadge JE, Dalal MK, Kurz SG, Chytil KM, Barry SE, Shand DG, Steiert M. Pharmacokinetics and biodistribution of RGD-targeted doxorubicin loaded nanoparticles in tumor-bearing mice. Int. J. Pharm. 2005; 293: 281-290.

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VIRAL HEPATITIS-B: STRUCTURE AND THERAPIES

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ABSTRACT

Hepatitis- B (HB) is a viral disease, which causes inflammation in the liver. HB generally passes from the infected person to another via various modes such as blood, sweat, semen, vaginal serum, and other body fluids. HB affects people with upregulated or deregulated liver enzyme levels and albumin, bilirubin levels. Immunomodulators, antiviral therapies, and various target base therapies either provided to the Chronic Hepatitis-B (CHB) patients to treatment them or to check the development of Hepatitis B virus (HBV) inside body serum of patients and hepatocytes. New therapies have been developed to cure HB infection within the population.

Key words **:** Hepatitis-B virus, Chronic Hepatitis-B, Hepatitis B surface antigen (HBsAg), Hepatitis B envelope antigen (HBeAg), Sodium taurocholate Co-transporting Polypeptide (NTCP)

INTRODUCTION

Hepatitis is a "Greek" term which splits in two - firstly "hepar" and secondly "itis" meaning inflammation. Therefore, Hepatitis is considered as an inflammation of the liver that is caused by multifactor either via variety of infectious viruses and non-infectious agents leading to liver damage. The two main causes of non-viral hepatitis are alcohol consumption and ingestion of toxins/ drugs. Viral hepatitis is caused by designated hepatitis A, B, C, D, E viruses Among all viral hepatitis, Hepatitis-B and hepatitis-C causes more casualty in India than Hepatitis-A and Hepatitis-D. Hepatitis- B is caused by Hepatitis- B Virus which belongs to *hepadnaviridae* family. and with negligible rate. Hepatitis B virus (HBV) has infected humans for at least the past 40000 years and is the 10th leading global cause of death. HBV infection results in substantial human morbidity and mortality, predominantly through the consequences of chronic infection (MacLachlan & Cowie, 2015). Transmission of acute viral hepatitis occur to humans either enterically or percutaneously via blood borne route. Chronic hepatitis caused by blood borne viruses is associated with prolonged viremia, advanced liver disease, end-stage carcinoma and excessive mortality. In contrast to the acute HBV infection which usually causes self-limiting and transient hepatitis, the persistent HBV infection can lead to a wide span of liver disease, like chronic hepatitis of different grades, which can progress to liver fibrosis, cirrhosis and culminate in decompensated liver disease and/or hepatocellular carcinoma (HCC) (Zheng *et al*., 2022).

Morphology of Hepatitis-B Virus (HBV)

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HBV is a hepatotropic virus and most of the time does not cause a cytopathic effect (Bassit *et al*., 2021). HBV is a partially double-stranded DNA virus and a member of the *Hepadnavirus* family. HBV is a 3200 bp consisting of relaxed circular DNA (rcDNA) for replication (Susluer *et al*., n.d.,2018). Seven proteins are produced from the translation of HBV RNA transcripts: X protein, HBeAg, HBcAg (core), RT-polymerase, and HBsAg (surface big [preS1+preS2+S domains], middle [preS2+S domain], tiny [S domain]). The three sizes of surface proteins (big, middle, and small) that make up the outer envelope of HBV virion particles enclose the double-stranded DNA genome of the capsid. (Bassit *et al*., 2021)

HBeAg (envelope protein)

High amounts of HBV DNA are detected by the serologic marker known as the hepatitis B "e" antigen (HBeAg). Although it seldom happens in moms who are negative, perinatal transmission can happen in mothers who have blood levels of HBV DNA that are > 200,000 IU/ml. An infant born to a mother who has HBeAg is at up to 100% risk of contracting HBV at birth if they are not immunized. Given the magnitude of the global public health burden from hepatitis B, the World Health Organization (WHO) has outlined ambitious hepatitis B elimination targets of a 65% reduction in mortality and a 90% reduction in incidence from baseline (2015) by 2030.

The HBV genome is a partly double-stranded DNA molecule, and its X ORF codes for the 154 amino acids long regulatory protein HBx. By transactivating cellular promoters and enhancers crucial for ongoing viral infection, HBx encourages the production and replication of viral genes. By blocking a number of cell signal transduction pathways, HBx also has an impact on cell survival, proliferation, migration, and transformation. The pathogenesis of HBV-mediated HCC is effectively aided by HBx.

HBsAg

One of the most crucial factors in determining the level of HBV infection is the monitoring of serum hepatitis B surface antigen (HBsAg). Patients who test positive for serum HBsAg have either an acute or chronic HBV infection and have been exposed to the virus. Serum HBsAg has long been known to have predictive significance. The hallmark of acute HBV infection resolution is loss of serum HBsAg, which is generally accompanied by seroconversion, or the seroconversion of anti-HBsAg antibodies. Serum HBsAg levels fluctuate throughout chronic HBV infection, but are typically substantially lower in the HBeAg negative illness phase. The change from chronic HBV infection to "occult HBV infection" or cured hepatitis B is characterised by HBsAg loss and/or seroconversion. The term "functional cure" and the ideal aim of antiviral therapy both relate to loss of HBsAg (Meier *et al*., 2021).

HBcAg

The HBV pgRNA that also codes for the viral polymerase encodes the core protein, which acts as a template for rcDNA production through reverse transcription. In order to control these two apparently antagonistic processes, the viral polymerase functions as a molecular switch, which

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enables the virus to replicate effectively. The HBV genome-containing icosahedral capsid is built from the 21 kDa HBc protein, or capsomer. LncRNAs in particular are associated with and regulated by HBc, which functions as an RNA-interacting protein. RNA metabolism, capsid building and transport, subcellular trafficking and release of the HBV genome, reverse transcription, and practically every other phase of the HBV life cycle are all affected by HBc (Diab *et al*., 2018) .

HBV replication

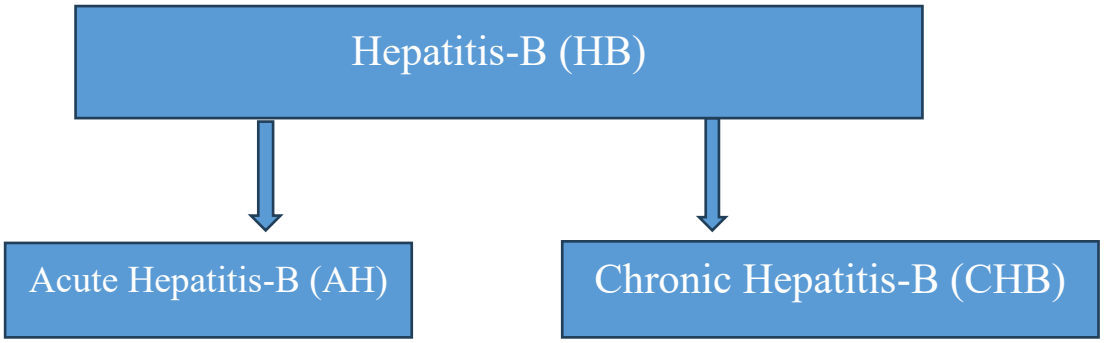
An important intermediate form (occurring in the nucleus of infected cells) is the covalently closed circular DNA (cccDNA) that is the template for pregenomic RNA (pgRNA) transcription and produces the template for reverse transcription and viral genome replication (Inoue & Tanaka, 2019).The sodium taurocholate co-transporting polypeptide (NTCP) receptor on the hepatocyte serves as a platform for HBV binding and cell entry (Wei & Ploss, 2021).

The pgRNA is reverse-transcribed into viral minus strand DNA after being transported to the cytoplasm where it is encapsidated with the viral polymerase. The partially double-stranded relaxed circular DNA is then created by synthesizing the plus-stranded DNA. The mature nucleocapsid can either be exported as infectious virions to infect additional cells or recycled back to the nucleus to maintain the pool of cccDNA (Yuen *et al*., 2018).

Intrahepatic cccDNA is the episomal virus template in the nucleus of HBV-infected hepatocytes. It is considered an important cause of viral persistence and a key obstacle for a cure of chronic hepatitis B. (Bassit *et al*., 2021)

Types of Hepatitis-B

HBV infection is a global public health issue since it can cause both acute and chronic liver disorders. One of the most frequent reasons for liver transplantation is liver illness connected to HBV (Susluer *et al*., n.d.).



Adults in India who develop acute hepatitis (AH) are often infected with the hepatitis E virus (HEV), however other significant causes of AH in adults include the hepatitis B virus (HBV), alcoholic hepatitis, and drug-induced liver injury (DILI) (Mandal, Das and Chaudhary.,2022). AH persists only for less than six months.

Chronic hepatitis B (CHB) can lead to cirrhosis and hepatocellular carcinoma, resulting in premature death. In addition to increased mortality from liver-related causes, chronic hepatitis

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B has been associated with premature mortality and elevated mortality rates from all causes. Elevated mortality and premature death among persons with chronic hepatitis B has been associated with coinfection with hepatitis C virus (HCV), HIV, or hepatitis D virus (HDV), diabetes, metabolic syn- drome, alcohol use disorder, and smoking (Netw *et al*., 2022).

CHB infection can be categorized into four different phases: Immune tolerant [IT, also termed hepatitis B envelope antigen (HBeAg)-positive chronic infection], immune active (IA, also termed HBeAg-positive chronic hepatitis), inactive carrier (IC, also termed HBeAg-negative chronic infection), and HBeAg-negative hepatitis (ENEG) phases. Furthermore, the levels of serum hepatitis B surface antigen (HBsAg), HBV-DNA and intrahepatic covalently closed circular DNA (cccDNA) differ markedly across the four phases. However, the underlying mechanisms or immune features distinguishing these phases are still obscure.

Modes of transmission

HB transmission takes place via various ways but majorly these are categorized in two forms -

a. Vertical Transmission
b. Horizontal Transmission

S.No.	Vertical Transmission	Horizontal Transmission
1.	Mother to neonate	Mother to child
2.		Child to child
3.		Father to child
4.		Inadequate sterilization of health care instruments
5.		Administration of contaminated blood products
6.		Male-to-male sex
7.		Heterosexual sexual contact by an individual with multiple partners

(Nguyen *et al*., 2020).

Therapies

HBV treatment includes number of therapies few of the recent and old therapies have been discussed here-

Immunomodulators such as interferon Alpha2b, peginterferon alpha-2a and anti-viral drugs (nucleos(t)ide analogs for CHB patients. These immunomodulators enhances T cell differentiation and maturation which cope with the scarcity of T cell number and thus maintain the functional effect within the immune system. These immunomodulators administered subcutaneously and causes adverse effects leading to elongation in treatment duration up to 48 weeks. For instance, Thymosin alpha-1 (Zadaxin) is an immune modulator, administered

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subcutaneously with minimal side effects approved as monotherapy for chronic hepatitis B in Asian countries by increasing the number of T cell within the patients's body.

As per studies CHB patients show seroconversion of HBeAg in their blood serum levels. Various nucleotide analogs, consumed orally, such as Lamivudine (approved in 1998), adefovir (approved in 2002), entecavir (approved in 2005), telbivudine (approved in 2006), tenofovir (approved in 2008), and tenofovir alafenamide (approved in 2016) are used and are proved effective against viral load development but not very effective for loss of HBsAg. These nucleoside analogs treatment duration lasts for several years hence the CHB patients may develop resistance against drug by mutating. Fewer drugs clevudine (approved for HBV in South Korea and the Philippines) and besifovir (nucleotide approved in South Korea) act as anti-viral drug for HBV with no significant toxicity for six months but later after 14 months causes reversible mitochondrial myotherapy (Bassit *et al*., 2022). Long term use of clevudine was found to exhibit reversible skeletal myopathy therefore discontinued for treating CHB patients.

With current available antiviral therapies for chronic hepatitis B, it is possible to control HBV replication. However, treatment is non-curative and therefore requires long-term continued use which has resulted in concerns for the development of antiviral resistance and adverse events, such as renal impairment or gastrointestinal disorders (important issue when considering adherence to treatment).

New therapies

The current preventative vaccine has little impact on a chronic infection that has already been established. Because the viral covalently closed circular DNA (cccDNA) transcriptional template persists in infected hepatocytes. Replication of HBV in chronically infected patients are unable to mount an immune response that is sufficiently strong, functional, and sustained to eradicate the infection. Thus, currently available treatments only suppress viral replication and are not curative. Therefore, treatment is typically required to last a lifetime. However, even patients who have their viral infection successfully controlled may still develop liver cancer, particularly if their livers are cirrhotic (Revill *et al*., 2019).

The drugs can be divided according to their strategies and target sites to eradicate chronic HBV infection.

1. Virologic (direct-acting agents or DAAs)
2. Host immune approaches (indirect-acting agents or immune therapy)

Virologic (direct-acting agents or DAAs)

New treatments known as virologic antiviral drugs, or DAAs, have the potential to directly target the processes of HBV replication without destroying infected cells. Nucleoside analogues (NA) blocks HBV replication, by inhibiting the viral reverse transcriptase enzyme and needed to be used over an extended period of time but would not entirely eradicate HBV from hepatocytes. Therefore, new DAAs are developed.

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Capsid Assembly Effectors or Modulators (CAM)

Chronic infection and viral replication are maintained by HBV nucleocapsid. In the viral replication cycle involves HBV genome packaging, reverse transcription, intracellular trafficking of relaxed circular DNA (rcDNA) into the nucleus. Therefore, to regulate the functioning of HBV via nucleocapsid, new drugs were introduced and named as Capsid assembly modulators (CAM). CAM are characterized by two types:

(1) **class I or hetero-aryl-pyrimidines (HAP)** are core protein allosteric modulators (CpAM) that upon binding to HBV capsids promote their misassembled to aberrant non-capsid core polymers, and

(2) **class II or phenyl-propan-amides (PP), sulfamoyl-benzamides (SBA)**, or derivatives are capsid assembly modulators that upon binding to the capsid form normal but empty nonfunctional capsids devoid of pgRNA/rcDNA.

Both types of HBV capsid effectors have the ability to disrupt a number of HBV replication cycle. Capsid effectors disrupt pre- and post-capsid formation, preventing capsid assembly, disrupting the integrity of incoming virus particles' capsids. These allow HBV capsid and core particles to enter the cell nucleus, and pregenomic RNA encapsidation and later causes reverse transcription. All of these alterations to the HBV replication cycle could eventually set the stage for suppression of cccDNA synthesis and/or amplification.

Entry Inhibitors

HBV enters the cell by attaching the receptor binding region of pre-S1 to the NTCP receptor at the membrane of the hepatocyte. Drugs such as Bulevirtide (Myrcludex B) binds irreversibly to NTCP inhibiting the HBV entry into the hepatocyte were administered subcutaneously to CHB patients. Drug binding to NTCP prevents infection but also inhibits hepatic bile salt uptake leading to the transiently elevated bile salt level. Previous studies had shown, 2mg Bulevirtide plus PEG-IFN had undetectable HBsAg, and HBsAg seroconversion was recorded in CHB patients (Bassit *et al*., 2021).

Small Interfering RNA (siRNA)

RNA interference (RNAi) is the mechanism through which double-stranded RNAs silence cognate genes. It is characterized by the presence of RNAs about 22 nucleotides homologous to the gene that is being suppressed. Dicer is the cellular nuclease that cleaves double-stranded RNAs and can produce putative guide RNAs or small interfering RNA (siRNA). After the sense strand is removed and the antisense strand is loaded on the RNA-induced silence complex (RISC), it hybridizes to a complementary region of a target mRNA, which results in its degradation. This phenomenon provides effective agents for inhibiting infectious, metabolic, cancer, and genetic diseases. A critical issue in the development of siRNA-based drugs is to avoid toxicity such as (1) immunogenic reactions to dsRNA (2′-O-methyl base modifications have largely avoided this issue), (2) toxicity of excipients (work continues on developing potent and nontoxic nanoparticles), (3) unintended RNAi activity (avoided by detailed screening target

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sites against human genome sequences), and (4) on target RNAi activity in nontarget tissues (selection of highly diseased selective genes and delivery routes which reduce accumulation in nontarget tissues).

Nucleic Acid Polymers (NAPs)

NAPs are phosphorothioate oligonucleotides (PS-ONs) that inhibit HBV via a post-entry mechanism blocking the assembly/release of HBV subviral particles. The universal model for NAP pharmacology is based on the interaction of the amphipathic protein domain and the hydrophobic side of NAPs, preventing the conformational changes in the target or its interaction with other amphipathic helices. In this class of antivirals, there are the HBsAg inhibitors and the STOPs (s-antigen transport inhibiting oligonucleotide polymers).

HBsAg Inhibitors

HBsAg has direct immunoinhibitory action against both innate and adaptive immune responses. HBsAg loss is infrequently achieved with the current therapy; therefore, antivirals targeting the inhibition of HBsAg are being developed. NAPs have the ability to interact with hydrophobic surfaces of proteins and have emerged as the first therapy to be able to achieve rapid HBsAg loss is a phosphorothioate oligodeoxyribonucleotide (PS-ONs) with the sequence (dAdC)20.

Antisense Molecules

Antisense oligonucleotides (ASO) are small single-stranded nucleic acid sequences that bind with high selectivity to their target RNAs. This triggers degradation via an RNAse H-dependent pathway. GSK 3228836 is a 2′-O-methoxyethyl free ASO currently in development for the treatment of chronic hepatitis B. treatment with ASO shows ALT elevations with transient concurrent HBsAg decline with no changes in liver function.

Nucleoside Analogs

As previously studied, clevudine development was stopped after it was discovered that long-term use of the drug caused reversible skeletal myopathy in a limited number of people. The first phosphorylation step, where the 5′-monophosphate is changed in the liver to the active 5′-triphosphate, was avoided by ATI-2173 by altering clevudine. In contrast to capsid inhibitor resistance mutations, 25 viral polymerase mutations linked to entecavir, lamivudine, and adefovir resistance reduced the efficacy of ATI-2173. This substance may act as a non-nucleoside antiviral agent, it has been suggested.

RNAseH Inhibitors

RNAseH is one of the two enzymatically active domains on HBV polymerase and destroys the HBV RNA after it has been copied into DNA by the reverse transcriptase. RNAseH is a potential target for antiviral drugs, and over 150 RNAseH inhibitors are divided in four compound classes: (1) α-hydroxytropolones (αHT), (2) N-hydroxyisoquinolinediones (HID), (3) N-hydroxypyridinediones (HPD), and (4) and N-hydroxynapthyridinones [76–81]. Novel amide

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αHT were studied with EC50 values from 0.31 to 54 μM. Studies in chimeric mouse showed that an HPD and an αHT suppressed HBV replication to up to 1.4 log10 after two weeks of treatment followed by a rebound in the viral titers.

Indirectly Acting Antiviral Agents (Immune Therapy)

Specific immune therapy can maintain the HBV replication under control of a functional host antiviral response. For instance, Pegylated interferon alpha (PEG IF-alpha) alone or in combination therapy can achieve sustained off-treatment control but in only a small portion of individuals. One tactic to accomplish the effective treatment of HBV is the therapeutic restoration of protective immunity. Numerous strategies are taken into consideration for treatment, including gene editing, innate immune activation (TLR-8 and TLR-7 agonists), therapeutic vaccinations, host acting route (apoptosis inducer and cyclophilin inhibitor), and many more.

Therapeutic Vaccines

There is a renewed interest in therapeutic vaccines with the development of novel formulations, suitable immunization routes for designed adequate antigens, and adjuvant strategies. In addition, it is important to consider adequate strategies, including combination therapy with other antivirals, either concomitant or sequential strategies.

Innate Immune Stimulation

The host immune responses to HBV dictate whether a person can successfully eradicate the virus (functional cure) or not (chronic hepatitis B). The actions of the toll-like receptor (TLR) family provide one means of controlling the host's immune system. Members of the endosomal TLR family, TLR8 and TLR7, share many sequence and functional similarities. They recognize pathogen-associated molecular patterns (viral single-stranded RNA fragments) and trigger innate and adaptive immune responses. Agonist ligands of Toll-like receptors 7 and 8 have immune stimulating activity allowing to intervene several diseases and to be valuable vaccine adjuvant candidates.

Host Acting Pathway

Cellular inhibitor of apoptosis proteins (cIAPs) impairs clearance of hepatitis B virus (HBV) infection by preventing TNF-mediated killing/death of infected cells. Animal studies showed that drug inhibitors of cIAPs were able to reduce serum HBV DNA, hepatitis B surface, and core antigens. APG-1387 is an apoptosis inducer; it is a second mitochondria-derived activator of caspase (SMAC) mimetic, and it targets inhibitors of apoptosis proteins (IAPs) (Bassit *et al*., 2021). CRV 431 is a small molecule under clinical development for the treatment of liver diseases including fibrosis and hepatocellular carcinoma. Antiviral activity of CRV 431 has been reported against hepatitis B reducing HBV DNA and HBsAg levels in transgenic mice (Gallay *et al*., 2019).

Gene Editing

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Clustered regularly interspaced short palindrome repeats (CRISPR)/Cas9-based antiviral strategy is one of the most versatile gene-editing tools, discovered as a bacterial adaptive immune system. The CRISPR/Cas9 system can specifically destruct HBV genomes in vitro and in vivo, mediating specific cleavage of cccDNA. Several optimal targets in HBV genome have been described, such as the surface and polymerase overlap region; the YMDD RT motif and the HBV enhancer I, II, X protein; and pre-core regions with high efficacy. However, CRISPR/Cas system inevitably targets integrated HBV DNA and induces double-strand breaks (DSBs) of host genome, raising concerns of genome instability and carcinogenicity. To avoid DSBs of the host genome, recently it was described a permanently Cas9-mediated base editors that effectively introduced nonsense mutations that generated premature stop codons of surface gene in both integrated and cccDNA reducing HBsAg secretion. **EBT107** is a gene-editing CRISPR/Cas 9 drug that uses a duplex gRNA excision knockout as a candidate for HBV in preclinical studies. ARCUS genome-editing technology is another platform of gene editing being developed for chronic hepatitis B. The ARCUS technology is based on the properties of a naturally occurring gene-editing enzyme – the homing endonuclease I-CreI—and reduces the risk of additional off-target DNA edits (Yang YC., 2020).

Farnesoid X receptor (FXR) agonist:

HBV enters the hepatocyte by binding to NTCP, the genome of which contains two active farnesoid X receptor (FXR)α response elements that participate in HBV transcriptional activity. In vitro studies showed that FXR agonists inhibited viral mRNA, DNA, and protein production and reduced the cccDNA pool size. Vonafexor (EYP001) is a farnesoid X receptor (FXR) agonist with anti-HBV effects is under study.

T cell immunotherapy:

LTCR-H2–1 is a preclinical drug that boosts adaptive immune response through T cell receptor (TCR) gene transfer [119]. It is engineered to target virus-derived peptides presented on MHC class I on the surface of virus-infected cells. This technology is based on leukapheresis to isolate white blood cells, followed by T cell expansion; HBV targeting TCR are introduced into the activated T cells by viral transduction or electroporation, and then after phenotypic and functional validation, the TCR-engineered T cells are infused back into the individual (Bassit *et al*., 2021)

Conclusion

HBV affects a majority of the population and has high risk of transmission within the family. This intrafamilial transmission can leads to explode in chronic liver diseases and liver damage patients within the society. As it was studied and concluded from the previously reported data that no actual cure of hepatitis b is possible therefore only preventive measures can restrict the prognosis and development of the disease.

HBV prevention and treatment strategies should be incorporated instantly in order to control its transmission. Prevention strategies for the development of HCC in patients with HBV are of

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the utmost importance. Accordingly, there is a need to investigate further the role of HBsAg in mechanisms underlying HCC recurrence and to evaluate the impact of neutralizing HBsAg. Mutant HBV should be monitored and therapies must be developed to inhibit the mutation of the virus and inhibit the replication with no side-effects within the patient.

REFERENCES

1. Bassit, L., Ono, S. K., & Schinazi, R. F. (2021). Moving Fast Toward Hepatitis B Virus Elimination. In *Advances in Experimental Medicine and Biology* (Vol. 1322). https://doi.org/10.1007/978-981-16-0267-2_5

2. Diab, A., Foca, A., Zoulim, F., Durantel, D., & Andrisani, O. (2018). The diverse functions of the hepatitis B core/capsid protein (HBc) in the viral life cycle: Implications for the development of HBc-targeting antivirals. In *Antiviral Research* (Vol. 149, pp. 211–220). Elsevier B.V. https://doi.org/10.1016/j.antiviral.2017.11.015

3. Gallay, P., Ure, D., Bobardt, M., Chatterji, U., Ou, J., Trepanier, D., & Foster, R. (2019). The cyclophilin inhibitor CRV431 inhibits liver HBV DNA and HBsAg in transgenic mice. *PLoS ONE*, *14*(6). https://doi.org/10.1371/journal.pone.0217433

4. Inoue, T., & Tanaka, Y. (2019). The role of hepatitis B core-related antigen. In *Genes* (Vol. 10, Issue 5). MDPI AG. https://doi.org/10.3390/genes10050357

5. MacLachlan, J. H., & Cowie, B. C. (2015). Hepatitis B virus epidemiology. *Cold Spring Harbor Perspectives in Medicine*, *5*(5). https://doi.org/10.1101/cshperspect.a021410

6. Meier, M. A., Calabrese, D., Suslov, A., Terracciano, L. M., Heim, M. H., & Wieland, S. (2021). Ubiquitous expression of HBsAg from integrated HBV DNA in patients with low viral load. *Journal of Hepatology*, *75*(4), 840–847. https://doi.org/10.1016/j.jhep.2021.04.051

7. Netw, J., Jun, O., & Ly, K. N. (2022). *JAMA Network Open Regional Differences in Mortality Rates and Characteristics of Decedents With Hepatitis B Listed as a Cause of Death , United States , 2000-2019*. *5*(6). https://doi.org/10.1001/jamanetworkopen.2022.19170

8. Nguyen, M. H., Wong, G., Gane, E., Kao, J. H., & Dusheiko, G. (2020). Hepatitis B virus: Advances in prevention, diagnosis, and therapy. *Clinical Microbiology Reviews*, *33*(2), 1–38. https://doi.org/10.1128/CMR.00046-19

9. Revill, P. A., Chisari, F. V., Block, J. M., Dandri, M., Gehring, A. J., Guo, H., Hu, J., Kramvis, A., Lampertico, P., Janssen, H. L. A., Levrero, M., Li, W., Liang, T. J., Lim, S. G., Lu, F., Penicaud, M. C., Tavis, J. E., Thimme, R., Arbuthnot, P., … Zoulim, F. (2019). A global scientific strategy to cure hepatitis B. *The Lancet Gastroenterology and Hepatology*, *4*(7), 545–558. https://doi.org/10.1016/S2468-1253(19) 30119-0

10. Susluer, S. Y., Kayabasi, C., Yelken, B. O., Asik, A., Celik, D., & Okcanoglu, T. B. (n.d.). *Analysis of long non-coding RNA (lncRNA) expression in hepatitis B patients*. 150–161.

11. Wei, L., & Ploss, A. (2021). Mechanism of hepatitis b virus cccdna formation. In *Viruses* (Vol. 13, Issue 8). MDPI AG. https://doi.org/10.3390/v13081463

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MARINE QUORUM SENSING AND MECHANISM

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ABSTRACT

In both natural and artificial environments, microbial communities play a variety of roles. Bacteria can communicate with one another via a technique called quorum sensing to exchange data about gene expression and cell density. It also controls a number of other processes, including the creation of biofilms, the synthesis of antibiotics, and environmental adaptation. The topic of this chapter is quorum sensing and the growing interest in marine ecosystems that began in the 1970s.

Key words **:** Quorum sensing, Quorum quenching, Marine science, Biofilm, Bacteria, Fungus.

INTRODUCTION

In natural or artificial environment, the microbial community is formed by a group of microorganisms and makes complex microbial interaction. It also help to adapt changing environments and microbial diversity (Zeng *et al*., 2023). The microbial community is important in the biogeochemical cycle, biodegradation, and species evolution etc. For example, the stability of the microbial population in the gut might improve resistance to diseases by preventing the attack of pathogenic organisms (Khan Khattak *et al*., 2021). The microbial community was also important in the creation of flavor compounds, biodegradation of herbicide and nitrate/nitrite compounds. From these we can understand the microbial population leads to a wide range of applications such as wastewater treatment, human health, food fermentation, and synthetic biology (Zeng *et al*., 2023).

Quorum sensing is a type of chemical communication between bacteria that relies on the synthesis, detection, and response to extracellular signalling molecules known as autoinducers. Quorum sensing enables groups of bacteria to adjust their behaviour in response to changes in the vicinal community's population density and different species. Currently, it is accepted that quorum sensing mediated communication is the norm in the microbial world (Mukherjee and Bassler, 2019). Four decades ago, Kenneth Nealson was the first to report on quorum sensing (QS). He noticed something and claimed to be a conditioning of the media used to cultivate the marine bioluminescent bacteria Aliivibrio fischeri. QS, which is regulated by signals such as acylated homoserine lactones (AHLs) or furanosyl-borate diesters [autoinducer-2 (AI-2) molecules], has been shown to be involved in important processes within the marine carbon cycle, health of coral reef ecosystems, and trophic interactions between a variety of eukaryotes and their bacterial associates. Bacteria are the most abundant organisms in the ocean and mediate biogeochemical transformations essential to major nutrient cycles in complex

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ecosystems. Although planktonic bacteria numerically dominate the ocean, the most metabolically active bacteria are attached to surfaces (Smith *et al*. 1992, 1995); thus, the impact of surface-attached bacteria on the chemical and biological properties of the ocean is disproportionate to their numerical abundance (Hmelo, 2017a).

The first detection of QS in the marine environment occurred over four decades ago (Nealson *et al*., 1970), but its significance in marine microbial communities and possible consequences on marine biogeochemistry and ecology have just been studied thoroughly in the last decade. Many great studies have delves into the molecular aspects of QS in many bacterial species (Fuqua *et al*., 2001)(Miller and Bassler, 2001).

Bacteria are the most common creatures in the water, and they conduct biogeochemical transformations that are critical to large nutrient cycles in complex ecosystems. Although planktonic bacteria predominate in the ocean in terms of numerical abundance, the most metabolically active bacteria are attached to surfaces thus, the impact of surface-attached bacteria on the chemical and biological properties of the ocean is disproportionate to their numerical abundance (Smith *et al*., 1992). Bacteria that live on surfaces commonly form complex colonies known as biofilms. Bacteria are embedded in a biological matrix that links them to surfaces and to each other to form dense populations called biofilms. (Costerton *et al*., 1995). Bacteria are arranged together at densities up to three orders of magnitude higher in marine biofilms than in planktonic equivalents. Cell-cell interactions are strengthened at these concentrations. Quorum sensing (QS) is one of the well studied types of bacterial community interaction, with observable effects on a variety of marine microbial systems. (Koren and Rosenberg, 2006) (Sheridan *et al*., 2002) (Simon *et al*., 2002).

The finding that farnesol regulates filamentation in the pathogenic polymorphic fungus *Candida albicans* 10 years ago showed the presence of fungal QS systems. Farnesol has been found to serve numerous roles in *C. albicans* physiology throughout the last decade, including signaling molecule and protecting fungus against oxidative stress and immune modulation. In *Candida albicans*, influencing proliferation, morphogenesis, and biofilm formation. Two more aromatic alcohols, phenylethanol and tryptophol, were discovered to be QSMs in *Saccharomyces cerevisiae*, controlling morphogenesis addition to farnesol, the aromatic alcohol tyrosol was discovered to be a QS molecule (QSM) in during nitrogen deprivation circumstances. Several additional fungal species have also been documented as having population density-dependent behaviour similar to QS (Padder *et al*., 2018a). Although research on fungal QS is still in its early stages, its discovery has altered our perceptions of the fungus world and may ultimately lead to the development of novel antifungal medicines.(Albuquerque and Casadevall, 2012).

1. WHAT IS QUORUM SENSING

In nature, microorganisms are everywhere, and they play a crucial role in both our micro- and macroenvironments. Numerous bacteria reside in the human body, particularly in mucosal areas. Quorum-sensing (QS) was first suggested as a method of cell-to-cell communication in bacterial populations in 1965 (Wu and Luo, 2021). It is a method by which bacteria employ signal

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molecules to control gene expression in response to population density. Quorum sensing, which is frequently used by pathogens (disease-causing organisms) in disease and infection processes, enables bacterial populations to communicate and coordinate group behavior. In the mid-1960s, Hungary-born microbiologist Alexander Tomasz made the discovery of quorum sensing-related bacterial activity while studying the ability of Pneumococcus (later known as Streptococcus pneumoniae) to absorb free DNA from its environment.(González and Keshavan, 2006).

2.1 APPLICATIONS OF QUORUM SENSING

Biosensors

Engineering whole cell microbial biosensors to distinguish between harmful microorganisms prevalent in the environment and host organisms is an intriguing application of quorum sensing. In addition, Quorum sensing has been used to produce bacteria that could attack cancer cells. Novel anti-cancer medications could be developed by combining cancer-destructing elements with these microbial biosensors. In order to create transgenic plants that can defend themselves against common bacterial diseases, QS and quorum quenching serve another purpose.(Aziz *et al*., 2019)

Bioluminescence and Pigment Synthesis

Bioluminescence is an essential marine bacterial feature that is controlled by quorum sensing. QS has been shown to modulate pigment synthesis (particularly purple violacein) (McClean *et al*., 1997). A QS dependent synthesis of violacein has been observed in the marine strain *Pseudoalteromonas ulvae* TC14 (Mireille Ayé *et al*., 2015). It's remarkable to note that the strains do not seem to produce AHLs in planktonic conditions; instead, the addition of C6-, C12-, 3-oxo-C8-, and 3-oxo-C12-HSL boosts and lowers violacein production. It was found that, in sessile conditions, the 3-oxo-C8 upregulates the emission of the purple pigment. These findings imply that violacein synthesis in this strain is mediated by an orphan LuxR receptor.

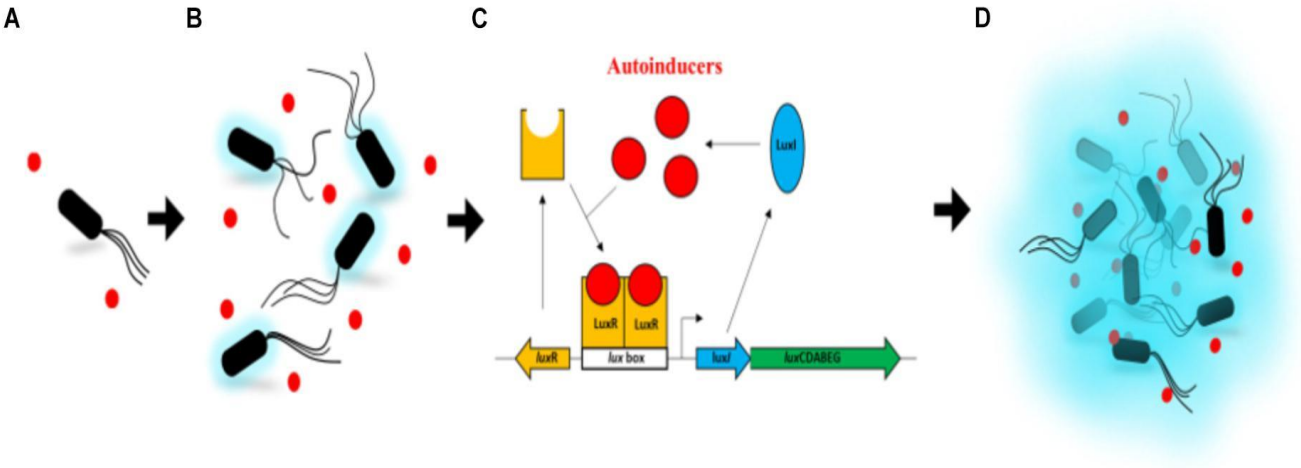

Fig1: **Bioluminescence** (Activation of the *lux* operon by LuxR and LuxI in *Aliivibrio fischeri*. **(A)** At low cell density, the autoinducers (3OC6-HSL – red dots), produced by LuxI,

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diffuse through the cell membrane into the growth medium. **(B)** As the cell growth continues, the autoinducers in the medium start to accumulate in a confined environment. A very low intensity of light can be detected. **(C)** When enough autoinducers have accumulated in the medium, they can re-enter the cell where they directly bind the LuxR protein to activate *lux*ICDABEG expression. **(D)** High levels of autoinducers activate the luminescent system of *A. fischeri*. A high intensity of light can be detected) (Tanet *et al*., 2019)

Treatment and diagnosis for pathogens

Gram-negative cells can produce AH, which can be detected by the majority of whole cell QS biosensors that have been described many years ago. An AHL sensitive transcriptional regulator and a homologous promoter, which control the reporter gene's transcription, are both included in a typical AHL biosensor. The use of QS signals as indicators of the presence of harmful microorganisms in clinical and environmental samples has been advised. It is still possible to design biosensing circuits using quorum sensing-based amplification circuits to detect the presence of dangerous bacteria in contaminated dairy and meat products.(Kumari *et al*., 2008)

Biocontrol

The rhizosphere is a small area of soil that surrounds a plant's roots and is influenced by local soil bacteria and root secretions. The rhizosphere community is mostly composed of bacteria that use quorum sensing. Rhizobia, or bacteria that nodulate legumes, typically produce N-acyl homoserine lactones, which control the induction of gene expression in a way that is dependent on quorum sensing or population density (Sanchez-Contreras *et al*., 2007).

Quorum-suppressing enzymes have also been used by scientists to lessen bacterial pathogenicity against plants. However, QS systems also regulate crucial rhizosphere-beneficial bacterial processes as biofilm formation, antibiotic synthesis, and nitrogen fixation.(Müller *et al*., 2009)

Prevention of biofouling

The growth of bacteria, algae, and other organisms, such as protozoa and crustaceans, on surfaces that have had prolonged contact with water is referred to as "biofouling". Biofouling can occur on a variety of surfaces, including those found on pipelines, tanks, ship hulls, membrane bioreactors, dental or medical implants, and catheters. In addition to pollution and colonization, this unwanted development of living things and their activities also causes machine parts to corrode when they are exposed to water, which lowers machine efficiency.

Quorum incorporation one method for reducing *P. aeruginosa* biofouling of surgical implants is to detect inhibitors on the device surface. QS suppression can be used to offer protection because many pathogens depend on QS to start the biofilm formation.

Gene recombination and expression

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One of the most exciting directions in quorum sensing research may be the creation of recombinant gene products through metabolic engineering. Quorum sensing has been used to control gene expression and cellular growth (Aziz *et al*., 2019).

Managing pathogens and pests

The majority of the current uses for quorum-sensing technologies are in the management of pathogens and pests, or any organism whose presence in a certain environment is unwanted. The obvious and, in reality, most valued use of quorum-sensing information is the inhibition of quorum signaling (Aziz *et al*., 2019).

Application in Food industry

The equivalent biofilm can be created by a range of harmful microbes that are found in food. This behaviour is common in many different food-borne pathogenic organisms and has a significant impact on the preparation and safety of food. QS has a crucial role in controlling the development of biofilms.

QS is crucial for the development of biofilms, and the development of biofilms has an impact on QS regulation as well, creating a feedback loop. One of the most significant public health issues in the world, *Salmonella* Sp. related food-borne illness poses a substantial threat to human health. On equipment and containers used in food preparation, *Listeria monocytogenes* may quickly build biofilms. More powerful mixed biofilms, which are difficult to remove and frequently cause food-borne illnesses, can be produced by using this Défense mechanism (Daneshvar Alavi and Truelstrup Hansen, 2013). When consumed with raw or poorly cooked food, the widely prevalent food-borne pathogen *V. parahaemolyticus c*an result in gastroenteritis or food poisoning. Studies have demonstrated that the activity of the QS signal molecule AI-2 and the expression of the luxS gene in *V. parahaemolyticus* may both be inhibited by brominated furanone. Similar effects are also seen on its biofilms and extracellular enzymatic activity (Phuvasate *et al*., 2012). Therefore, by influencing the development of biofilms, the QQ approach can prevent bacteria from being harmful.

A QS signal can be interrupted in numerous ways: (1) halting signaling molecule synthesis; (2) inactivation or enzymatic destruction of signaling molecules, preventing accumulation to a threshold value; (3) interfering with signal receptor binding in a bacterial cell or competing with signal molecules—receptor analogs; and (4) inhibiting target genes that should have been activated by the QS signal (Moradi and Hadi, 2021)

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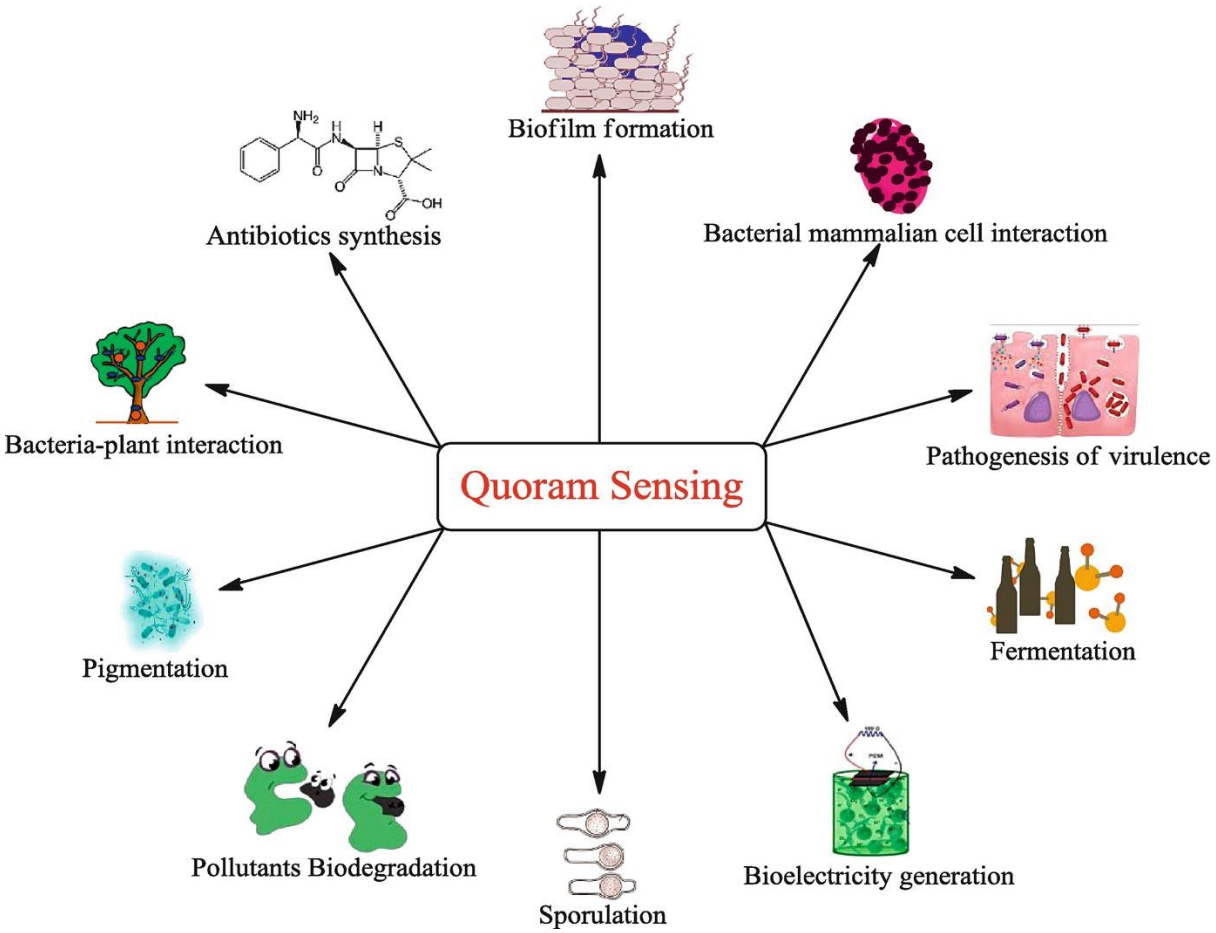

Fig 2: Applications of Quorum Sensing

2. Quorum quenching

One way of interspecies and even cross-kingdom contact is quorum-quenching (QQ), or the interruption of QS signaling. It is an antagonistic mechanism used by bacteria, and an immune system used by eukaryotes to fight off infections. Any disruption in interpersonal communication is referred to be QQ in general. (Prazdnova *et al*., 2022).

2.1 APPLICATIONS QUORUM QUENCHING

Aquaculture

Aquaculture is among the food production systems that is expanding the quickest in the world. On the other side, disease outbreaks have impeded its further development. Antibiotics are considered an effective treatment for bacterial diseases. The widespread and frequent use of antibiotics in aquaculture has sped up the development of antibiotic-resistant bacteria, and if adequate action is not taken quickly, the situation might get significantly worse. Finally, it might endanger people's health and safety. In order to protect human health and ensure the sustainable growth of the aquaculture business, new approaches to managing bacterial infections are needed. Potential application benefits of QQ technology have been discovered in the prevention and treatment of serious aquatic illnesses (Harms *et al*., 2016).

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Preventing Environmental Pollution

Marine environmental contamination has significantly harmed marine life and put people's lives in danger. According to (Dobretsov *et al*.,2011), biofouling is a significant issue for maritime enterprises and the marine ecosystem. To reduce biofouling in the past, We used antifouling substances such as tributyltin (TBT), which worked well, but was prohibited because to its high toxicity and pollution. Given that QQ is an eco-friendly control method, it has emerged as a key technique for preventing biological pollution in its early phases.

Enzymes that break down AHLs or QSIs help lessen the biological pollution. According to reports, QSIs can stop biofouling when used in coatings. According to reports, kojic acid is a non-toxic QSI When applied to paints, it can minimize marine biofouling by inhibiting bacterial and diatom contamination within a month. (Dobretsov *et al*., 2011).

3. QUORUM SENSING IN MARINE ENVIRONMENT

Marine prokaryotes represent abundant and diverse communities across oceans. These organisms are free living, symbionts, parasites or produce biofilm on very diverse types of marine surfaces. These surfaces include micro- and macro-algae leaves (phycosphere), marine particles, seashore rocks, buoys, microplastics and many others. Such niches favor bacterial population and thus different types of bacterial interactions, including quorum sensing. This consists of a mechanism that allows cells to coordinate their gene expression and physiology when a defined number of cells is reached. It will first focus on the wide range of chemical compounds involved in these processes and will describe the diversity of bacterial functions regulated by quorum sensing. Then, the implications of quorum sensing at a larger and integrated scale will be examined, including potential biogeochemical implication of quorum sensing expression. Also the importance in quorum sensing in the functioning of diverse holobionts will be addressed, as well as potential industrial applications.

Quorum sensing is suspected of causing remarkable phenomena in marine environments. For example, the bioluminescent Vibrio harveyi has been attributed to an area of 15400 km2 in the Arabian Sea, known as "Milkysea". According to theory, these cells exhibit quorum sensing, which is what makes them glow and bloom when there is a phytoplankton bloom (Miller *et al*., 2005). Quorum sensing, however, has been shown to function not only in this spectacular form of bioluminescence but also in a wide range of bacterial activities and in phylogenetically diverse marine cells since its discovery in the 1970s.

4. HISTORIC BACKGROUND OF QUORUM SENSING

In the 1990s, (Fuqua *et al*., 1994) established the idea of quorum sensing, which refers to a population density-based physiological response of bacterial cells. However, the majority of the discoveries that led to the development of this notion came from tests done by marine biologists Kenneth Nealson, Terry Platt and J. Woodland Hastings in the 1970s. During this decade, a large amount of information was gathered on *Vibrio fischeri* strains that were able to colonize the light organ of the Hawaiian bobtail squid *Euprymna scolopes* and produce bioluminescence (Nealson

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et al., 1970) (Fuqua *et al*., 1994). This symbiotic bacterial community was first discovered to have a density-dependent phenotype. These cells are free-living and sparse in the surrounding saltwater, and they do not create light. They can bioluminesce, however, when they reach large populations as in lab cultures, or when they invade the squid's light organ.

1990s–2010s: Research on quorum sensing in marine environments saw an increase in interest. For nearly ten years after these first findings and the subsequent thorough characterization of the quorum sensing genetic system, the scientific world had little interest in this mechanism. At the time, quorum sensing appeared to be a specific kind of regulation for bioluminescence. This interest was rekindled in the 1990s with the development of DNA sequencing technologies and the discovery of a vast diversity of luxI and luxR homologs in several bacterial species. It gradually became apparent that a large variety of bacterial strains may be employed with the luxI-luxR paradigm developed for *V. fischeri* (figure 3) ("Whiteley *et al*., 2017," n.d.). These discoveries led to the development of the quorum sensing concept in 1994 (Fuqua *et al*., 1994).

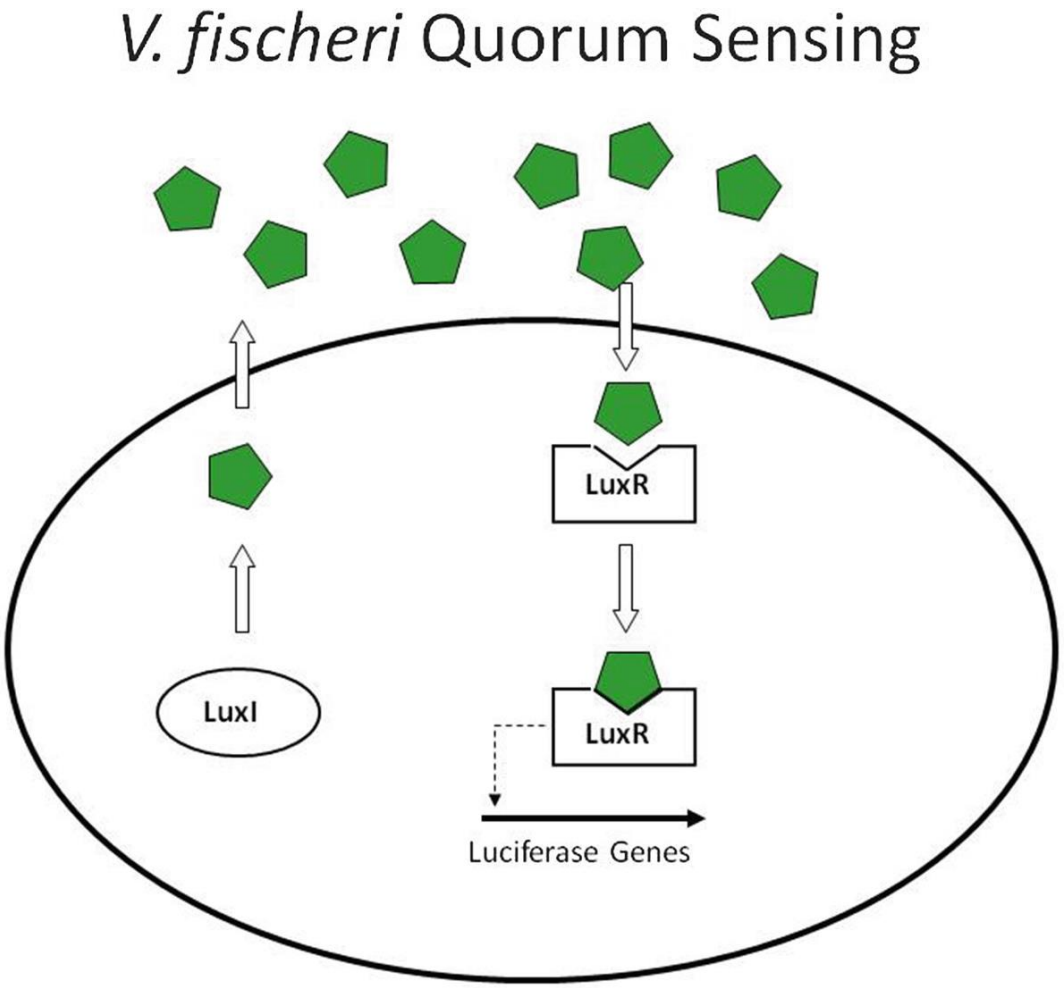

Fig3: luxI-luxR model

5. QUORUM SENSING IN A MODERNIZING OCEAN

Acidification of ocean

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The ocean has slowed the increase in atmospheric carbondioxide since the beginning of the modern era by absorbing around a quarter of anthropogenic emissions (Feely *et al*., 2009). Ocean acidification (OA), a phenomenon brought on by the absorption of CO2, is a process in which the chemistry of the ocean is drastically altered. According to (Orr *et al*., 2005). Ocean acidification (OA) is one of the global issues caused by rising atmospheric CO2. The rising pCO2 and resulting pH decrease has altered ocean carbonate chemistry. Microbes are key components of marine environments involved in nutrient cycles and carbon flow in marine ecosystems The precise processes by which OA could affect heterotrophic bacteria are, however, still poorly understood(Liu *et al*., 2010)(Orr *et al*. 2005), (Decho *et al*. 2009), (Hmelo and Mooy, 2009), (Yates *et al*., 2002).

The half-life of AHLs is directly impacted by pH changes. The quorum number necessary to start QS behaviours and the spreading distance (the greatest distance over which QS is effective) are both impacted by the half-life of AHLs. The expression of QS-regulated traits may be impacted by a rise in calling distance because to the slower molecular breakdown at lower pHs. Similarly, signals that fade more slowly and accumulate more quickly may reduce the quorum required to start transcription. Even though certain QS populations, such those within stromatolites, have evolved to accept rather short and abrupt changes in pH, it is less clear how QS populations that have not originated in such an environment will adapt to long-term changes in ambient pH. (Decho *et al*., 2009). Key microbial systems, such those connected to POC, may be impacted if changes in ambient pH influence the time of induction of QS-regulated characteristics.

In addition to chemically affecting how QS is expressed, pH changes can cause changes in the composition of the microbial community, which may have an effect on the number and variety of QS communities. Lowering pH, for example, can alter the proportion of *Proteobacteria* and *Bacteroidetes* in some microbial communities, such as those connected to corals.(Thurber *et al*., 2009) (Witt *et al*., 2011). Because AHL-QS is only known to exist in the *Proteobacteria,* a change from a Proteobacteria-dominated community to a *Bacteroidetes*-dominated community suggests a change away from ecosystems supported by AHL-QS-regulated processes. In addition to potentially affecting the variety of bacterial services offered to the environment, Ulva and other species that contain QS are likely to be impacted by this. Although this may have some effect on the variety of environmental services offered by bacteria, it is also likely to have an influence on creatures like Ulva that use QS signals as a component of their environmental sensing repertoire. Particularly, OA-associated changes away from QS bacteria in biofilms may result in lower settling of eukaryotes on surfaces, which may have significant negative effects on coral reefs and other ecosystems. This is because AHLs can act as recruitment cues for eukaryotic larvae. ("Sharp: Multi-partner interactions in corals in the... - Google Scholar," n.d.).

Rising Temperature

Sea surface temperatures are anticipated to rise together with the climate as a result of rising atmospheric CO2 concentrations (IPCC 2013). Temperature-induced instability is predicted to

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have an impact on the effectiveness of AHLs since the rate of AHL degradation accelerates as temperatures rise (Yates *et al*. 2002).

Coral habitats might be especially susceptible to the effects of increasing sea surface temperatures on the QS-responsive population. It is previously established that increasing sea surface temperatures and a rise in sea surface temperature anomalies contribute to coral illnesses and bleaching (Bruno *et al*., 2007) ("Sharp: Multi-partner interactions in corals in the... - Google Scholar," n.d.). Changes in host vulnerability and QS-regulated virulence might provide light on this occurrence. The expression of AHL-dependent regulons in coral-associated bacteria appears to be sensitive to temperature fluctuations in the range of natural intersessional variability (Kimes *et al*., 2012)(Tait *et al*., 2010).

Kimes *et al*., (2012) showed that the coral pathogen *V. coralliilyticus* enhances its synthesis of AHL and AI-2 as well as upregulating many QS proteins at higher temperatures. Because Tait *et al*., (2010) showed that short-chain AHL production was hindered at high temperatures in several *Vibrio* strains, this may be wide spread phenomena. The phenotypic effects of these temperature-induced alterations to the QS system require further investigation.

Temperature-induced coral illness is caused by *Vibrio* species (Munn, 2015). Because QS is believed to be implicated in the virulence of *Vibrio* strains, especially at high temperatures, extended periods of peak temperatures or more frequent temperature anomalies allow for higher expression of QS-regulated disease components. Corals and related bacteria may boost synthesis of QS inhibitors in response to increasing abundance of QS pathogens during bouts of environmental stress (caused by temperature or pH). There is now increasing evidence that temperature may directly influence QQ production in some bacteria (Tait *et al*., 2010). Although it is presently hard to anticipate the overall effect of rising temperatures on QS and QQ behaviours within the coral microbiome, it is apparent that temperature may alter the QS community in a variety of ways. Temperature stress on QS-regulated behaviours is predicted to become more obvious if sea surface temperature anomalies grow more frequent and peak temperature periods persist longer.

6. MARINE ORGANISMS PERFORMING QUORUM SENSING

6.1 QUORUM SENSING IN MARINE FUNGUS

Marine fungal strains are powerful makers of polyketide-derived alkaloids, terpenes, peptides, and mixed biosynthetic chemicals, which represent chemical categories of fungi secondary metabolites (Hasan *et al*., 2015). In the past 15 years, the research of quorum sensing (QS) mechanisms in fungi has been an immensely popular subject, there are currently more than 8000 citations to the more than 230 works on this subject that are indexed in the web of science(WOS) (Barriuso *et al*., 2015). The filamentation in the human fungus *Candida albicans* was controlled by cell density. This was the initial explanation of microbial QS regulation (Tian *et al*., 2021). Incredibly, the phenomena of QS in fungi were first discovered by farnesol fifteen years ago while studying the filamentation regulation in the pathogenic polymorphic fungus *C. albicans*. A reduced tendency for the yeast-to-hyphal flip has been seen in dense cultures of the human

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opportunistic pathogenic fungus *C. albicans* due to the accumulation of the sesquiterpene alcohol farnesol, suggesting that farnesol plays a role in preventing hyphae production (Padder *et al*., 2018b). In *C. albicans*, tyrosol functions as another QSM, shortening the lag phase of development and promoting filamentation and biofilm formation (figure3). When farnesol is present, these effects are reduced, indicating a precise QS-mediated regulation (Chen *et al*., 2004).

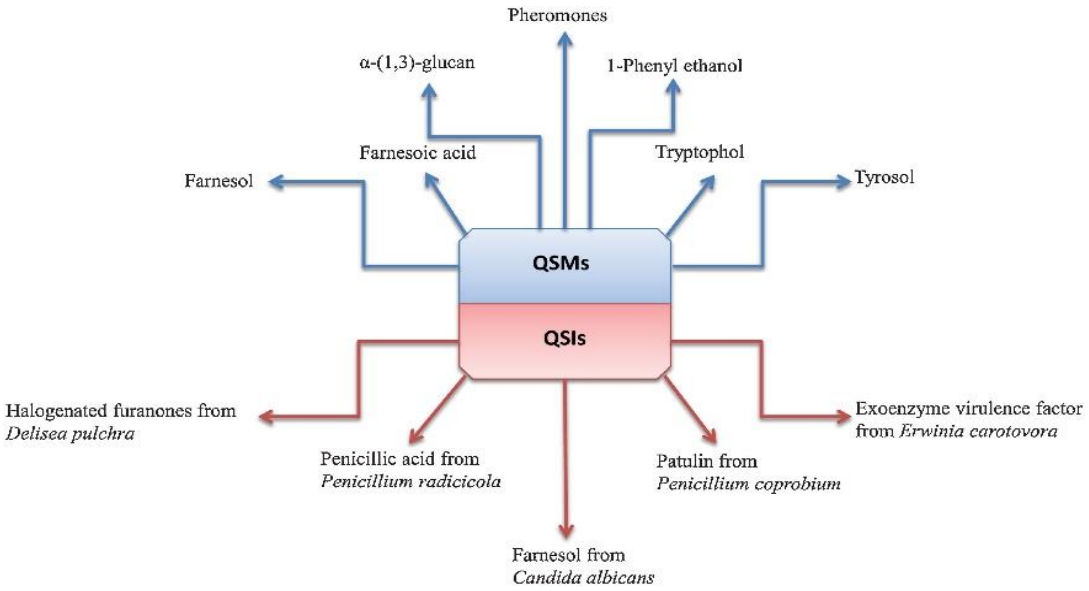

Fig 4: Fungal quorum sensing molecule and quorum sensing inhibitors

Despite the recent explosion of knowledge regarding bacterial QS, QS in eukaryotic organisms remained unknown until the discovery of farnesol as a QSM in the pathogenic fungus *Candida albicans*(Jacob M. Hornby *et al*., 2001). In the ten years since this revolutionary work was published, it has spawned almost 90 PubMed publications including the phrases 'Candida' and 'farnesol,' as well as many more concerning the effects of this chemical in other organisms. Other known fungal QSMs, in addition to farnesol, include all alcohols produced from aromatic amino acids tyrosine (tyrosol), phenylalanine (phenylethanol), and tryptophan (tryptophol). Tyrosol was the second QSM identified in *Candida albicans* (Chen *et al*., 2004), whereas the other two were discovered in the 1960s as autoantibiotics suppressing filamentation in *Candida albicans* (Lingappa *et al*., 1969), and were subsequently shown to be Saccharomyces cerevisiae QSMs (Chen and Fink, 2006).

Farnesol

Farnesol, an exogenous autoregulatory substance released by *Candida albicans*, suppresses hyphal development and synthesis of a variety of morphology-specific genes required for effective biofilm formation. Farnesol affects the framework of mature biofilms, the scattering of cells from biofilms, and many other stages of biofilm development (Deveau and Hogan, 2011).

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In 2001 Hornby *et al* was identified farnesol as the first QS molecule in eukaryotic species. it is a sesquiterpene alcohol (3,7,11-trimethyl-2,6,10-dodecatriene-1-ol) that contains three isoprene units. Ergosterol, which is crucial for maintaining the integrity of tiny fungi's membranes, is created as a byproduct of this biosynthesis. A adverse modulator of filamentous development is farnesol (Dizová and Bujdáková, 2017).

Three different investigations attempted to determine the amounts of farnesol, a secreted chemical, in various growth media under aerobic and anaerobic circumstances (J. M. Hornby *et al*., 2001). Under anaerobic condition farnesol was not detected. The reported amounts in planktonic cultures produced under aerobic conditions differed greatly between research. the reported farnesol concentrations to be in the range of 10–50 μM and nM. These results are remarkable because farnesol concentrations in both secreted and generated by cells are three orders of magnitude lower here than in several other investigations (Krom *et al*., 2015).

Farnesol suppresses hyphal development in C. albicans by controlling the cyclic AMP (cAMP) signaling pathway, and CYR1 and PDE2 control two enzymes that are directly involved in the creation and breakdown of cAMP. The molecular mechanism is unclear (Chen *et al*., 2018).

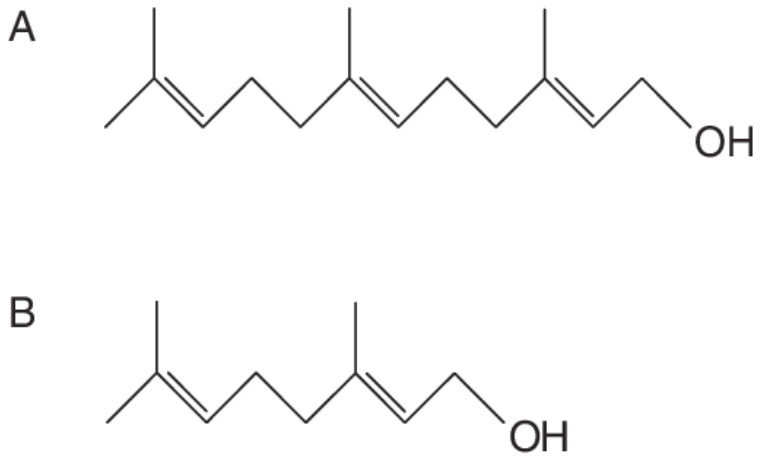

Fig 5: Structure of farnesol

There are at least four useful benefits from using farnesol analogs, in addition to learning more about the mechanism of fungal quorum detection. (1) Early studies using a mouse model don't indicate any clear toxicity from farnesol. However, it would be preferable to produce less toxic analogs that yet maintain QSM action if farnesol toxicity were to turn into an issue at greater doses or with prolonged administration. (2) There is little water solubility for farnesol. Farnesol's structural changes may produce active substances that are more soluble and hence easier to distribute to animal systems. (3) Farnesol analogs may also have superior pharmacokinetics, such as a greater capacity to enter the bloodstream through the peritoneum or gastrointestinal tract. Alternately, specific structural alterations may increase retention because farnesol is typically expelled from animals after being converted to farnesoic acid and dicarboxylic acids. Both possibilities could support keeping the analog in the host in a usable state. (4) It is currently unknown whether farnesol will be used as a preventative measure in an

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animal model, despite our in vitro experiments' suggestion that it might. The factor that causes virulence for *C. albicans* may be farnesol (Shchepin *et al*., 2003).

Tyrosol

Tyr, (2-(4-hydroxyphenyl)-ethanol, is a member of the class of phenolic substances known as phenylethanoids (Chung *et al*., 2017). It help to promote hyphal development and germ-tube production during the early and middle stages of biofilm generation (Cordeiro *et al*., 2015). Tyrosine's derivative, tyrosol (2-[4-hydroxyphenyl] ethanol), was later discovered to be C. albicans' second quorum-sensing molecule. Similar to farnesol, this substance constantly releases growth medium during growth, eliminating the lag phase that typically occurs when overnight colonies are diluted into medium. Germ tubes quickly form thanks to tyrosol. Therefore, it would seem that complicated positive and negative control of morphogenesis in C. albicans is exerted by tyrosol and farnesol, respectively (Alem *et al*., 2006). Additionally, it has been demonstrated that tyrosol inhibits neutrophils, perhaps by interfering with their oxidative burst (Albuquerque and Casadevall, 2012).

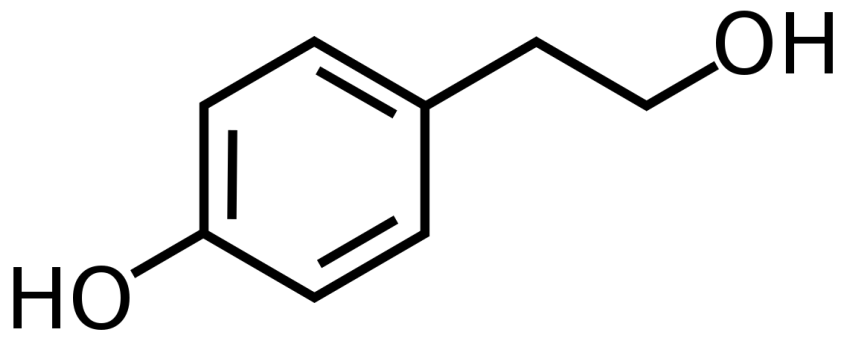

Fig 6: Structure of Tyrosol

6.2 QUORUM SENSING IN MARINE BACTERIA

The most numerous species in the ocean are bacteria, which also mediate important biogeochemical changes necessary for significant nutrient cycles in complex ecosystems. The majority of the ocean's bacteria are planktonic, however they are attached to surfaces, where they are more metabolically active. In comparison to their planktonic counterparts, bacteria in marine biofilms can be found at concentrations up to three orders of magnitude higher In order to determine the density of their clonal population and to start group-beneficial activities at high population densities, QS bacteria use tiny, diffusible chemical signals. The synthesis of extracellular hydrolytic enzymes, the creation of antibiotic compounds, and the production of exopolysaccharides necessary for adhesion and biofilm formation are all phenotypes that are regulated by QS. These phenotypes support colonization, nutrition uptake, and group defence (Hmelo, 2017b).

QS controls genes that direct beneficial activities when carried out by groups of bacteria working in concert. Activities controlled by QS include bioluminescence, sporulation,

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competencs, antibioyic production, biofilm formation and secretion of virulence factors (Rutherford and Bassler, 2012). How is quorum sensing implemented? Individual bacteria produce autoinducers as part of their reproduction process. Acyl-homoserine lactone autoinducers are made by gram-negative bacteria, and they can passively diffuse through their fragile cell walls. In contrast, the ATP-binding cassette (ABC) transporter system is required for the active transport of gram-positive bacterial autoinducers through their peptidoglycan cell wall. In both situations, autoinducers leave individual cells as they develop. Since the bacteria are multiplying, gradually more individual cells are manufacturing autoinducers, and their extracellular concentration rises until it reaches a "critical mass." This threshold prevents intracellular autoinducers from leaving the cell (whether by diffusion or transport) indefinitely, leading to a rise in their intracellular concentration. Autoinducers bind to their targets once intracellular concentration rises. Autoinducers bind to their receptors when their intracellular concentration rises, initiating signaling cascades that change the activity of transcription factors and, consequently, gene expression. For many bacteria, a negative feedback loop in which autoinducer synthesis is downregulated results in a shift in gene expression (figure 6)("How Quorum Sensing Works," n.d.).

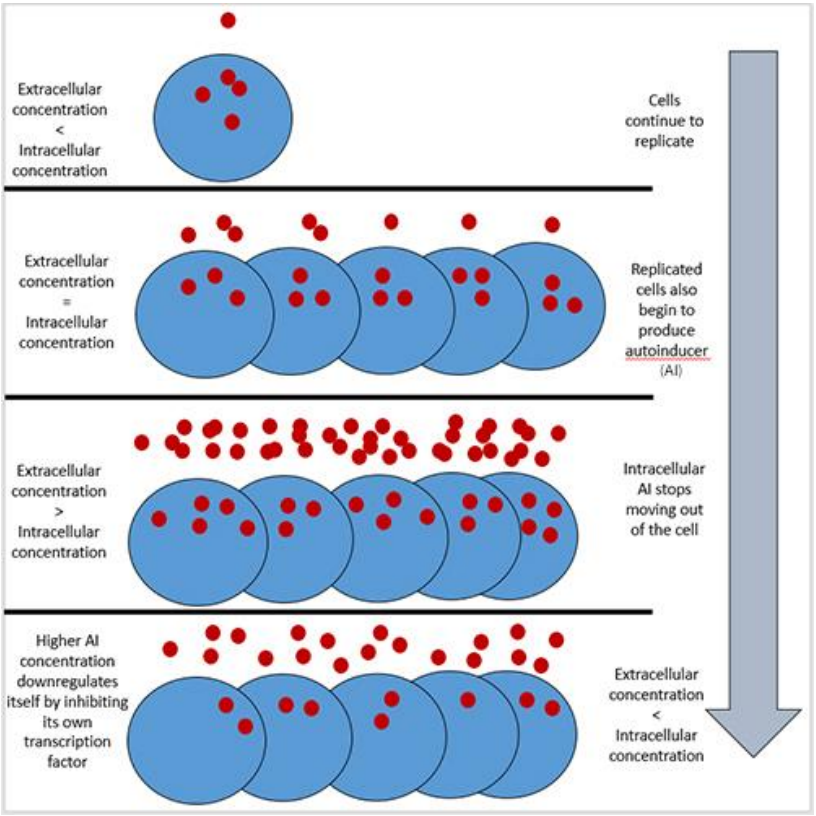

Fig 7: Overview of bacterial quorum sensing

A bacterial community can mount a cooperative response using quorum sensing, which facilitates better nutritional accessibility, competitive defense, and environmental survival. Many different bacterial species use quantum sensing (QS) to control the production of biofilms, bioluminescence, pathogenicity, DNA exchange, and other elements of life.("Quorum sensing | Bacterial Communication, Signaling & Regulation | Britannica," n.d.)

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The creation of tiny autoinducers (AIs), which resemble pheromones and are continuously created and detected by the cells, is essential for bacterial qs. Three elements are crucial to QS: (i) the signal molecule itself; (ii) the signal molecule synthase, which creates the signal molecule; and (iii) the AI receptor, which starts the transcriptional control of genes of interest when AIs reach an acceptable amount. N-acylhomoserine lactones (AHLs), furanosyl-borate diester (AI-2), and 2-heptyl-3-hydroxy-4-quinolone (Pseudomonas quinolone signal, PQS) are a few of the signaling molecules that are classified as AIs, while there is a much wider variety of signaling molecules (Urvoy *et al*., 2022).

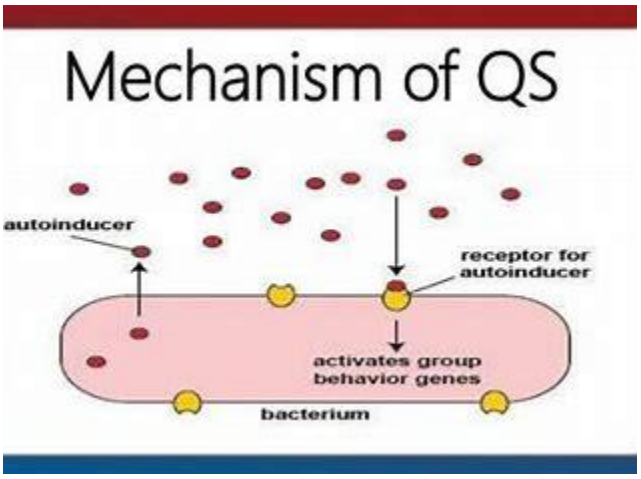

Fig 8: Mechanism of quorum sensing

Acylhomoserine lactones (AHLs), which are produced by an autoinducer synthase (LuxI) and recognized by an autoinducer regulator (LuxR), are the most common type of autoinducers (Fei *et al*., 2020). AHLs are created by an AHL synthase (luxI, ainS, or hdtS family) and released into the environment either through passive diffusion for short-chain AHLs or active transport for long-chain AHLs. More recently, it was found that hydrophobic AIs (such as long-chain AHLs, CAI-1, and PQS) could also be carried through outer membrane vesicles, allowing their solubilization and targeted distribution. The cytoplasmic LuxR-type and membrane-bound LuxN-type receptors are examples of AHL receptors. It's interesting to note that AHL synthases and receptors can both exist independently, without the other.Some bacteria shows four type of QS pathways of the LuxI/LuxR type (LasI/LasR and RhlI/RhlR systems), the quinolone-based QS system (PQS, 2-heptyl3-hydroxy-4-quinolone signal), and more recently the integrated QS system (IQS, 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde signal) are all independent or dependent QS pathways. These QS circuits are organized hierarchically (Lee and Zhang, 2015).

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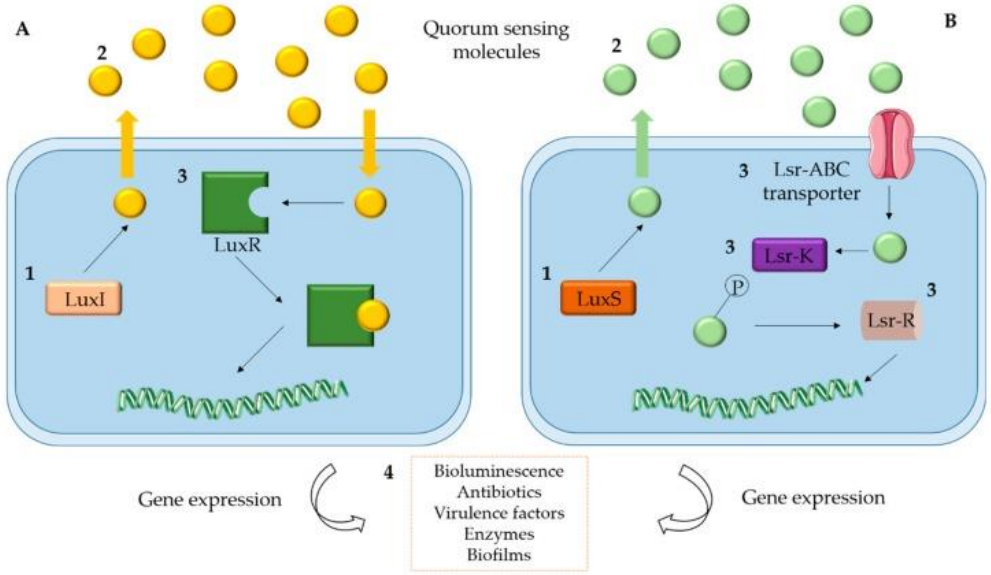

Fig 9: Schematic representation of bacterial quorum sensing

6.3 Some other of Marine organisms performing quorum sensing

Phycosphere of (micro)-algae and phyllosphere of marine plants

The phycosphere is the immediate environment around algae. It depicts a microbial environment that is heavily influenced by algae (Bell and Mitchell, 1972). Bacterial abundances in this milieu can approach 108-1011 cells per ml (Hannides *et al*., 2002), allowing quorum sensing to occur. Bachofen and Shenk's pioneering work published in 1998 indicated the generation of AHLs inside a cyanobacterial phytoplankton bloom at quantities up to 10 mg l-1 (Schaefer *et al*., 2008).Many findings on quorum sensing systems in the phycosphere of (micro)algae have been published since then(Rolland *et al*., 2016). A recent study employing transcriptomics methods discovered an increase in luxI and luxR gene expression in *Ruegeria pomeroyi* co-cultured with the microalgae *Alexandrium tamarense*. This study discovered potentially significant linkages between microalgae growth and quorum sensing-dependent physiological control within *Rhodobacteraceae* strains associated with certain microalgae (Landa *et al*., 2017).

Quorum sensing in microbial communities related to biofilm

QS is most likely to occur in biofilms in the water, any study of QS in marine environments must also include a discussion of biofilms. In the marine environment, biofilm-associated bacteria can be found on biotic surfaces like coral or phytoplankton as well as abiotic surfaces like detrital aggregates like marine snow. There are various benefits for bacteria that live in biofilms. Bacteria in biofilms show greater tolerance for a range of environmental challenges, including UV exposure or desiccation, as well as higher resistance to predation, antibiotics, and other drugs(Hall-Stoodley *et al*., 2004) (Matz and Kjelleberg, 2005). QS aids in the formation, growth, and dissemination of biofilms in model bacteria that generate them (Romeo, 2008) and biofilms in turn provide an extremely conducive environment for QS.

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Frequently, biofilms provide as a good example of QS-friendly circumstances. Signal diffusion may be physically constrained by the biofilm matrix, and the biofilm matrix may help to concentrate longer-chain AHL homologs (Horswill *et al*. 2007). The pH within biofilms is typically lower than that of the surrounding environment(Alldredge and Cohen, 1987); (Horswill *et al*., 2007); (Vroom *et al*., 1999); (Wild *et al*., 2005). According to (Charlton *et al*., 2000) and(Dobretsov *et al*., 2011), AHLs accumulate inside biofilms at significantly higher concentrations than are seen in planktonic cultures and are not in balance with their environment. As a result, biofilms may be able to lessen many of the chemical and physical difficulties caused by the marine environment (such as abiotic degradation or diffusion).

AHLs have been found in marine biofilms in several studies, and evidence shows that QS plays a key role in the formation of multispecies biofilms in the marine environment(Huang *et al*., 2007). Stromatolites, marine snow, and *Trichodesmium* colonies are only a few of the natural biofilm habitats where AHLs have been recorded (Decho *et al*., 2009) (Hmelo *et al*., 2011). Numerous studies (Gardères *et al*., 2012b) (Tait *et al*., 2005) (Ransome *et al*., 2014) (Wheeler *et al*., 2006) and other pieces of strong evidence indicate that QS is active not just in these habitats but also in biofilms that are connected with invertebrate creatures like corals and sponges. The next sections emphasize the supporting data and the part that QS plays in a number of these systems.(Hmelo, 2017c)

Corals and other cnidarian-associated communities

Many cnidarian species have bacterial-associated communities whose members interact via quorum sensing signals. *Anemonia viridis* and the *Gorgonacea Eunicella verrucosa*, for example, have extremely varied associated bacterial communities that include quorum sensing AIs producers(Ransome *et al*., 2014). However, the majority of study on cnidarian species in the field of quorum sensing has concentrated on corals, particularly in the context of pathogenicity (i.e., black band disease, white band disease, and so on).

Coral species have significant and dense related microbial communities that are 10-1000 times more concentrated than in ambient saltwater (Rosenberg *et al*., 2007). According to 2011 research, 30% of bacteria in these consortia are capable of quorum sensing.

Sponge-associated communities

The sponge (phylum *Porifera)* microbiome is complex and diverse (Reveillaud *et al*., 2014) (Webster and Thomas, 2016). It has been discovered that certain sponges are poorly colonized by bacteria whereas others are not (Hentschel *et al*., 2003). Bacteria inhabiting sponge tissues, on the other hand, can account for up to 35% of the sponge biomass(Vacelet and Donadey, 1977). Again, in this sort of micro-niche, bacteria may commonly achieve a sufficient abundance to create quorum sensing-based communication, and different sponge symbionts capable of producing AIs are discovered (Mohamed *et al*., 2008).Furthermore, some reports suggest that this type of communication between sponge bacterial symbionts is common, as 77% of studied Australian sponges can activate AHL-based biosensors (Lami, 2019), and 46% of sponge species are from the Mediterranean and Red Sea. Marine natural products with antibiotic activity have

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been a rich source of drug discovery; however, the emergence of antibiotic-resistant bacterial strains has turned attention towards the discovery of alternative innovative strategies to combat pathogens. In many pathogenic bacteria, the expression of virulence factors is under the regulation of quorum sensing (QS). QS inhibitors (QSIs) present a promising alternative or potential synergistic treatment since they disrupt the signaling pathway used for intra- and interspecies coordination of expression of virulence factors. This review covers the set of molecules showing QSI activity that were isolated from marine organisms, including plants (algae), animals (sponges, cnidarians, and bryozoans), and microorganisms (bacteria, fungi, and cyanobacteria). The compounds found and the methods used for their isolation are the emphasis of this review (Saurav *et al*., 2017) (Britstein *et al*., 2018). The chemical variety of AHLs implicated in these microbiota has been elucidated and found to be quite varied, with short and long acyl side chains and between 6 and 18 carbons (Saurav *et al*., 2017). C6-HSL, C7-HSL, and 3-oxoC12-HSL were discovered in the Celtic sea sponge *Suberites domuncula* (Gardères *et al*., 2012b), and an increasing number of AHLs from sponges are being identified (Britstein *et al*., 2016), (Bose *et al*., 2017). The strain *Ruegeria sp*. KLH11, isolated from *Mycale laxissima*, has been established as a model to better understand the cellular impacts of quorum sensing and to further describe the link between quorum sensing expression and bacterial sponge symbiotic characteristics. These studies revealed the presence of two pairs of quorum sensing luxI/R genes and an orphan luxI gene in this KLH11 strain model, which control biofilm formation (negatively regulated) and flagella-based motility (positively regulated) (Zan *et al*., 2011) (Zan *et al*., 2013)(Zan *et al*., 2015).

Such management may prevent bacterial aggregation inside the sponge and encourage its dispersion and discharge into the environment.The quorum sensing-dependent connections between sponges and their symbionts appear to be quite intricate. For example, bacterial 3-oxo-C12-HSL affects gene expression and suppresses the innate immune system in S. domuncula (Gardères *et al*., 2012a), and certain sponge chemicals can interfere with quorum sensing signaling (Costantino *et al*., 2017).

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ARTIFICIAL INTELLIGENCE IN DRUG DISCOVERY

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ABSTRACT

Artificial intelligence (AI) has made significant contributions to medicinal chemistry. It is used for drug discovery, virtual screening, predicting drug target interactions and optimizing chemical compounds. Drug discovery is a process with multi stage designed to confirm everything about a new potential treatment is better understood. Before any testing on humans or live animals to be conducted, a new drug needs to first be invented. It involves wide ranges of specific disciplines including biology, chemistry and pharmacology. AI algorithms analyze vast data sets and perform tasks like molecule generation and property prediction, which can accelerate drug development and reduce costs. AI became a powerful tool for designing and identifying potential drug candidates.

Keywords: Artificial intelligence, Drug discovery, Machine learning, Deep learning

INTRODUCTION

Artificial intelligence (AI) was making significant strides in the field of medicinal chemistry. AI has the potential to revolutionize drug discovery and development in several ways.

Focusing on the Drug Discovery Phase, the first thing is target identification, a gene, a protein or a particular disease related nominee. AI can assist in identifying potential drug candidates by analyzing vast datasets of chemical compounds, biological interactions, and disease mechanisms. Machine learning algorithms can predict the binding affinity of molecules to specific drug targets, helping researchers prioritize compounds for experimental testing. AI can help identify new drug targets by analyzing omics data (genomics, proteomics, etc.) and identifying genes or proteins associated with diseases. Once the target is identified, the target will get validated, which kind of compound or a molecule that bind/deactivate from mutation. This information can be crucial for designing targeted therapies.

The lead molecule comes from hundreds and thousands of different targets, that can be done experimentally i.e., efficacy, cytotoxicity and binding. The lead molecule is optimized to make sure that it can be produced as a small batch to test whether they are stable, whether the compound can be made into a medicine. AI can identify existing drugs that may have therapeutic effects for different diseases, a process known as drug repurposing or repositioning. This approach can save time and resources compared to developing entirely new drugs.

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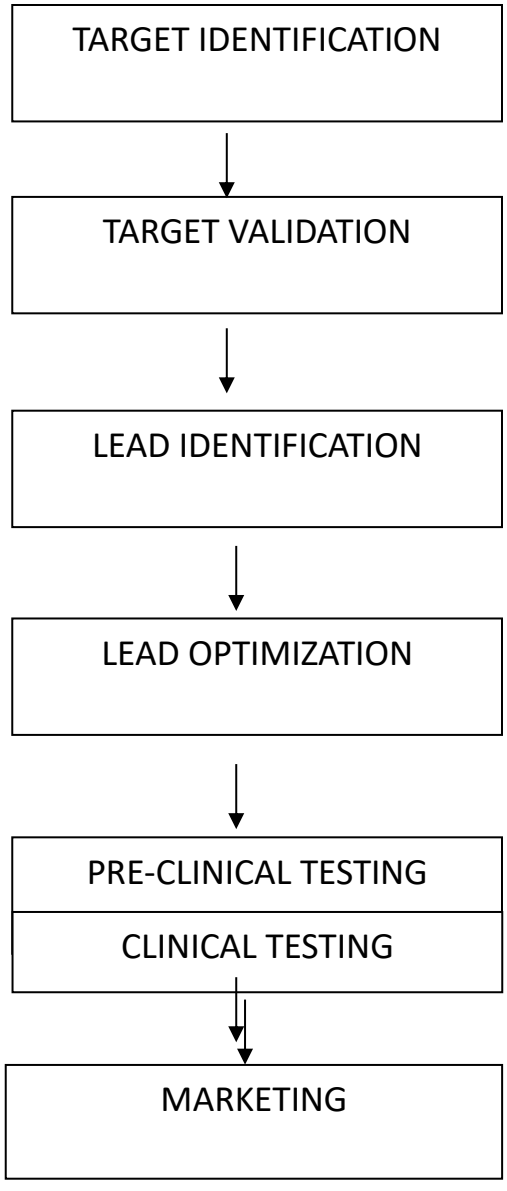
AI algorithms predict potential drug-drug interactions and adverse effects, helping clinicians make informed decisions about drug combinations and dosages. AI analyze biological data to discover biomarkers that can aid in patient stratification, disease diagnosis, personalized medicine and it can assist in optimizing drug formulations, such as dosage forms, delivery methods, and stability. Machine learning models can predict potential toxicities of drug candidates early in the development process, reducing the risk of costly failures in later stages. Later on, the lead compound undergoes pre-clinical testing and clinical testing, in which applied to tissues in-vitro, in-vivo or ex-vivo, in which animal models come into place.

The drug response and the drug interaction with micro disease environment are determined. AI can help design more efficient and patient-centric clinical trials, improving patient recruitment, retention, and data analysis. AI improves drug manufacturing processes, ensuring product quality and consistency while reducing production costs. AI systems continuously monitor real-world data for adverse drug reactions and safety concerns, enabling rapid response to emerging issues.



1. Predict the binding affinity of molecules
2. Generate variants using gene-disease association techniques
3. Identify new drug targets by analyzing omics data
4. Identify lead molecules by efficacy, cytotoxicity and binding
5. Ligand based compound screening
6. Designing the compound with desirable property
7. Tissue specific biomarker identification
8. Prediction of biomarkers
9. Determination of drug response
10. Determination of drug interaction with disease micro environment

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TARGET IDENTIFICATION

The most common approach is used in the target identification is genetic screening. There are two types of genetic screening. Forward genetic screening and reverse genetic screening, where, in forward genetic screening, we have an unknown gene, we understand what gene is being mutated for the disease to happen or particular side effects happen. In reverse genetic screening, the particular gene is known; the phenotypic study is done how the gene misbehaves from its normal functions. The target identification process is used to identify specific biological targets such as proteins, enzymes or receptors that are associated with a particular disease. It involves various approaches, including genetic studies, omics data analysis, and literature review. Once identified, the targets are validated to ensure their relevance and potential for therapeutic intervention.

Artificial intelligence (AI) has made significant contributions to the field of drug target identification, revolutionizing the drug discovery process. Drug target identification is a crucial early stage in drug development where researchers identify specific molecules (usually proteins) that can be targeted by drugs to treat a disease or condition. AI algorithms can analyze vast datasets from various sources, including genomics, proteomics, and electronic health records, to identify potential drug targets. These algorithms can find correlations and patterns that might not be apparent through traditional methods. Machine learning models, such as deep learning and random forests, can predict potential drug targets based on known drug-protein interactions, molecular structures, and biological pathways. These models can prioritize targets with the highest likelihood of success. AI performs virtual screenings of chemical compounds to identify molecules that have the potential to interact with a specific target. This reduces the number of compounds that need to be tested in the lab, saving time and resources.

AI techniques can analyze biological networks and pathways to identify key nodes or proteins that play crucial roles in diseases. These proteins can be potential drug targets. AI-powered natural language processing (NLP) can extract valuable information from scientific literature, patents, and clinical trial reports. This information can help researchers identify promising drug targets and understand their mechanisms of action. AI can help identify individualized drug targets by analyzing patient data, including genetics and clinical history. This can lead to the development of targeted therapies that are more effective and have fewer side effects. AI identifies existing drugs that could be repurposed for new indications by analyzing their interactions with various targets. This approach can significantly reduce drug development timelines and costs. AI analyzes images from various biological assays, such as high-throughput screening and microscopy, to identify potential drug targets or assess drug effects on cells and tissues. Generative AI models, like generative adversarial networks (GANs) and variational auto encoders (VAEs), can assist in generating novel molecules with desired properties, which can be potential drug candidates. AI-driven algorithms can optimize the chemical structure of drug candidates to enhance their efficacy, safety, and pharmacokinetic properties.

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HIT GENERATION

The hit generation usually search for chemical compounds or molecules that have the potential to interact with the identified target. Various methods includes high throughput screening, virtual screening and fragment based screening are employed to identify hits that bind to the target and show initial activity. Artificial intelligence (AI) is playing a significant role in drug screening, which is the process of identifying and evaluating potential drug candidates for their efficacy and safety. AI applications in drug screening are helping to streamline the process, reduce costs, and improve the success rate. AI algorithms and machine learning models are used to virtually screen large chemical libraries to identify potential drug candidates. These models predict the binding affinity and activity of compounds with specific drug targets, saving time and resources compared to traditional high-throughput screening. AI-powered molecular docking simulations predict how a drug candidate interacts with its target protein at the molecular level. This helps in understanding the binding affinity and the potential for therapeutic activity. AI models can establish relationships between the chemical structure of compounds and their biological activity. QSAR (Quantitative Structure Activity Relationship) models help in predicting the activity of new compounds, prioritizing them for further testing.

AI, combined with advanced imaging and microscopy techniques, can analyze and interpret complex high-content screening data. This is particularly useful in studying the effects of compounds on cells and tissues. AI models can predict the toxicity of potential drug candidates. This is critical for assessing safety and reducing the risk of adverse effects during clinical trials. AI can predict how a drug candidate will be absorbed, distributed, metabolized, and excreted in the body. This helps in understanding a compound's pharmacokinetic profile. AI can prioritize compounds based on their potential as drug candidates, helping researchers focus on the most promising leads. AI can assist in fragment-based drug design, where smaller molecular fragments are combined to form new drug candidates with specific properties.

LEAD IDENTIFICATION AND OPTIMIZATION

The hits identified in the previous stage undergo optimization to improve their potency, selectivity and pharmacological properties. This involves chemical modification of the hit compounds through medicinal chemistry techniques to enhance the activity and reduce potential off-target effects. The lead compounds selected based on the improved activity and specificity undergoes further optimization to improve their drug like properties. This involves extensive medicinal chemistry efforts to enhance potency, optimize pharmaco kinetics and minimize toxicity.

Artificial Intelligence (AI) plays a crucial role in lead identification and optimization in drug discovery. This phase of drug development involves the refinement of potential drug candidates, improving their efficacy, safety, and pharmacokinetic properties.

Here are some ways AI is employed in lead identification and optimization:

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- **Chemoinformatics and Structure-Activity Relationship (SAR) Analysis:** AI algorithms can analyze the structure and properties of compounds to identify structural features associated with desired or undesired activities. This aids in the selection and modification of lead compounds.
- **Generative Chemistry:** AI-driven generative models can create novel compound structures based on known leads and desired properties. This helps in the exploration of chemical space and the discovery of new lead candidates.
- **Virtual Screening:** AI can perform virtual screening of large compound libraries to identify potential lead compounds. Machine learning models can predict the binding affinity of compounds to specific target proteins.
- **Predictive Toxicology:** AI models can predict the potential toxicity of lead compounds, reducing the risk of late-stage failures. They analyze data on compound structure and biological interactions to assess safety.
- **Pharmacokinetics and Pharmacodynamics (PK/PD) Modeling:** AI is used to model and predict how lead compounds are absorbed, distributed, metabolized, and excreted in the body, as well as their effects on target proteins and pathways.
- **Multi-Objective Optimization:** AI-driven algorithms can simultaneously optimize multiple properties of lead compounds, such as potency, selectivity, solubility, and metabolic stability, to find the best candidates.
- **Protein-Ligand Interaction Prediction:** AI can predict how lead compounds interact with target proteins at the molecular level, helping to design more effective and specific drugs.
- **Structure-Based Drug Design:** AI can assist in the design of compounds by analyzing the three-dimensional structure of target proteins and suggesting modifications to improve binding affinity.
- **Clinical Trial Simulation:** AI can simulate clinical trials to predict the outcomes of different dosing regimens and patient populations, optimizing trial design and resource allocation.
- **Data Integration:** AI can integrate a wide range of data sources, including biological, chemical, and clinical data, to provide a comprehensive understanding of lead compounds and their potential.
- **Automation:** Robotic systems equipped with AI can automate the synthesis and testing of lead compounds, accelerating the optimization process.
- **Adaptive Design:** AI can guide adaptive clinical trial design, allowing for real-time adjustments based on accumulating data, increasing the chances of success.

AI in lead identification and optimization not only speeds up the drug development process but also enhances the quality and efficiency of lead candidate selection. It enables the design of more effective and safer drugs, reducing the risk of late-stage failures and increasing the success rate of drug development programs. However, the integration of AI with expert knowledge and experimental validation remains crucial in this process.

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PRE CLINICAL TESTING

The lead compounds that show from using characteristics in terms of efficiency and safety undergo clinical testing. This involves rigorous testing in various in vitro and invivo models to evaluate their pharmacological properties, toxicological profile and potential side effects. Testing is conducted to provide essential date of for assessing the compound's safety and determining its suitability for the development.

Acute Toxicity Studies: Acute toxicity studies assess the compound's safety by administering a single high dose to animals and monitoring for advance affects or mortallity.

Chronic and chronic toxicity studies: assess the safety and potential toxicity of the drug over time.

Efficacy Studies: measure parameters such as tumor growth inhibition, disease progression, or other relevant end points.

Safety pharmacological studies: assess the compound's effects on vital organ systems such as cardiovascular, respiratory or central nervous system to identify potential safety concerns.

Reproductive and development toxicity studies: investigate the compounds affect on reproduction, fertility and embryonic development using animal models.

AI is significantly impacting preclinical testing, the phase of drug development that precedes clinical trials. It plays a vital role in streamlining processes, enhancing efficiency, and reducing costs. Here are some key applications of AI in preclinical testing:

- **Toxicology:** AI models can predict the toxicity of new compounds by analyzing and correlating their chemical structures and properties with known toxic effects. This helps in the early identification of potentially harmful compounds, reducing the risk of failure in later stages of drug development.
- **ADME (Absorption, Distribution, Metabolism, Excretion) Prediction:** AI can predict how a compound is absorbed, distributed, metabolized, and excreted in the body. This information is crucial in assessing the pharmacokinetic properties of a drug candidate and helps in optimizing its bioavailability and efficacy.
- **Biological Data Analysis:** AI can process and analyze vast amounts of biological data from various sources, including genomics, proteomics, and transcriptomics, to identify potential drug targets and understand disease mechanisms better.
- **Image Analysis:** AI is used in the analysis of microscopic and imaging data, aiding in the interpretation of histopathological images, radiological scans, and other visual data used in preclinical studies.
- **Disease Modeling:** AI can be used to create complex computational models of diseases, enabling researchers to simulate disease progression and test the efficacy of potential treatments in a virtual environment before moving to animal studies.

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o **Data Integration and Analysis:** AI can integrate data from various preclinical studies, including in vitro and in vivo experiments, to provide a comprehensive understanding of the safety and efficacy of a drug candidate.

The integration of AI in preclinical testing enhances the efficiency and accuracy of data analysis, enabling researchers to make informed decisions about the viability of drug candidates before advancing to clinical trials. However, it's essential to validate AI-generated predictions through traditional experimental methods to ensure the safety and efficacy of potential treatments.

AI TOOL KIT IN DRUG DISCOVERY

The AI Drug Discovery Toolkit integrates multiple technologies, including machine learning (ML), deep learning (DL), and data analysis, and takes inspiration from a variety of academic fields. Additionally, this has resulted in a language for artificial intelligence that is getting wider. These phrases have distinct meanings and contexts, despite the fact that they are frequently used synonymously. These contexts include things like data requirements, complexity, transparency, and capacity. Through the identification of intricate patterns and the prediction of outcomes from unseen data, machine learning algorithms are built to create models that can learn from training data relevant to a given problem without the need for direct programming. For these reasons, they are frequently employed in preclinical drug discovery and have been shown to be effective in more accurately predicting physicochemical qualities, ADMET-related metrics, and bioactivity. The three main categories of machine learning algorithms are reinforcement learning (RL), unsupervised learning, and supervised learning.

Unlike supervised and unsupervised learning, RL systems continuously interact with their environment and use feedback from previous actions and experiences to achieve their goals. Every time an RL agent performs an action, it uses an objective function that rewards if the result is acceptable and penalizes otherwise. The goal of the RL algorithm is to identify the optimal policy that maximizes the reward function. RL algorithms such as Generative Tensorial Reinforcement Learning (GENTRL) and Reinforcement Learning for Structural Evolution (ReLeaSE) have been used to design molecules with desired properties during generative modelling. DL is a subset of ML and belongs to the larger family of artificial neural network (ANN) algorithms. It is currently a cutting-edge artificial intelligence technology and can be described as a class of representative learning techniques. ANN algorithms are loosely inspired by the structure of the human brain. Therefore, the ANN architecture contains many processing elements, called neurons, organized into multiple layers. The network consists of input nodes and a layer of output nodes connected by a layer of hidden nodes. Each hidden node has an associated weight, an activation function, and a bias function that transforms the input to produce predictions.

The adjective “deep” in DL refers to a multilayer ANN, and the number of hidden layers indicates the depth of the network. DL methods contain multiple hidden layers, unlike traditional “shallow learning” ML methods, which typically contain one or two hidden layers.

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While DL methods use deep, specialized architectures to automatically learn and extract higher-level features from unstructured data, they also require massive amounts of training data. Another key difference between DL and deep learning ML algorithms is that DL algorithms adapt to the data, while deep learning ML algorithms converge to a certain level of performance. Popular DL architectures used in drug discovery include Convolutional Neural Networks (CNNs), Graphical CNNs (GCNNs), Auto encoders (AEs), and Recurrent NNs (RNNs). CNNs are the most widely used DL method in drug discovery.7 A CNN architecture consists of multiple layers of neurons, each of which is fully connected to all neurons of the previous layer. It usually contains multiple layers of alternating convolution and pooling, which can learn any highly nonlinear function. Deep CNN models trained on 3D atomic networks extracted from experimental protein-Ligand complex structures are currently used for structure-based prediction of VS and its properties. They have been shown to effectively model the complex and nonlinear phenomenon of small molecule binding to proteins and have shown significant improvement in property prediction. Other DL architectures such as RNN have also been used for generative modelling and direct library generation.

APPLICATIONS OF AI IN DRUG DISCOVERY

1. Comparison of Drug Target and Binding Site

The availability of atomic-resolution structural information about small molecules that bind to drug targets provides opportunities for structure-based hit identification (structure-based VS), fragment screening (structure-based drug discovery fragments; FBDD), and ligand optimization (based drugs). Structural target information also provides insight into factors affecting selectivity, resistance mechanisms, mode of action, identification of allosteric pockets, and assessment of ligability of for new drug targets. Despite technological advances in X-ray crystallography, single-particle NMR spectroscopy, and cryoEM, structural coverage covers only about 35% of the human proteome. In many cases, this structural coverage is often limited to a single structural domain of a multidomain protein. Therefore, there is always a gap between the number of known protein sequences and the number of experimentally solved structures.Importantly, the structural coverage of pharmaceutically relevant target protein families such as G protein-coupled receptors (GPCRs) and ion channels is still underrepresented in databases such as the Protein Data Bank (PDB). An alternative approach to generate the 3D structure of proteins without experimental structure is to use computational methods for structure prediction. Homology modelling is a traditional approach to bridging the gap between sequence and structure. It predicts the 3D structure of an unknown (target) protein based on the experimental structure of a homologous (reference) protein using its amino acid sequence. Homology modelled structures with up to 30% sequence identity have generally been shown to be suitable for SBDD.48 For proteins without a homologous structure, accurate structure prediction remains a challenge; However, advances in DL-based methods and the incorporation of co evolution data into modelling have revitalized the field of protein structure prediction. DL-based algorithms such as CNNs, RNNs, Variational Auto encoders (VAEs), and

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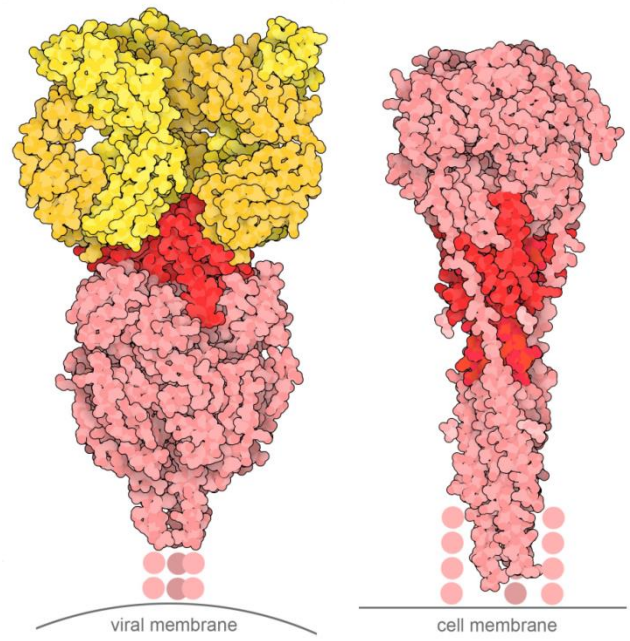
generative adversarial networks have shown higher performance in predicting protein structure even in the absence of a template structure.



Structural superposition of Glycoprotein structure

2. AI Augmented Virtual Screening

VS is a computational technique that provides a complementary and cost-effective approach to hit identification compared to experimental screening methods such as high-throughput screening. Instead of physically examining each compound in the screening set, VS uses computational techniques to prioritize a subset of compounds for evaluation in the primary test. The growing size of make-on-demand screening libraries and the increasing number of complex, high-value drug targets identified through functional genomics screening pose significant challenges to traditional VS techniques. Therefore, AI methods that improve the VS approach and help efficiently explore chemical space to identify hits are of great interest for drug development.

3. Virtual Screening using Ligand Based

Ligand-based VS techniques (LBVS) aim to identify active compounds from a chemical library based on the principle of molecular similarity. These include similarity search, pharmacophore screening, pattern matching and predictive modeling. Predictive modeling for VS is an extension of the classic QSAR modeling paradigm. Classic QSAR uses congeneric series statistical data modeling methods to create explanatory models that retrospectively quantify SAR trends. Access to large amounts of chemogenomic data (PubChem BioAssay and ChEMBL databases) and advances in ML and DL algorithms that can handle large data sets have opened new opportunities for QSAR modeling as a VS technique.As a result, many successful applications of QSAR-based VS workflows for result identification have been reported. The

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authors used two ML algorithms (SVM and kNN) to develop a binary (active or inactive) classification model trained on 3,133 compounds with known antimalarial activities. QSAR models were used to perform VS against from the ChemBridge database and resulted in the selection of 174 compounds for further screening for Plasmodium falciparum growth inhibition and cellular analyses.Experimental validation showed that 25 selected compounds were active, resulting in a success rate of 14.2%, with the strongest having an EC50 of 95.6 nM. Subsequently, many studies have shown the use of ML and DL-based QSAR workflows as promising VS tools.

CONCLUSION

AI in drug target identification has the potential to significantly accelerate the drug discovery process, reduce costs, and increase the success rate of bringing new drugs to market. However, it also comes with challenges, such as data quality, interpretability of AI models, and ethical considerations, which need to be carefully addressed in practice. By harnessing the power of AI, drug discovery efforts are becoming more efficient, cost-effective, and targeted, ultimately leading to the identification of hit compounds that have a higher probability of success in clinical development. This is especially important in addressing complex and challenging diseases where traditional drug discovery methods may fall short. AI is not a replacement for traditional drug discovery processes but rather a powerful tool that complements and accelerates these processes. It has the potential to significantly reduce the time and costs involved in drug development, leading to the discovery of more effective and safer drugs. However, it is essential to validate and refine AI-driven results through rigorous experimental and clinical testing to ensure their safety and efficacy in real-world applications.

REFERENCES

1. B.W.J. Irwin, J.R. Levell, T.M. Whitehead, M.D. Segall, G.J. Conduit, Practical applications of deep learning to impute heterogeneous drug discovery data, J Chem Inf Model 60 (2020) 2848–2857.
2. B.W.J. Irwin, T.M. Whitehead, S. Rowland, S.Y. Mahmoud, G.J. Conduit, M.D. Segall, Deep imputation on large-scale drug discovery data, Applied AI Letters 2 (2021) e31.
3. C.W. Coley, W.H. Green, K.F. Jensen, Machine learning in computer-aided synthesis planning, Acc Chem Res 51 (2018) 1281–1289.
4. D.B. Kitchen, H.Y. Decornez, Computational techniques to support hit triage, in: W. Czechtizky, P. Hamley (Eds.), Small Molecule Medicinal Chemistry, John Wiley & Sons, Inc., Hoboken, 2015, pp. 189–220.
5. D.G. Brown, H.J. Wobst, A decade of FDA-approved drugs (2010–2019): trends and future directions, J Med Chem 64 (2021) 2312–2338.
6. H. Dolgos, M. Trusheim, D. Gross, J.P. Halle, J. Ogden, B. Osterwalder, *et al*., Translational medicine guide transforms drug development processes: the recent Merck experience, Drug Discov Today 21 (2016) 517–526.
7. J. Jiménez-Luna, F. Grisoni, G. Schneider, Drug discovery with explainable artificial intelligence, Nat Mach Intell 2 (2020) 573–584.

ISBN : 978-81-966183-3-9

8. K. McCloskey, E.A. Sigel, S. Kearnes, L. Xue, X. Tian, D. Moccia, *et al*., Machine learning on DNA-encoded libraries: a new paradigm for hit finding, J Med Chem 63 (2020) 8857–8866.
9. M.D. Segall, Multi-parameter optimization: identifying high quality compounds with a balance of properties, Curr Pharm Des 18 (2012) 1292–1310.
10. M.M. Hann, G.M. Keseru, Finding the sweet spot: the role of nature and nurture in medicinal chemistry, Nat Rev Drug Discov 11 (2012) 355–365.
11. N. Fleming, How artificial intelligence is changing drug discovery, Nature 557 (2018) S55–S57.
12. O.J. Wouters, M. McKee, J. Luyten, Research and development costs of new drugs–reply, JAMA 324 (2020) 518.
13. P. Schneider, W.P. Walters, A.T. Plowright, N. Sieroka, J. Listgarten, R.A. Goodnow Jr, *et al*., Rethinking drug design in the artificial intelligence era, Nat Rev Drug Discov 19 (2020) 353–364.
14. P.A. Vignaux, E. Minerali, D.H. Foil, A.C. Puhl, S. Ekins, Machine learning for discovery of gsk3beta inhibitors, ACS Omega 5 (2020) 26551–26561.
15. Properzi, F, Steedman, M, Taylor, K, Ronte, H, Haughey, J. (2019) Intelligent drug discovery powered by AI. Deloitte Insights; www2.deloitte.com/us/en/ insights/industry/life-sciences/artificial-intelligence-biopharma-intelligent-drug-discovery.html [Accessed November 19, 2021]
16. R.M. Plenge, Disciplined approach to drug discovery and early development, Sci Transl Med 8 (2016) 349PS315.
17. S. De Cesco, J.B. Davis, P.E. Brennan, TargetDB: a target information aggregation tool and tractability predictor, PLoS ONE 15 (2020) e0232644.
18. S.M. Paul, D.S. Mytelka, C.T. Dunwiddie, C.C. Persinger, B.H. Munos, S.R. Lindborg, *et al*., How to improve R&D productivity: the pharmaceutical industry's grand challenge, Nat Rev Drug Discovery 9 (2010) 203–214.
19. T.M. Whitehead, B.W.J. Irwin, P. Hunt, M.D. Segall, G.J. Conduit, Imputation of assay bioactivity data using deep learning, J Chem Info Model 59 (2019) 1197– 1204.
20. T.S. Toh, F. Dondelinger, D. Wang, Looking beyond the hype: applied AI and machine learning in translational medicine, EBioMedicine 47 (2019) 607–615.
21. Webb, T.F. Jamison, Continuous flow multi-step organic synthesis, Chem Sci (2010) 675–680.
22. Y. Bian, X.Q. Xie, Generative chemistry: drug discovery with deep learning generative models, J Mol Model 27 (2021) 71.

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FOOD BORNE PATHOGENS AND ITS PATHOPHYSIOLOGY

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ABSTRACT

Food borne illness is most common in people due to their epidemic nature. The pathogens associated with these diseases are bacteria, fungi, viruses and parasites which affects the human system. The agents of illness are transmitted in our body through various types of food items, water and uncooked food. Pathogens cause severe illness in immunosuppressant people. This review gives general insights about harmful microbial pathogens involved in food borne illness. The development of a food-borne disease is due to specific conditions, such as the presence of virulence factors of microorganism in food, the microbial load present in the food and the conditions of the host's immune system. So, in this review, major issues are addressed such as reviewing the major food-related causes of disease. From this point of view, the relevant microorganisms involved in food contamination (bacteria, viruses, parasites, fungi and mycotoxins), are considered. In addition, potentially allergenic foods or foods most commonly associated with food intolerance, are also considered.

Key words: Food borne pathogens, Toxins, Infection, Diagnosis, Treatment.

1. Introduction

Outbreaks of food-borne illnesses have shown that food-borne bacterial pathogens significantly threaten public health. The most commonly infected foods include dairy products,beef, poultry, vegetables, and drinking water. Most food-borne diseases are infections caused by various bacteria, virus, and parasites (Figure 1). Foodborne diseases are caused by consuming food spoiled by pathogens or their toxins. These diseases are easily spread, and consequently, they are a worldwide public health problem. Detection of pathogens in food andprotection against food spoilage is a task of great social, economic and public health importance. To improve both sensitivity and selectivity of detecting diseases, different approaches can be combined with polymerase chain reaction (PCR)-based techniques. Although PCR methods possess relatively high sensitivity, they require expensive equipment and are likewise time-consuming.[1] Both immunoassay-based methods as well as DNA and fluorescent microarrays are capable of relatively fast detection concerning culture-based methods, yet they also require trained personnel and expensive equipment.[2]

2. Foodborne pathogens and their sources

Foodborne illness usually arises from improper handling, preparation, or food storage.Good

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hygiene practices before, during, and after food preparation can reduce the chances of contracting an illness (Table 1).

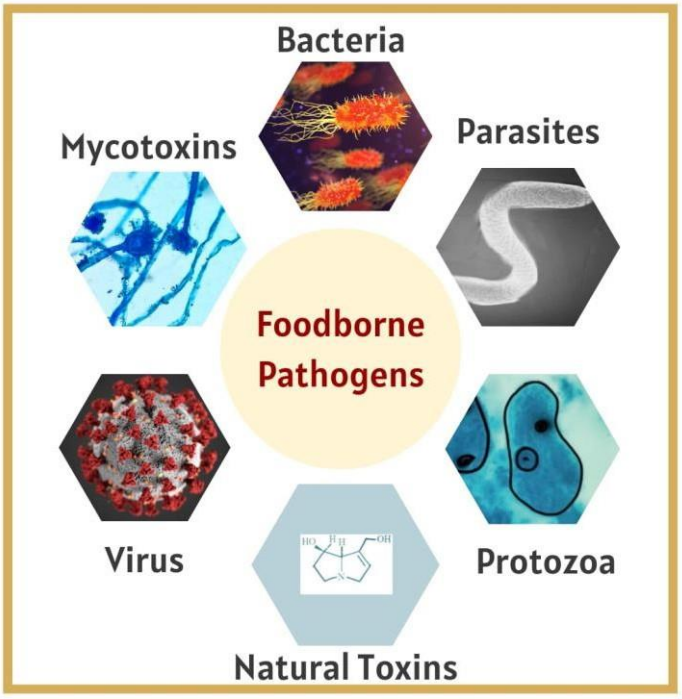

Figure 1. Pathogens associated with food borne diseases.

2.1 Bacteria

Bacteria are a common cause of foodborne illness. Toxins from bacterial infections are delayed because the bacteria need time to multiply. However, in some cases, such as Staphylococcal food poisoning, the onset of illness can be as soon as 30 minutes after ingestingcontaminated food.[3] Most common bacterial food borne pathogens are *Campylobacter jejuni* (secondary Guillain–Barre syndrome and periodontitis), *Clostridium perfringens*, *Salmonella typhimurium* (consumption of ineffectively cooked eggs or poultry), *Escherichia coli O157:H7* (hemolytic-uremic syndrome), *Bacillus cereus, Listeriamonocytogenes, Staphylococcus aureus, Vibrio cholerae, Yersinia enterocolitica* etc.

2.2. Mycotoxins and alimentary mycotoxicoses

The term alimentary mycotoxicoses refers to the effect of poisoningby Mycotoxins through food consumption. The common food-borne Mycotoxins include Aflatoxins (*Aspergillus parasiticus* and *A. flavus*). The pronounced forms of Aflatoxins are those of B1, B2, G1, and G2. Aflatoxin B1 predominantly targets the liver, which resultsin necrosis, cirrhosis, and carcinoma.[4]Altertoxins – are those of Alternariol (AOH), Alternariol methyl ether (AME), Altenuene (ALT), Altertoxin-1 (ATX-1), Tenuazonic acid (TeA) and Radicinin (RAD), originated from *Alternaria* sp. Other toxins are Citrinin, Citreoviridin, Cyclopiazonic acid, Cytochalasins, Ergot alkaloids / Ergopeptine alkaloids – Ergotamine, Fumonisins, etc.

2.3 Virus

Viral infections make up perhaps one-third of cases of food poisoning in developed countries.

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Foodborne viral infections are usually of intermediate (1–3 days) incubation period,causing illnesses that are self-limited in otherwise healthy individuals, e.g. Enterovirus,Hepatitis A, Hepatitis E, Norovirus, Rotavirus etc.

2.4 Parasites

Most food borne parasites are zoonoses, e.g. Platyhelminthes (*Diphyllobothrium* sp., *Nanophyetus* sp.), Nematode (*Anisakis* sp., *Ascaris lumbricoides, Eustrongylides* sp. etc.).

2.5 Protozoa

It includes *Acanthamoeba* and other free-living amoebae like *Cryptosporidium parvum, Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia lamblia* etc.

2.6 Natural toxins

Most animal poisons are not synthesized by the animal, but acquired by eating poisonous plants to which the animal is immune, or by bacterial action.[5] Natural toxins include Alkaloids, Ciguatera poisoning, Grayanotoxin (honey intoxication), Mushroom toxins, Phytohaemagglutinin, Pyrrolizidine alkaloids etc.

2.7 Mechanisms of food-borne pathogens

2.7.1 Incubation period

The delay between the consumption of a contaminated food and the appearance of thefirst symptoms of illness is called the incubation period. This ranges from hours to days (and rarely months or even years. If symptoms occur within one to six hours after eating the food, it suggests that it is caused by a bacterial toxin or a chemical rather than live bacteria.[6]

2.7.2 Infectious dose

The infectious dose is the amount of agent that must be consumed to give rise to symptoms of foodborne illness and varies according to the agent and the consumer's age and overall health. Pathogens vary in minimum infectious dose; e.g. *Shigella sonnei* has a low estimated minimum dose of < 500 colony-forming units (CFU).

Table 1: Infect to the microorganisms in food

Food	**Organism**
Dairy	*Campylobacter, Salmonella species*
Eggs	*Salmonella sp.*
Meats	*Clostridium perfringens, Bacillus cereus (diarrhea), Aeromonas,Campylobacter, and Salmonella sp.*
Ground beef	*E coli O157:H7*
Poultry	*Campylobacter sp.*
Pork	*C. perfringens, Yersinia enterocolitica*
Seafood	*Astrovirus, Aeromonas, Plesiomonas, and Vibrio sp.*
Oysters	*Caliciviru, Plesiomonas and Vibrio sp.*

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Vegetables	*Aeromonas sp., C. perfringens*
Mayonnaise containing salads and highly processed foods (creampuffs)	*Staphylococcus aureus*
Rice; starchy foods	*Bacillus cereus (vomiting)*
Canned foods; honey (children under 1 year of age)	*Clostridium botulinum*

3. Bacterial gastroenteritis gram-positive bacteria

Bacterial gastroenteritis causing gram positive bacteria are *Staphylococcus aureus* (gram$^+$, aerobic, coccus), *Bacillus cereus* (gram$^+$, aerobic, rod), *Clostridium perfringens* Type A (gram$^+$, anaerobic, rod), *Clostridium botulinum* (gram$^+$, anaerobic, rod) etc.[7] *S. aureus* enterotoxins are of 8 distinct antigenic typeslabeled SEA, SEB, SEC, SEE, SEG, SEH, SEI, and SEJ. They are water-soluble, low molecularweight proteins that bind to the emetic reflex center causing nausea and vomiting. The spore germination process of *B. cereus* produces two enterotoxins which cause either vomiting (emetic form) or diarrhea (diarrheal form). The enterotoxin stimulates the adenyl cyclase- cyclic AMP system.[8] *C. perfringens* enterotoxin causes watery diarrhea. The toxin is formed when the vegetative cells become spores. Refrigeration prevents the growth of organisms in the meat and reheating the meat destroys the heat-labile enterotoxin.[9]*C. perfringens* Type C beta-toxin-producing strains of this bacterium cancause a rare disease called necrotizing enteritis or enteritis necroticans (pig-bel). So far, *C. botulinum* neurotoxin presents seven distinct antigenic types labeled A, B, C, D, E, F, and G. Improperly canned foods are the most common source of this form of food poisoning. *C. perfringens* toxemia can rarely with heavily contaminated foods produce a diffuse, necrotizingenteritis of the jejunum, ileum and colon. Clinical symptoms usually occur within 12 hours of toxin ingestion as compared to an incubation period of 24-72 hours for infections.[10] Botulism can take from 1-2 days before symptoms are manifest. *S. aureus* causes vomiting (often projectile) little or no diarrhea, no fever. *C. perfringens* causes abdominal cramping and watery diarrhea. The diarrhea generally lasts less than 24 hours. No fever. In most instances, the actual cause of poisoning by *C. perfringens* is temperature abuse ofprepared foods. *C. botulinum* induces symptoms between 1-2 days after ingestion of improperly canned green beans, peppers, chili, or sausage. Bilateral descending weakness of the peripheral muscles develops in patients with flaccid paralysis. Death is usually attributed to respiratory failure.

Diseases caused by *clostridia* include Botulism (due to *C. botulinum*), *C. difficile*– induced colitis, Gastroenteritis, Soft-tissue infections, Tetanus (due to *C. tetani*) etc. Host factors include toxin A antibody production, interleukin-8 levels, and intestinal toxin receptors.[11] Serum antitoxin antibodies are the best-described host factor in *C. difficile* pathogenesis. Mounting a serum

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antibody response to toxin A during an initial episode of CDAD was associated with relative protection against recurrent CDAD.[12] The frequency of an IL-8 allele with a single nucleotide polymorphism (genotype AA, responsible for increased IL-8 production) was significantly associated with thedevelopment of *C. difficile* diarrhea in comparison to the two control groups (39 versus 16 and17 percent, respectively).[13] In all but botulism symptoms occur relativelysoon after ingestion of the toxin and do not include a fever.

In diagnosis, oftentimes the contaminated food is cultured or immunoassays are performed to detect the enterotoxins in the food.[14] The only fatal toxemia in this group is botulism; the emphasis should be on ruling out botulism in the diagnosis.[15] Anaerobic culture of the organism from the food source and demonstration of toxin production using a mouse bioassay can be performed however the sample must be sent to a Public Health lab.[16]

For treatment, in toxemia due to *S. aureus, B. cereus, C. perfringens* no treatment is usually given. If the patient becomes dehydrated intravenous replenishment of fluids and electrolytes is administered. Patients are to be admitted to permit monitoring of respiratory and cardiac function. Airway patency should be guaranteed by insertion of an endotracheal tube or tracheostomy before respiratory impairment becomes severe.[17] Induction of vomiting or gastric lavage is recommended if exposure has occurred within severalhours.[18]

4. Bacterial Gastroenteritis- Noninflammatory (no fecal WBCs)

4.1 *Escherichia coli* infection

The etiological agent for *Escherichia coli* infection is Enterotoxigenic *E. coli* (ETEC) -infantile diarrhea and Traveler's diarrhea; Enteropathogenic *E. coli* (EPEC) - diarrhea ininfants less than 6 months of age; Enteroaggregative *E. coli* (EAEC) - a major cause of Traveler's diarrhea, a more persistent diarrhea etc. (Figure. 2).

In pathology, EPEC produces no demonstrable toxin. They cause an attaching-and- effacing histopathology in the small intestine. They then intimately adhere to the host cells andinject (type III secretory system) bacterial factors into the host cells causing alterations in the glycocalyx of the small bowel epithelial cells.[19] ETEC strains colonize the small intestine and produce a cholera-like (heat-labile; LT) toxin and a heat-stable toxin (ST).Following endocytosis of the bound toxin, the A subunit is released into the cytoplasm and contains the enzymatic function that ADP ribosylates the GTP-binding protein.[20] The GTP-binding protein then permanently activates adenylate cyclase resulting in increased intracellular levels of cAMP. The cAMP activates cAMP-dependent protein kinase (A kinase), which will cause supranormal phosphorylation of chloride channel.[21] Stimulation of chloride ion secretion from secretory crypt cells and inhibition of Sodium chloride absorption by villus tip cells causes an increase in luminal ion content drawing water passively through the paracellular pathway and an osmotic diarrhea.[22] The *E. coli* ST is quite different from the LT. This toxin is about 18-19 aminoacids in length. ST binds to a membrane-spanning enzyme called guanylate cyclase. Guanylatecyclase is located in the apical membrane of intestinal epithelial cells and binding of the ST to the extracellular domains of the protein stimulates its intracellular enzymatic activity.[23] Enteroaggregative *E. coli* (EAEC)- Involves three stages that include adherence to the mucosa, enhanced mucus production that encases the bacteria forming a biofilm, followed by

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elaboration of a cytotoxin, which damages the intestinal cells. They may have the ability to colonize both the small and large intestine.

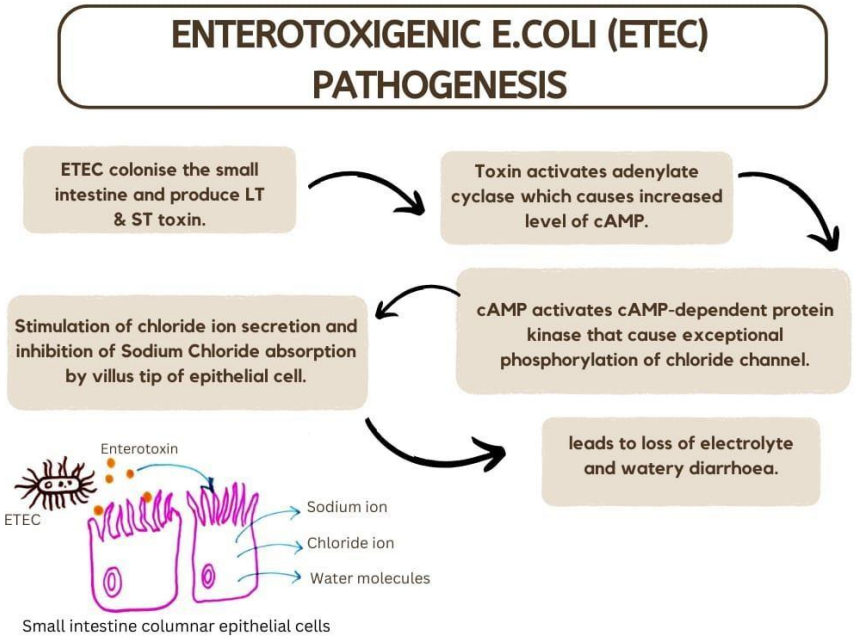

Figure 2. Pathogenesis of enterotoxigenic *E. coli*

Severe diarrheal disease caused by ETEC is generally characterized by the abrupt onset of watery diarrhea. The non-enterotoxin-producing, noninvasive *E. coli* (EPEC) have thus far been incriminated only in mild diarrheal disease in infants less than 6 months of age primarily. EAEC is a common cause of more persistent diarrhea. Diagnosis is made by isolating *E. coli* on MacConkey's agar. In treatment intravenous or peroral replacement of the fluid and electrolytes lost in feces. Peroral therapy is almost always adequate. Tetracycline and trimethoprim - sulfamethoxazole are effective in shorteningthe duration of symptoms but are not essential. Bismuth subsalicylate may provide symptomatic relief.[24]

4.2 *Vibrio*

V. cholerae is acid sensitive and the majority of ingested organisms are killed by stomach acidity; it takes ingestion of 10^8-10^{10} cells to cause disease. Symptoms and metabolicderangements in cholera result from the rapid loss of liquid from the gut.On exposure to small bowel epithelial cells, each B subunit rapidly binds to GM1 monosialoganglioside in the gut cell wall. Following binding, the A moiety migrates through the epithelial cell membrane. The A1 subunit contains ADP-ribosyltranferase activity and catalyzes the transfer of ADP-ribose from NAD to a guanosine triphosphate (GTP) - binding protein that regulates adenylate cyclase activity. The ADP-ribosylation of GTP bindingprotein inhibits the GTP turnoff reaction and causes a sustained increase in adenylate cyclase activity.[25] The resultant increased intracellular cyclic AMP acts at 2 sites to cause net secretion of isotonic liquid within the small bowel lumen.[26] The increased cyclic AMP inhibits neutral sodium chloride absorption across the glycocalyx via the co-transport mechanism; it also stimulates active chloride secretion into thegut lumen.

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The symptoms are characterized by abrupt, watery diarrhea and vomiting. Several liters of liquid may be lost within a few hours, rapidly leading to profound shock. The patient is cyanotic and has sunken eyes and cheeks, a scaphoid abdomen, poor skin turgor, and threadyor absent peripheral pulses. The voice is high-pitched or inaudible; the vital signs include tachycardia, tachypnea, and low or unobtainable blood pressure. The heart sounds are distant and often inaudible, and bowel sounds are hypoactive.

The diagnosis of cholera is made based on the clinical presentation, especially the presence of "rice water" stools. Confirmative diagnosis is made by plating a stool sample on TCBS (thiosulfate-citrate-bile salt-sucrose) agar, which is selective for *Vibrio*, and the adrenalcell assay. Successful therapy for Cholera requires only prompt replacement of fluids and electrolytes. Ringer's solution is the most used. It is given rapidly by IV injection - 50 to 100 ml per minute - until a strong radial pulse is restored. Tetracycline reduces the severity and length of disease. Chloramphenicol and furazolidone are slightly less effective.[27] Vaccines seem to provide somewhat better immunity and fewer side-effects than the previously available vaccine.

4.3 Infant *botulism*

C. botulinum can colonize the gastrointestinal tract of an infant less than 1 year of age. *C. botulinum* spores in honey are used to sweeten infants' milk or water, when ingested, geminate in the infants' intestinal tract, colonize it, and produce toxin *in vivo*. Constipation is the first sign of disease; the same neurological signs seen in the adult follow the constipation.[28] Therapy is the same as for adult botulism except that antitoxin is generally not used because the disease is milder in children.

4.4 Giardiasis

Ingestion of water containing *Giardia lamblia* (*duodenalis*) cysts causes Giardiasis. The cyst then develops into a trophozoite in the duodenum. Acute infections can be asymptomatic or result in bloating, flatulence, and watery diarrhea. Chronic infections can leadto malabsorption and fatty diarrhea-steatorrhea.[29]

In pathology, ingested organisms colonize the duodenum and jejunum where they adhere to the epithelium of the microvillus, without causing significant amounts of damage. Pathologic changes are mild in most cases, but shortening and thickening of the villi associatedwith acute focal inflammatory changes in the mucosal epithelium may be seen initially and arefollowed by chronic inflammation.

Disease onset is sudden and consists of foul-smelling, watery diarrhea, abdominal cramps, flatulence, and steatorrhea. Spontaneous recovery can occur after 10-14 days. However, a chronic disease may develop.

Presumptive diagnosis is made based on a history of drinking non-chlorinated waterand the expression of classical clinical symptoms.[30] Confirmative diagnosis requires the finding of *Giardia lamblia* trophozoites or cysts in the feces or an intestinal biopsy. Asymptomatic carriers and those with symptoms of infection should be treated with either quinacrine hydrochloride or metronidazole (Flagyl; 250 mg, PO, every 8 hours for 10 days). Properly maintaining the filtration systems at water plants and boiling of drinking wateris essential.

4.5 *Cryptosporidium parvum*

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Cryptosporidium parvum is a coccidium parasite causing Cryptosporidiosis.Ingestion of oocysts of *Cryptosporidium parvum* in immunocompromised persons is more likely to result in persistent chronic diarrhea. Loss of cell-mediated immunity increases the risk of infection and is a common cause of chronic diarrhea in AIDS patients.[31]

In pathology, it is known that the parasite affects intestinal ion transport and causes inflammatory damage to the microvilli resulting in malabsorption.[32] In people with normal immune function, an asymptomatic carrier state can occur as well as a self-limiting watery diarrhea. The preventive measures include avoiding contaminated water sources and fecal-oral routes. Other parasitic infections causing watery diarrhea in immune-compromised patients are *Cyclospora cayetanensis, Isosporabelli,* and *Microsporidia* (*Enterocytozoon bieneusi*).

4.6 *Pseudomembranous colitis*

Clostridium difficile is an anaerobic, Gram-positive, spore-forming rod that produces toxin A and toxin B. Both toxins are cytophilic but only toxin A is active against intestinal epithelial cells.[33]

In pathology, *C. difficile* overgrows and produces toxin A and B which bind to and kill cells in the bowel wall. These toxins cause the cells to round up and die by stimulation of host cell mutagen-activated protein kinases (MAP-kinases) and inactivating proteins that regulate actin filament assembly (small GTP-binding Rho proteins).[34] These toxinscause depolymerization of actin filaments, which then cause the cells to round up and detach forming shallow ulcers. Acute inflammation with pus and mucus formation results in pseudomembrane formation.

Watery diarrhea- the most common symptomatic disease is watery diarrhea (5-15stools per day). Symptoms include crampy bilateral lower quadrant pain that decreases after bowel movements, low-grade fever, and mild peripheral blood leukocytosis. Fulminant colitis-develops in 2-3% of patients. This disease has severe morbidity and high mortality. Diarrhea is usually present however the patient can be constipated with abdominal pain/distension/guarding associated with hypoactive bowel sounds. Complications can include toxic megacolon and bowel perforation.[35]

Diagnosis is generally made based on a history of antibiotic therapy within the pastmonth. ELISA for toxins A and B is performed on the feces and is rapid and relatively sensitive(70-90%). Endoscopy revealing the classical pathology can make the diagnosis if the patient isunable to produce stool or if an immediate diagnosis is required. The antibiotic and replacement of the intestinal flora generally suffice. If dehydrated, intravenous fluids and electrolytes are provided. If antibiotic treatment is needed, metronidazole will kill *C. difficile*. The important prevention measures are thorough hand washing and avoiding prolonged use of broad-spectrum antibiotic treatment.

5. Inflammatory Gastroenteritis

This form of intestinal infection affects the large intestine. Frequently the stool volumeis small, contains mucus and white blood cells, and if invasion is deep enough can be heme- positive.[6] Symptoms are fever, complains of abdominal pain, and pain while attempting to defecate (tenesmus). Antimicrobial treatment of these infections can in most cases be beneficial.[24] Treatment releases more shiga-like toxin and makes the patient more likely to develop HUS (hemolytic uremic syndrome).[36] Thethree most common causes of this

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form of gastroenteritis are *Salmonella*, *Shigella*, and *Campylobacter*.

5.1 *Campylobacteriosis*

These organisms are comma-shaped (seagull-shaped). It is relatively fragile and sensitive to environmental stresses (e.g., 21% oxygen, drying, heating, disinfectants, and acidicconditions).[37] Eight different species of *Campylobacter* cause gastrointestinal infections. *C. jejuni* is the most common cause of gastroenteritis worldwide. These are gram-negative, comma-shaped rods that commonly occur in pairs and are microaerophilic and motile.[38]

In pathology, *Campylobacter* adheres to intestinal epithelial cells and M cells. If theheat labile enterotoxin is produced a watery diarrhea occurs. After adhering to the host cells, the bacteria use a type III secretory system to inject bacterial proteins into the host cells. Meanwhile, some strains of the bacteria produce a toxin called Shiga toxin or serotonin that gets into the host cells' cytoplasm and stops protein synthesis by removing an adenine residuefrom the 28S rRNA in the 60S ribosomal unit.[39]

Initial symptoms include periumbilical cramping, intense abdominal pain that mimics appendicitis, malaise, myalgias, headache, and vomiting.[40] Symptoms include watery diarrhea, malaise, fever, abdominal cramps, tenesmus, bloody stools, and fecal leukocytes on light microscopy.

Presumptive diagnosis is based on the finding of gull-shaped bacteria in feces with darting motility. Definitive diagnosis requires isolation of the organism from the stool or othersites of infection. Campy-BAP or Skirrow media contain antibiotics that reduce the growth ofother enteric microorganisms. For treatment, correction of electrolyte abnormalities and oral rehydration are usually sufficient. *C jejuni* is usually sensitive to erythromycin, gentamicin, tetracycline, ciprofloxacin, and clindamycin.[41]

5.2 *Shigellosis- Bacillary*

Shigellosis is primarily transmitted by the fecal-oral route. Fifty *Shigella* species fall into one of four serological groups. *Shigella* is a nonmotile, nonlactose fermenting gram- negative rod whose natural habitat is the intestine of humans and other primates. The disease occurs when virulent *Shigella* organisms attach to and penetrate, epithelial cells of the intestinal mucosa. After the invasion, they multiply intracellular, and spread to contiguous epithelial cells resulting in tissue destruction.[42] Shigellosis is mainly adisease of children between 1 and 4 years of age.[43]

In pathology, *Shigella* after adhering to the host cells the bacteria use a type III secretory system to inject bacterial proteins into the host cells. When the bacteria reach the periphery of the cell it pushes outward to form membrane projections that are then ingested byadjacent cells.[44] The bacteria produce a toxin called Shiga toxin orverotoxin that gets into the host cells cytoplasm and stops protein synthesis by removing an adenine residue from the 28S rRNA in the 60S ribosomal unit.[45]

After an incubation period of 36-72 hours, the initial non-specific symptoms of fever(39-39^{o}C) and cramping abdominal pain are prominent. Sigmoidoscopy reveals intense hyperemia, multiple small bleeding sites, loss of transverse mucosal folds, and thick, purulent mucous

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secretions. Tenesmus is present and the feces are bloody, mucoid, and small in volume. Fluid and electrolyte loss may be quite significant.

Presumptive diagnosis is based on the acute onset of fever and diarrhea with bloodyand mucoid feces. Definitive diagnosis requires the isolation of *Shigella* from the feces.Microabscesses in a rectal biopsy are suggestive of shigellosis.[46] Diffuse involvement of the mucosa with multiple shallow ulcers 3-7 mm in diameter is suggestive of shigellosis.[47] A rectal swab of an ulcer will reveal clumps of neutrophils, macrophages, and erythrocytes. The stool usually contains white bloodcells and is positive for lactoferrin. Fluid and electrolyte replacement is necessary in severe cases.

5.3 *Salmonellosis*

S. enterica presents over 2460 unique serogroups (ex. *S .enterica* serovar *typhimurium* or *S. enterica* serovar *enteritidis*). *S. paratyphi* and *S. typhi* cause enteric fever.[48]

In pathology, *Salmonella* is sensitive to killing by gastric acid. Therefore, it requires a large number of organisms to cause an infection. Most infections result in fever, abdominalpain, and diarrhea.[49] *S. typhi* and *S. paratyphi* easily get into the bloodstream. They both cause enteric fever or typhoid fever (*S. typhi*) and paratyphoid fever (*S. paratyphi*).

The symptoms include Enteritis (nausea, abdominal cramps, vomiting and non bloodydiarrhea) and septicemia (risk is higher in pediatric/geriatric patients/immune compromised patients like AIDS). The clinical presentation is just like any other gram-negative sepsis. Enteric fever caused by *S. typhi* produces typhoid fever. *S. paratyphi A, S. schottmuelleri, and S. hirschfeldii* cause paratyphoid fever. Isolation of the organisms from the stool using S-S agaris necessary for a definitive diagnosis. Patients with enteric fevers warrant immediate antibiotic therapy (ciprofloxacin or ceftriaxone). Fluid and electrolyte replacement is necessary in severe cases. Antidiarrheal compounds which inhibit peristalsis, are contraindicated.[50] Vaccination is encouraged.

5.4 Viral gastroenteritis

The Norwalk virus is in the *Caliciviridae* family, i.e., it is a naked single-stranded RNA-containing virus with a single icosahedral capsid. Noroviruses (i.e., Norwalk-like viruses or NLV) are members of the family *Caliciviridae* and are well-recognized etiologic agents of nonbacterial acute gastroenteritis. Adenoviruses serotypes 40 and 41 are most associated with diarrhea in infants. Food-borne and water-borne epidemics of viral gastroenteritis are common.[6] Rotavirus disease (winter diarrhea) is most common among infants and youngchildren while Norwalk virus disease (summer diarrhea) affects older children and adults.[51] Noroviruses are the most common cause of gastroenteritis outbreaks in industrialized countries.

In pathology, the virus invades and destroys mature epithelial cells in the middle and upper villus, causing a decreased absorption of sodium and water from the bowel lumen.[52]

Rotaviruses usually cause vomiting diarrhea and fever in babies less than 2 years of age. The elderly can also have problems with this virus. Adenovirus complications can include intussusceptions.[53] Astroviruses usually cause symptoms of diarrhea in children less than 5

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years of age. Norovirus symptoms last 12--60 hours and are characterizedby sudden onset of low-grade fever, nausea, vomiting, and watery diarrhea; the incubation period is 12-48 hours. A rapid antigen test of the stool, either by EIA (>98% sensitivity and specificity) or latex agglutination tests can be used to aid in the diagnosis of rotavirus infection.[54] Viral gastroenteritis is a self-limiting disease but it is often necessary to administer fluids and electrolytes.[55]Oral rehydration therapy is recommended for preventing and treating early dehydration. Shock, severe dehydration, and decreased consciousness require intravenous therapy. It has consistently shown that probiotics, such as *Lactobacillus casei* GG and *Saccharomyces boulardii,* reduce the frequency and/or duration of diarrhea in acute infantile gastroenteritis by 30-70%.

REFERENCES

1. Postollec F, Falentin H, Pavan S *et al*. Recent advances in quantitative PCR (qPCR) applications in food microbiology. Food microbiology. 2011; 28(5):848-861.

2. Mead PS, Slutsker L, Dietz V *et al*. Food-related illness and death in the United States. Emerging infectious diseases. 1999; 5(5): 607-625.

3. Chapman CE. Improving nutrient utilization and growth in dairy heifers (Doctoral dissertation, 2016; University of New Hampshire.

4. "GASGA Technical Leaflet – 3 Mycotoxins in Grain". Food and Agriculture Organization of the United Nations. 2007; 3:1-12.

5. Adejumo TO, Hettwer U, Karlovsky P. Occurrence of *Fusarium* species and trichothecenes in Nigerian maize. International Journal of Food Microbiology. 2007; 116 (3): 350-357.

6. Bhunia AK. Foodborne microbial pathogens: mechanisms and pathogenesis. Edn 2, Springer New York, NY, 2018, pp365.

7. Khardori N. (Ed.). Bench to Bedside: Diagnostic Microbiology for the Clinicians. Edn 3, CRC Press, 2018, pp170.

8. Elshaghabee FM, Rokana N, Gulhane RD *et al*. *Bacillus* as Potential Probiotics: Status, Concerns, and Future Perspectives. Frontiers inmicrobiology. 2017; 8:1490.

9. Said HM. (Ed.). Host-Pathogen Interactions in Pathophysiology of Diarrheal Disorders. Physiology of the Gastrointestinal Tract. Edn 6, Academic Press, 2018, 1547-1577.

10. Evans AS. Epidemiological Concepts. Bacterial Infections of Humans. 2009; 29:1–50.

11. Poxton IR, McCoubrey J, Blair G. The pathogenicity of *Clostridium difficile*. Clinical microbiology and infection. 2001; 7(8): 421-427.

12. Cohen SH, Gerding DN, Johnson S *et al*. Clinical practice guidelines for *Clostridium difficile* infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). Infection Control & Hospital

ISBN : 978-81-966183-3-9

Epidemiology. 2010; 31(5): 431-455.

13. Yun J, Xu CT, Pan BR. Epidemiology and gene markers of ulcerative colitis in the Chinese. World Journal of Gastroenterology. 2009; 15(7): 788-803.

14. Carranza L. Standardization and internal validation of a bacteria identificationmethod utilizing focal-plane-array Fourier transformed infrared spectroscopy. 2012; McGill University (Canada).

15. Katirji B, Kaminski HJ, Ruff RL (Eds.). Botulism. Neuromuscular Disorders in Clinical Practice. Springer, New York, NY, 2014, 1101-1109.

16. Schechter R, Aureli P, Franciosa G. *Clostridium botulinum*. InternationalHandbook of Foodborne Pathogens. CRC Press, 2003, 81-110.

17. Alibrahim IJ, Kabbani MS, Abu-Sulaiman R *et al*. Outcome of tracheostomy after pediatric cardiac surgery. Journal of the Saudi Heart Association. 2012; 24(3): 163-168.

18. Goel A, Aggarwal P. Pesticide poisoning. National medical Journal of India. 2007; 20(4): 182-191.

19. Sansonetti P. Host–pathogen interactions: the seduction of molecular crosstalk. Gut, 2002; 50(suppl 3): iii2-iii8.

20. Falnes PØ, Sandvig K. Penetration of protein toxins into cells. Current opinion in cell biology. 2000; 12(4): 407-413.

21. El-Armouche A, Zolk O, Rau T *et al*. Inhibitory G-proteins and their role in desensitization of the adenylyl cyclase pathway in heart failure. Cardiovascular research. 2003; 60(3): 478-487.

22. Freeman DE. Small intestine. Equine surgery. 2006; 4: 416-453.

23. Liu D. Diarrhoeagenic *Escherichia coli*. Molecular Medical Microbiology. Edn 2, Vol. I, Academic Press, 2015, 1133-1146.

24. DuPont HL, Pickering LK. Infections of the gastrointestinal tract: microbiology, pathophysiology,and clinical features. Springer New York, NY, 2012, pp 274.

25. Aktories K (Ed.). ADP-ribosylating toxins. Edn 1, Vol. 175, Springer Berlin, Heidelberg, 2012, pp 148.

26. Wapnir RA, Teichberg S. Regulation mechanisms of intestinal secretion: implications in nutrient absorption. The Journal of nutritional biochemistry. 2002; 13(4): 190-199.

27. Ryabokon OV, Savelyev VG, Ushenina NS *et al*. Particularly dangerous infections: cholera, plague, contagious hemorrhagic fevers. Zaporizhzhia State Medical University. 2016, pp78.

28. Lindström M, Myllykoski J, Sivelä S *et al*. *Clostridium botulinum* incattle and dairy products. Critical reviews in food science and nutrition. 2010; 50(4): 281-304.

ISBN : 978-81-966183-3-9

29. Müller N, Von Allmen N. Recent insights into the mucosal reactions associated with Giardia lamblia infections. International journal for parasitology. 2005; 35(13): 1339- 1347.

30. Ezzat SM. An Approach Using Non-Conventional Indicators for Detecting Microbial Water Pollution. CLEAN–Soil, Air, Water. 2014; 42(9): 1223-1231.

31. Huang DB, White AC. An updated review on Cryptosporidium and Giardia. Gastroenterology Clinics. 2006; 35(2): 291-314.

32. Cotton JA, Beatty JK, Buret AG. Host-parasite interactions and pathophysiology in Giardia infections. International journal for parasitology. 2011; 41(9): 925-933.

33. Kayser FH, Bienz K, Eckert J, Zinkernagel R. Color Atlas of Medical Microbiology. New York: Thieme. 2005: 3(4): 231-233.

34. Hofs S. Identification of Candida lysin: A Candida Albicans Peptide Toxin Involved in Epithelial Damage. Doctoral dissertation, 2016, Friedrich-Schiller-Universität Jena.

35. Anemias M, Loss AB, Coagulation I. Hematologic Diseases. Anesthesia and Uncommon Diseases: Expert Consult-Online and Print, 2012, 350.

36. Scheiring J, Andreoli SP, Zimmerhackl LB. Treatment and outcome of Shiga-toxin-associated hemolytic uremic syndrome (HUS). Pediatric nephrology. 2008; 23(10): 1749-1760.

37. Bicudo JR, Goyal SM. Pathogens and manure management systems: a review. Environmental technology. 2003; 24(1): 115-130.

38. Graeter L, Hertenstein E, Accurso C *et al*. Elsevier's Medical Laboratory Science Examination Review-E-Book. 2014, Elsevier Health Sciences.

39. Ibrahim OO. Food Borne Pathogen Escherichia coli O157: H7 History, Sources of Transmission, Symptoms, Detection and Prevention. EC Microbiology. 2015; 2: 214-222.

40. Solomkin J, Husted TL, Wacha H. Clinician's Manual on Intra-abdominal Infections. Edn 1, Springer Healthcare Tarporley, 2011, pp 81.

41. Mehta N, Saha S, Chien EK *et al*. Disorders of the gastrointestinal tract in pregnancy. Edn 5, de Swiet's Medical Disorders in Obstetric Practice, 2010, 256-292.

42. Butler SM, Camilli A. Going against the grain: chemotaxis and infection in *Vibrio cholerae*. Nature reviews Microbiology. 2005; 3(8): 611-620.

43. Kotloff KL, Nataro JP, Blackwelder WC *et al*. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): aprospective, case-control study. The Lancet. 2013; 382(9888): 209-222.

44. Rapisarda C, Fronzes R. Secretion Systems Used by Bacteria to Subvert Host Functions. Current issues in molecular biology. 2017; 25:1-42.

45. Melton-Celsa AR. Shiga toxin (Stx) classification, structure, and function. Microbiology spectrum. 2014; 2(2): 1-21.

ISBN : 978-81-966183-3-9

46. Abee CR, Mansfield K, Tardif S (Eds.). Bacterial and mycotic diseases of nonhuman primates. Nonhuman Primates in Biomedical Research. Edn 2, Academic Press, 2012, 105-172.

47. Warren KS, Mahmoud AA. Geographic medicine for the practitioner. Edn 2, Springer-Verlag New York Inc., 2012, pp 212.

48. Peterson GJ. Factors that affect horizontal gene transfer in enteric bacteria. Doctoral dissertation, 2011, Kansas State University.

49. Zaharik ML, Gruenheid S, Perrin AJ *et al*. Delivery of dangerous goods: type III secretion in enteric pathogens. International journal of medical microbiology. 2002; 291(8): 593-603.

50. O'Connor DP, Fincher AL. Clinical Pathology for Athletic Trainers: Recognizing Systemic Disease. Edn 2, Slack Incorporated, 2008, pp 429.

51. Coffey R, Cummins E, Cormican M *et al*. Microbial exposure assessment of waterborne pathogens. Human and Ecological Risk Assessment. 2007; 13(6): 1313-1351.

52. Lundgren O, Svensson L. Pathogenesis of rotavirus diarrhea. Microbes and infection. 2001; 3(13): 1145-1156.

53. Anderson EJ. Prevention and treatment of viral diarrhea in pediatrics. Expert review of anti-infective therapy. 2010; 8(2): 205-217.

54. Ciccarelli S, Stolfi I, Caramia G. Management strategies in the treatment of neonatal and pediatric gastroenteritis. Infection and drug resistance. 2013; 6: 133-161.

55. Tribble DR, Baqar S, Pang LW *et al*. Diagnostic approach to acute diarrheal illness in a military population on training exercises in Thailand, a region of *Campylobacter* hyperendemicity. Journal of clinical microbiology. 2008; 46(4): 1418-1425.

HISTORY AND FORMULATION DEVELOPMENT OF ANTIBIOTICS

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ABSTRACT

The antibiotics are defined as the medicine that are fight against to bacteria either by inhibiting the growth of bacteria or by killing bacteria.This chapter detailed the history of antimicrobial development, showing how the challenges in discovering new classes of drugs have been with us for the last 80 years.The present state of antimicrobial discovery and development is shaped by these challenges as well asby the economic realities of the pharmaceutical industry.Although the discovery and development of new classes of antimicrobials through the 1960s presented an array of treatment options, these options for some serious and life-threatening infectious diseases may now be more limited.The antibiotics used in the chemotherapy of microbial infections are considered with respect to their antimicrobial spectra and their mechanisms of action, respectively. The sites of action of the different groups of antibiotics interfering with the same cellular processes are discussed. According to their primary actions on sensible cells the antimicrobial antibiotics are divided into different groups.

Key words: β-lactam antibiotics, Aminoglycoside antibiotics, Tetracycline antibiotics, Polypeptide antibiotics, Macrolide antibiotics

INTRODUCTION

The antibiotics are defined as the medicine that are fight against to bacteriaeither by inhibiting the growth of bacteria or by killing bacteria. The word of the antibiotics was derived from the word Antibiosis that means against to the life. In the ancient Era the Antibiotics were considered as the Organic compound that were produced by the one microorganism which are the enemy to the other microbes.As a result of this notion, an antibiotic was originally, broadly defined as a substance, produced by one microorganism, or of biological origin which at low concentrations can inhibit the growth of, or are lethal to other microorganisms. However, this definition has been modified in modern times, to include antimicrobials that are also produced partly or wholly through synthetic means.Whilst some antibiotics can completely kill other bacteria, some are only able to inhibit their growth. Those that kill bacteria are termed bactericidal while those that inhibit bacterial growth are termed bacteriostatic. Although antibiotic generally refers to antibacterial, antibiotic compounds are differentiated as antibacterial, antifungals and antivirals to reflect the group of microorganisms theyantagonize.

MICRO-ORGANISM

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Microbes are tiny living things that are found all around us and are too small to be seen by the naked eye. They live in water, soil, and in the air. The human body is home to millions of these microbes too.

Bacteria are single-cell organisms. Some Bact need oxygen to survive, and others do not. Some love the heat, while others prefer a cold environment. Well-known examples of bacteria include salmonella and staphylococcus bacteria.

Most are not dangerous for humans. Many of them even live on or in our body and help us to stay healthy. For instance, lactic acid bacteria in the bowel help us to digest food. Other bacteria help the immune system by fighting germs. Some bacteria are also needed to produce certain types of food, like yogurt, sauerkraut, or cheese.

Less than 1% of all are responsible for diseases – but this is just a rough estimate because there are no exact numbers. Tuberculosis, for instance, is caused by bacteria. Bacterial infections can be treated with. These are medicines that kill the bacteria or at least stop them from multiplying.Many other infections – including diarrhoea, colds, or tonsillitis – can also be caused by, but viruses are usually responsible for them. Antibiotics are not effective against viruses. So,it is not a good idea to start using them too soon if it is only suspected that bacteria are causing the infection.

Some microbes make us sick; others are important for our health. The most common types are viruses and fungi. There are also microbes called protozoa. These are tiny living things that are responsible for diseases such as toxoplasmosis and malaria.

A few bacteria can be dangerous to our health by causinginfections and even death.

- We can get them from outside the body:Other humans, animals, food, water
- Sometimes our "own" bacteria can cause disease
- Examples of bacterial infections: –Pneumonia, Blood stream infections, Urinary tract infections,

Wound infections, The sexually transmitted disease gonorrhoea.

HISTORY OF ANTIBIOTICS

Before the discovery of antibiotics, infectious diseases were one of the leading causes of morbidity and mortality among humans. Before the commencement of the modern antibiotic era (more than 2000 years ago), microbes that produce antibiotics were used as interventions to treat infectious diseases in Serbia, China, Greece, and Egypt. The Eber's papyrus, an Egyptian medical papyrus dated 1550 BC, is the oldest document describing the use of moldy bread and medicinal soils in treating infections. Similarly, traces of tetracycline, an antibody with chelating effects, were found in human bones collected from the Dakhleh Oasis, Egypt.

Phylogenetic reconstruction analysis has identified the persistent presence of several antibiotic-resistant genes since ancient times. Phylogenetic analysis of β-lactamase and housekeeping genes has revealed that these genes are highly conserved in Klebsiella oxytoca and have been evolving in the host for more than 100 million years. Similarly, analysis of metagenomic clones

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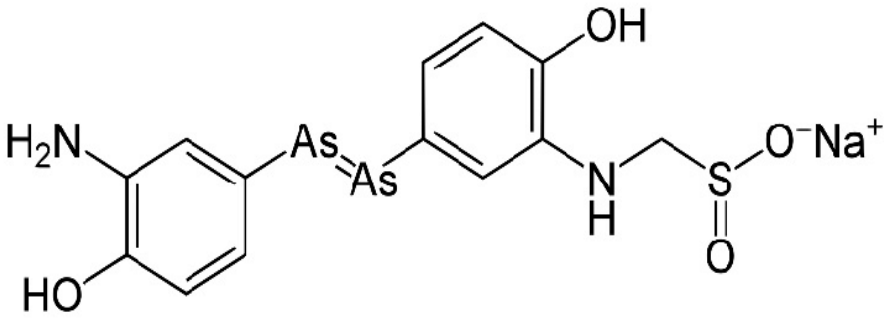
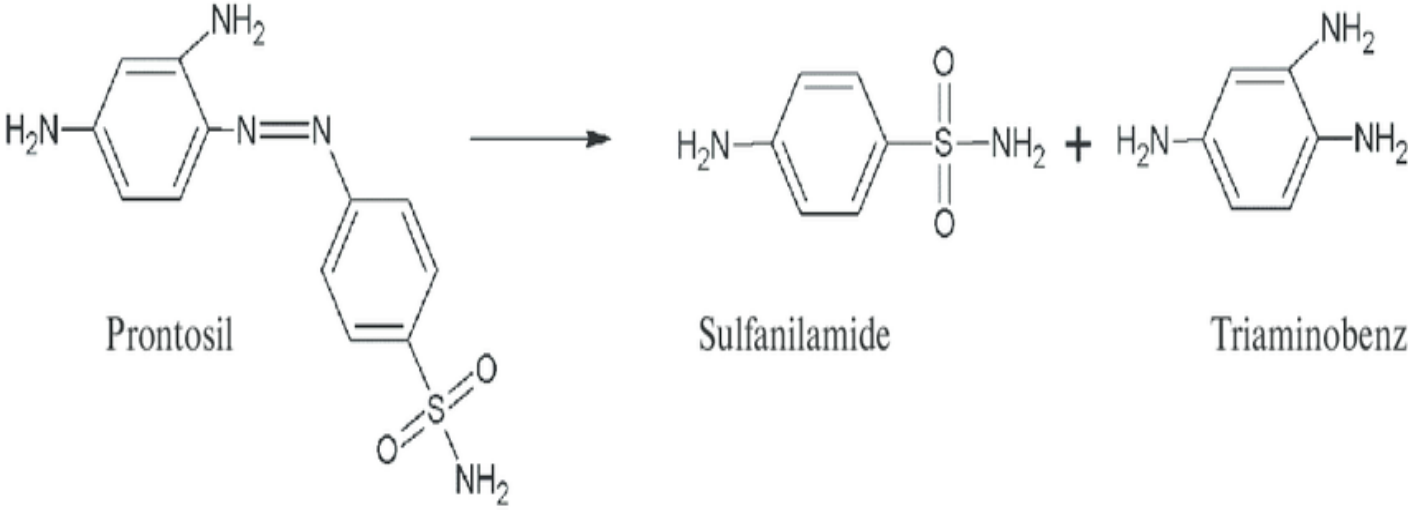
obtained from 10,000-year-old ocean samples has revealed that the diversity of β-lactamases is mostly associated with ancient evolution.

The beginning of the modern antibiotic era can be marked by the discovery of a synthetic prodrug salvarsan and neosalvarsan by Paul Ehrlich in 1910 to treat Treponema pallidum, a spirochaete bacterium that causes the sexually transmitted disease syphilis. Inspired by his own discovery of dyes that specifically stain bacterial cells, Paul Ehrlich started screening a panel of synthetic drugs and subsequently identified salvarsan.



Later, salvarsan was gradually replaced by a sulphonamide prodrug prontosil, which was discovered by bacteriologist Gerhard Domagk. Although sulphonamides are still in clinical use as broad-spectrum antibiotics, large-scale use of these drugs was gradually replaced by the discovery of penicillin by Alexander Fleming in 1928. Fleming isolated and purified penicillin from a fungus Penicillium notatum that had accidentally contaminated a culture plate of Staphylococcus bacteria.



Later on, the large-scale purification of penicillin was conducted by a team of Oxford scientists (Howard Florey, Ernst Chain, and Norman Heatley), which helped penicillin mass production and distribution in 1945. In the same year, Alexander Fleming together with

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Howard Florey and Ernst Chain received the Nobel Prize in medicine "for the discovery of penicillin and its curative effect in various infectious diseases."

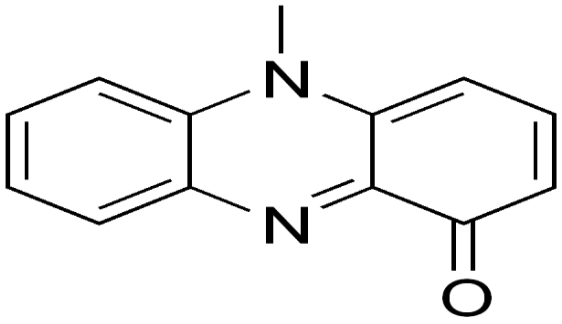
The systemic evaluation of microbes and their ability to produce anti-microbial compounds was first initiated by Selman Waksman in the 1930s. He defined an antibiotic as "a compound made by a microbe to destroy other microbes" and discovered multiple antibiotics from soil-dwelling filamentous actinomycetes, including neomycin and streptomycin (antibiotics against tuberculosis).

The period between the 1940s and 1960s was considered the golden age of antibiotic discovery. The majority of antibiotics discovered during this period are still in clinical use today. However, a marked decline in their efficacy has been observed over time because of antibiotic resistance.

An abrupt decline in the rate of antibiotic discovery after the 1970s together with excessive use of existing antibiotics is the primary cause of antibiotic resistance. A few antibiotics that are currently under investigation are synthetic antibiotics or the derivatives of known classes of antibiotics. This calls for an urgent need of discovering new classes of antibiotics to treat multidrug-resistant bacterial infections.

FIRST CLINICAL USE OF ANTIBIOTICS

Pyocyanase, an extract of Pseudomonas aeruginosa, was the first antibiotic that was used in a hospital to treat hundreds of patients in the 1890s. Pyocyanase, discovered by Emmerich and Löw, was found to be effective against a variety of pathogens and was in use until the 1910s. Although initially considered an enzyme, pyocyanase could be a combination of pyocyanin, quorum-sensing phenazine, and 2-alkyl-4-hydroxy-quinolones.



DEVELOPMENTS IN ANTIBIOTIC DISCOVERY

New classes of antibiotics are primarily identified through large-scale screening of antibiotic-producing soil organisms. The path of antibiotic discovery has been rejuvenated by the

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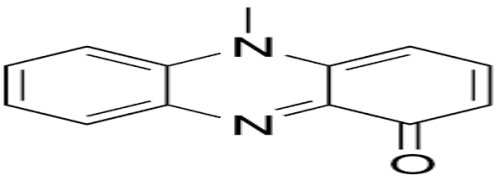
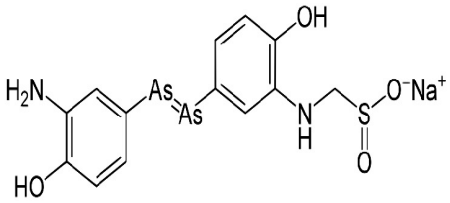
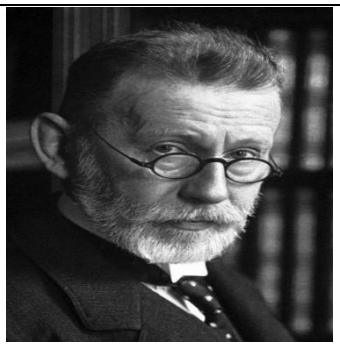
identification of novel organisms from under-explored environments. In addition, the development of new techniques for genome mining and heterologous pathway expression has accelerated the discovery of new antibiotics.

The actinomycete genus Salinospora isolated from the marine environment has been identified as a good source of structurally novel antibiotics including salinosporamide A, which is currently under phase III clinical trial for the treatment of glioblastoma.

In recent years, awareness of the issue of antibiotic resistance has increased, including in the political field: In 2017, the G20 countries decided to intensify global collaboration on this issue to stimulate the R&D of antimicrobial molecules, also starting from existing antibiotics. Since 2017, eight new antibiotics have been approved by the FDA, including one for the treatment of multidrug-resistant tuberculosis: Most of these drugs were developed from traditional molecules and target Enterobacteriaceae resistant to carbapenems and other pathogens considered dangerous by WHO.

OVERVIEW OF HISTORY

Antibiotics	Discovery	**Scientist Name**	Uses
1. Pyocyase 	In 1890s	 **Rudolph Emmerich and Oscar low**	Treatment of Diphtheria and against meningococci.
Salvarsan 	In 1910s	 **Paul Ehrlich**	Treatment of Syphilis Caused by the bacteria Treponima pallidum, Spirochaete bacterium.

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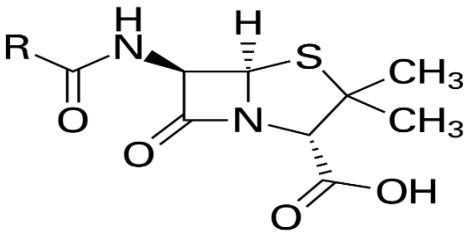
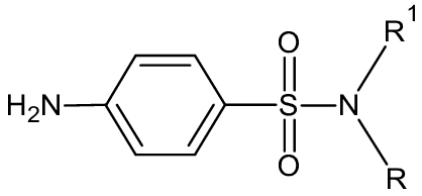
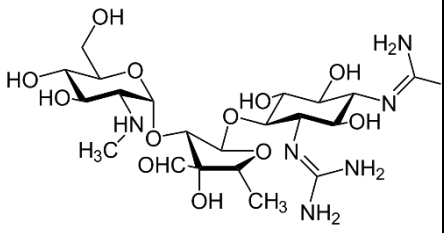
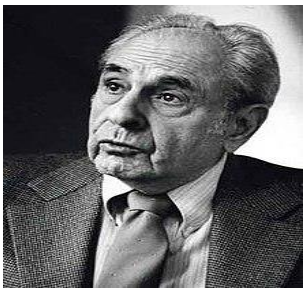
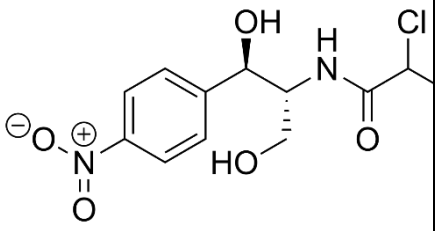
2. Penicilline 	In 1928s	 **Alexander Fleming**	treatment of ear infections, throat infections, skin infections and preventing rheumatic fever.
3. Sulphonamide 	In 1935s	 **Gerhard Domagk**	Treatment of urinary tract infections, inflammatory malaria; skin, vaginal, and eye infections, burns.
streptomycin 	In 1944s	 **Albert Schatz**	It is used to treat certain kinds of bacterial infections.
chloramphenicol 	In 1949s	 **Mildred Rebstock**	management and treatment of superficial eye infections such as bacterial conjunctivitis, and otitis externa.

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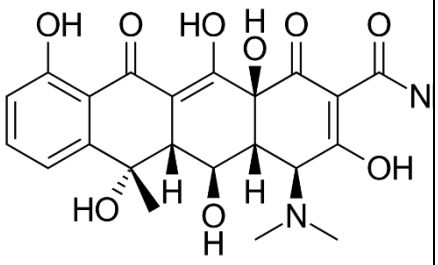
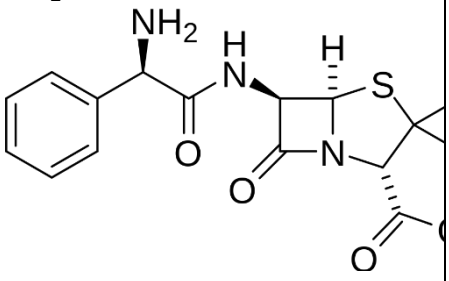
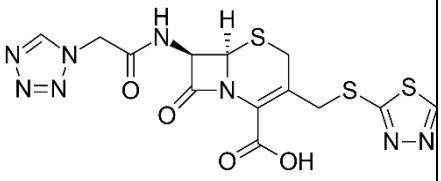
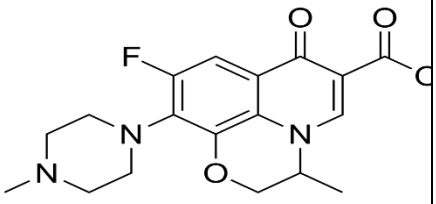
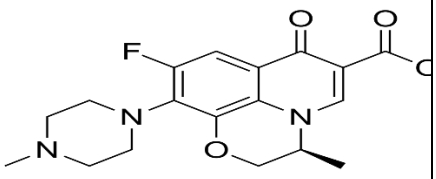
oxytetracycline 	In 1950s	**Pfizer laboratories**	Treatment infections of the respiratory tracturinary tract, soft tissues, and skin.
ampicillin 	In 1961s	 **Alexander Fleming**	Ampicillin is a medication used to manage and treat certain bacterial infections.
cefazolin 	In 1971s	**Boehringer Ingelheim and Eli Lilly and Company**	Treatment bacterial infections in many different parts of the body.
ofloxacin 	In 1985s	**Daiichi Seiyaku.**	Treatment infectionsof pneumonia, and infections of the skin, bladder, reproductive organs.
levofloxacin 	In 1993s	**Daiichi Pharmaceutica in JAPAn**	It is also used to treat anthrax infection after inhalational exposure.

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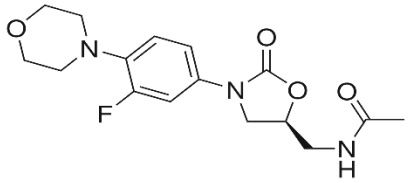
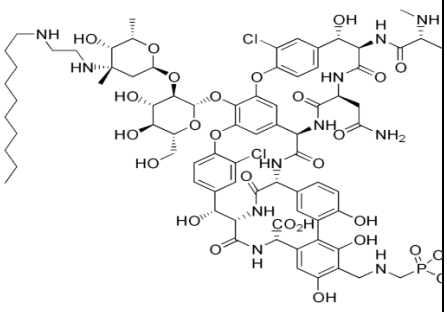
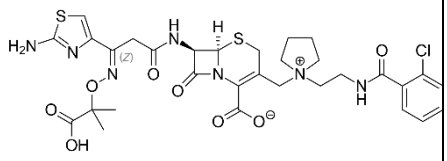
linezolid 	In 2000s	**Douglas Hutchinson, and Dr. Michael Barbachyn**	Treatment bacterial infection of skin infections or pneumonia.
Telavancin 	In 2009s	**Cumberland Pharmaceuticals**	It is also used alone or with other medications to treat certain types of pneumonia.
cefiderocol 	In 2019s	**Shionogi & Co., Ltd., Japan.**	It is also used to treat pneumonia in adults who are on ventilators or who were already in a hospital

CLASSIFICATION

Antibiotics are classified on the basis of their mechanism of action and by itschemical nature.

Classification Based on Mechanism of Action

1. Agents that inhibit the synthesis of bacterial cell wall: These include the **penicillins** and cephalosporins that are structurally similar and dissimilar agents,
such as cycloserine, vancomycin, bacitracin and the imidazole antifungal agents.

2. Agents that act directly on the cell membrane of the microorganisms, affecting permeability, and leading to leakage of intracellular compounds: These includepolymyxin, polyene antifungal agents, nystatin, and amphotericin B that bind to cell wall sterols.

3. Agents that affect the function of 30s and 50s ribosomal subunits to cause reversible inhibition of protein synthesis: These include tetracyclines, erythromycins, chloramphenicol, and clindamycin.

4. Agents that bind to the 30s ribosomal subunit and alter protein synthesis: These include aminoglycosides that leads to cell deaths eventually.

5. Agents that affect nucleic acid metabolism: Such as rifamycins, which inhibitDNA dependent RNA polymerase.

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Classification Based on Chemical Structure

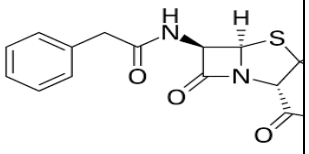
1. β-lactam antibiotics
2. Aminoglycoside antibiotics
3. Tetracycline antibiotics
4. Polypeptide antibiotics
5. Macrolide antibiotics
6. Lincomycins
7. Other antibiotics

1. β-lactam antibiotics

β-Lactam antibiotics are currently the most used class of antibacterial agents in the infectious disease armamentarium. As shown in Figure 1, β-lactams account for 65% of all prescriptions for injectable antibiotics in the United States. Of the β-lactams, cephalosporins comprise nearly half of the prescriptions (Table 1). The β-lactams are well tolerated, efficacious, and widely prescribed. Their major toxicity is related to an allergic response in a small percentage of patients who react to related side chain determinants; notably, these reactions are most common with penicillins and cephalosporins with minimal reactivity caused by monobactams (Saxon *et al*. 1984; Moss *et al*. 1991). The bactericidal mechanism of killing by β-lactams is perceived to be a major advantage in the treatment of serious infections. When these agents were threatened by the rapid emergence of β-lactamases, β-lactamase-stable agents were developed, as well as potent β-lactamase inhibitors (BLIs

Mechanism of Action - β-Lactam antibiotics are bactericidal agents that interrupt bacterial cell-wall formation as a result of covalent binding to essential penicillin-binding proteins (PBPs), enzymes that are involved in the terminal steps of peptidoglycan cross-linking in both Gram-negative and Gram-positive bacteria.

A.Penicillin derivatives

Name	Marketing Example	Route of Administration	Approval Date	Uses
1.Benzylpenicilline 		IM or IV	1946	septicaemia, meningitis, pericarditis, endocarditis and severe pneumonia.

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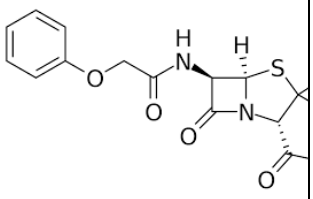
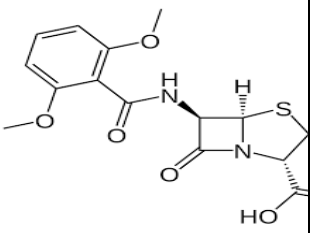
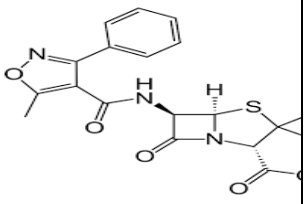
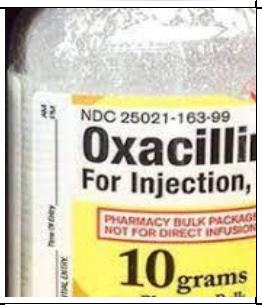
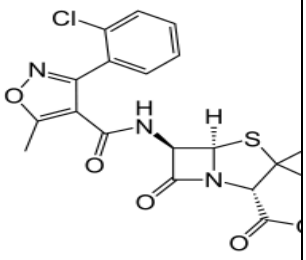
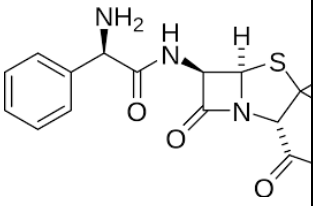
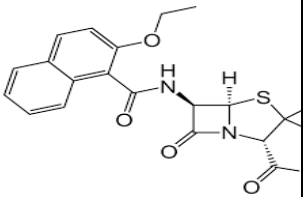
2.Pheoxymethylpenicilline 		ORAL	1968 ss	It's used to treat bacterial infections, including ear, chest, throat and skin infections.
3.Methicillin 		IV	1960	skin structure infections, osteomyelitis, and endocarditis.
4.Oxacillin 		ORAL, IV	1962	Oxacillin injection is used to treat infections caused by certain bacteria.
5.Cloxacillin 		ORAL, IV	1974	Cloxacillin is indicated for the treatment of beta-hemolytic streptococcal, pneumococcal, and staphylococcal infections.
6.Amphicllin 		ORAL, IV	1963	treatment of infection Respiratory, GI, UTI and meningitis.
7.Nafcillin 		IV	1970	used to treat moderate-to-severe bacterial infections caused by penicillinase-producing bacteria.

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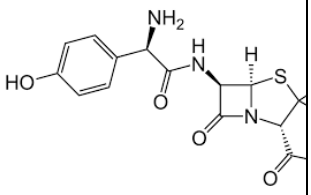
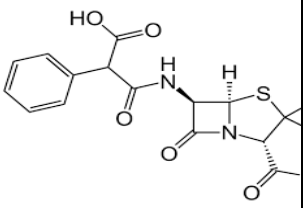
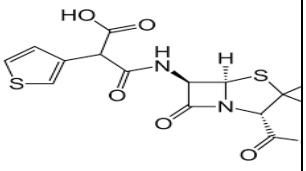
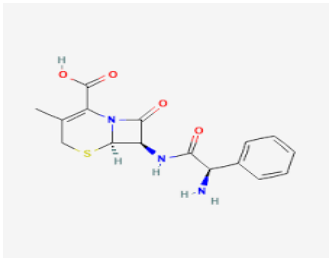
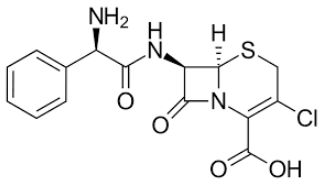
8.Amoxicillin 		ORAL, IV	1972	It is used to treat bacterial infections, such as chest infections (including pneumonia) and dental abscesses.
9.Carbenicillin 		ORAL	1972	Carbenicillin is used for the treatment of urinary tract infections when oral therapy is needed and other antibiotics are ineffective.
10.Tecarcillin 		IV	1976	treat moderate-to-severe infections due to susceptible organisms.

B. Cephalosporins.

1.Cephalexin 		Oral	1971	Cephalexin is used upper respiratory infections, ear infections, skin infections, urinary tract infections and bone infections.
2.Cefaclor 		Oral	1979	Cefaclor is used to treatment of such as pneumonia and other lower respiratory tract (lung)

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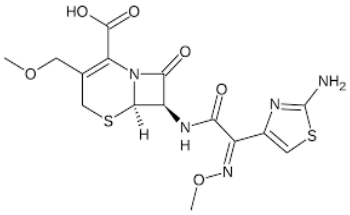
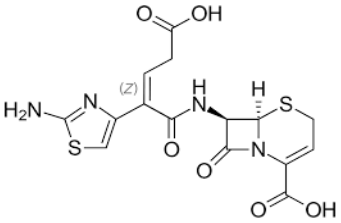
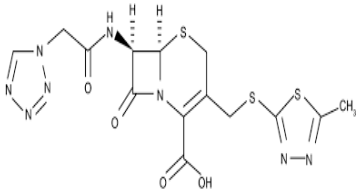
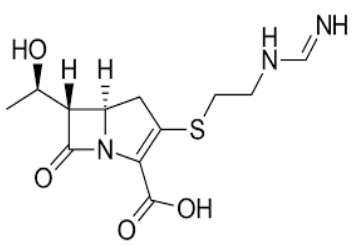
				infections; and infections of the skin, ears, throat, tonsils, and urinary tract.
3.Cefpodoxime 		Oral	1992	Cefpodoxime is used to treatment ofbronchitis ;pneumonia; gonorrhea ; and infections of the skin, ear, sinuses.
4.Ceftibutin 		Oral	1995	Indicatedforthe treatment of acute bacterial exacerbations of chronic bronchitis (ABECB), acute bacterial otitis media, pharyngitis,and tonsilitis.
5.Cefazolin 		IV	1973	Mainly used to treat bacterial infections of the skin.

C.Carbapenems.

1.Imipenem 		**IV**

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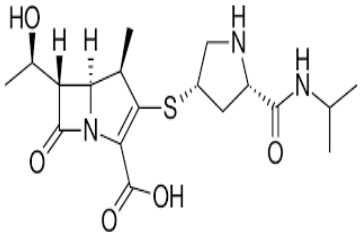
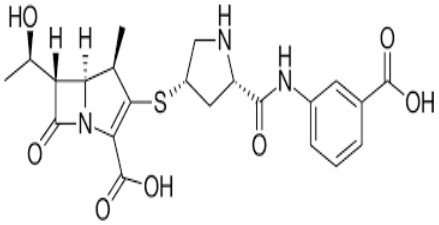
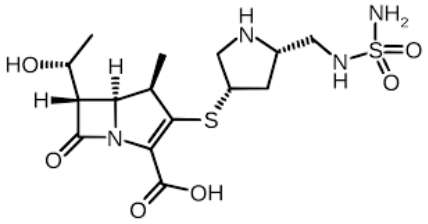
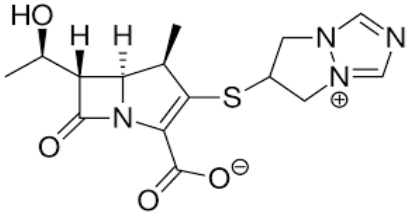
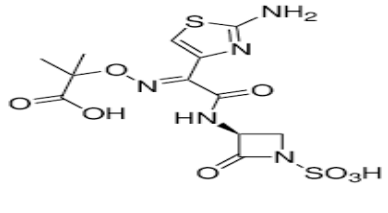
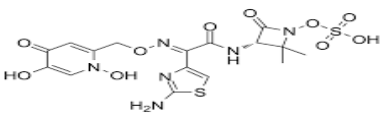
2.Meropenem 		IV
3.Ertapenem 		IV
4.Doripenem 		IV and IM
5.Biapenem 		IV

D. Mono cyclic Bete lactams

1.Aztreonam monobactam  11, Aztreonam		IM, IV
2.BAL30072 monosulfctam 		Topically

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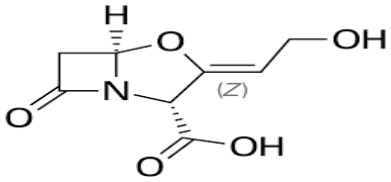
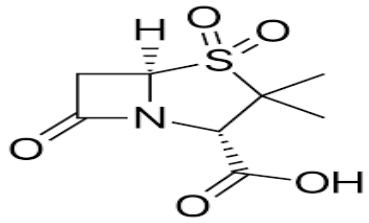
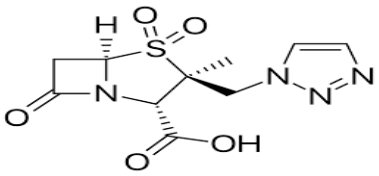
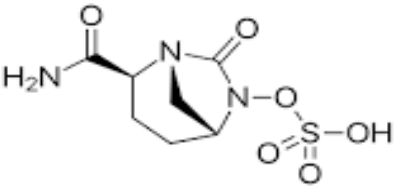
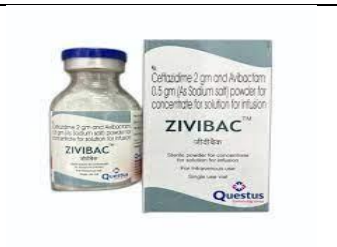
E.Bete Lactams Inhibitors.

1.Clavunalic Acid 		It is use in conjunction with amoxicillin to treat certain bacterial infections.
2.Sulbactam 		Sulbactam is currently available in combination products with ampicillin. Used to various type infection.
3.Tazobactam 		Piperacillin and tazobactam is used to treat pneumonia and skin,gynecological, and abdominalinfections.
4.Avibactam 		The combination of ceftazidime and avibactam injection is used with metronidazole to treat abdominal infections.

2.Aminoglycosides

Aminoglycosides are potent, broad-spectrum antibiotics that act through inhibition of protein synthesis. The class has been a cornerstone of antibacterial chemotherapy since streptomycin wasfirst isolated from Streptomyces griseus and introduced into clinical use in 1944. Several other members of the class were introduced over the intervening years including neomycin , kanamycin , gentamicin , netilmicin , tobramycin , and amikacin . A shift away from systemic use of the class began in the 1980s with the availability of the third generation cephalosporins, carbapenems, and fluoroquinolones, which were perceived to be less toxic and/or provide broader coverage than the aminoglycosides. However, increasing resistance to these classes of drugs, combined with more extensive knowledge of the basis of aminoglycoside resistance, has led to renewed interest in the legacy aminoglycosides and the development of novel aminoglycosides such as arbekacin and plazomicin . These latter agents were designed to

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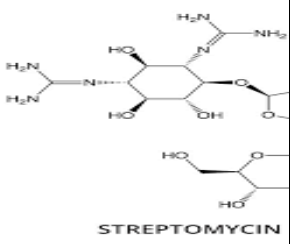
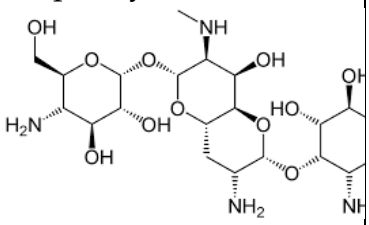
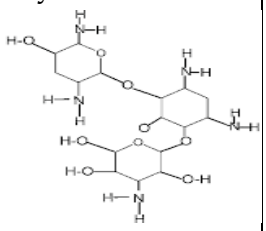
overcome common aminoglycoside resistance mechanisms thereby maintaining potency against multidrug-resistant (MDR) pathogens.

Machanism of Action - Aminoglycosides inhibit protein synthesis by binding, with high affinity, to the A-site on the 16S ribosomal RNA of the 30S ribosome. Although aminoglycoside class members have a different specificity for different regions on the A-site, all alter its conformation.

1.Streptomycin 		IM, IV	1944	treatment ofmycobactermtuberculosis,it is now largely a second line option due to resistance and toxicity.
2.Apramycin 		Oral	1940	Treatment of bacterial infections in animals.s
3.Tobramycin 		Drop	It was patented in 1965, and approved for medical use in 1974.	treat various types of bacterial infections, particularly Gram-negative infections.

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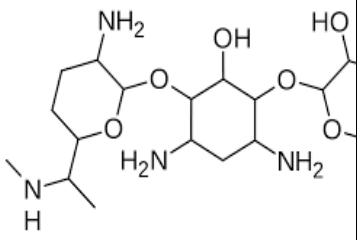
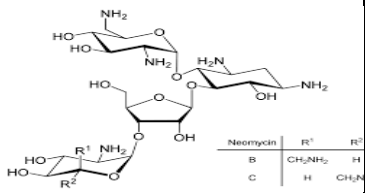
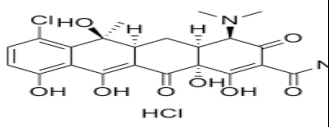
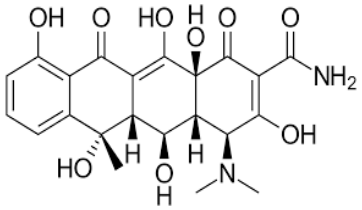
4.Gantamycin 		IV, IM	1963	Used for moderate-to-severe bacterial infections caused by sensitive agents, primarily gram negative bacteria.
5.Neomycin 		Topically	1949	Neomycin is a medication used to treat and manage hepatic coma and perioperative prophylaxis.

3.Tetracycline antibiotics

Tetracyclines were discovered in the 1940s and exhibited activity against a wide range of microorganisms including gram-positive and gram-negative bacteria, chlamydiae, mycoplasmas, rickettsiae, and protozoan parasites. They are inexpensive antibiotics, which have been used extensively in the prophlylaxis and therapy of human and animal infections and also at subtherapeutic levels in animal feed as growth promoters.

Mochanism of Action - It is well established that tetracyclines inhibit bacterial protein synthesis by preventing the association of aminoacyl-tRNA with the bacterial ribosome (44, 263). Therefore, to interact with their targets these molecules need to traverse one or more membrane systems depending on whether the susceptible organism is gram positive or gram negative.

1.7-chlortetracycline 		Oral	1948	In veterinary medicine, chlortetracycline is commonly used to treat conjunctivitis in cats, dogs and horses.
2.5-  Hydroxytetracycline		Oral, IV	1948	treat infections caused by bacteria including pneumonia and other respiratory tract infections

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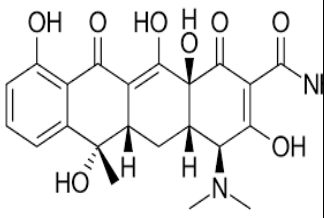
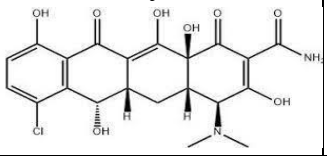
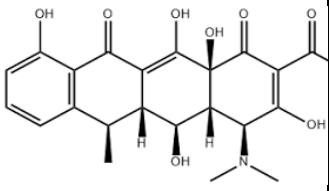
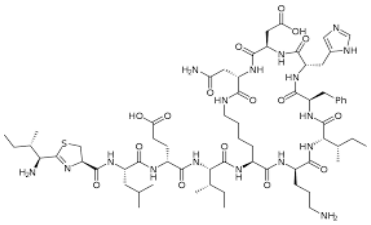
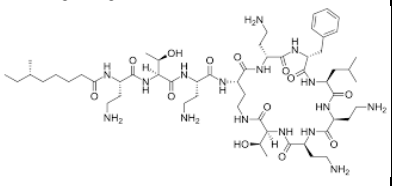
3.Tetracycline 		Oral	1953	Tetracyclin is used to treat infections caused by bacteria including pneumonia and other respiratory tract infections.
4.6-demethyl-7-chlortetracycline 		Oral	1957	treat infections caused by bacteria including pneumonia and other respiratory tract infections
5.6-Deoxy-5-hydroxytetracycline 		Oral, IV	1967	Treatment of infections caused by bacteria including pneumonia and other respiratory tract infections

4.**Polypeptide antibiotics**

The compounds have complex polypeptide structure. These are resistant to animal and plant proteases. These contain lipid moieties besides amino acids that are notfound in peptides of animal and plant origins. Examples: bacitracin, polymycin,amphomycin, tyrothricin, and vancomycin.

1.Bacitracin 		Widely used by both medical professionals and the general public to treat minor skin injuries, including cuts, scrapes, and burns.
2.**Polymycin** 		Treat minor wounds (such as cuts, scrapes, burns) and to help prevent or treat mild skin infections.

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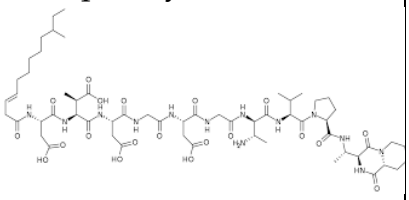
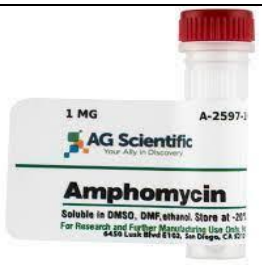
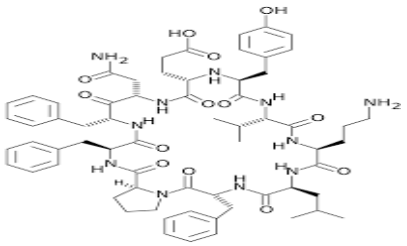
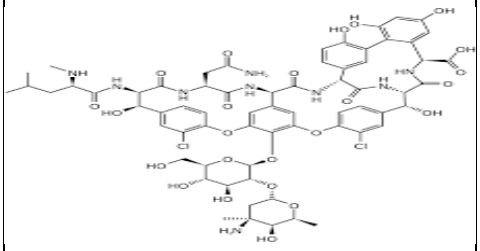
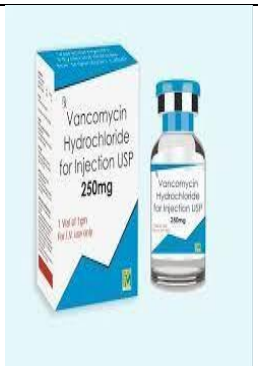
3.Amphomycin 		amphomycin was shown having anti-fungal activity
4.Tyrothricin 		It is used in sore throat medications and in agents for the healing of infected superficialand small-area wounds.
5.Vancomycin 		It is not work for colds, flu, or other virus infections. Vancomycin injection is also used to treat serious infections for which other medicines may not work.

5. MACROLIDE ANTIBIOTICS

The macrolide antibacterial agents are extremely useful chemotherapeutic agents forthe treatment of a variety of infectious disorders and diseases caused by a host ofgram-positive bacteria, both cocci and bacilli; they also exhibit useful effectivenessagainst gram-negative cocci, specially, neisseria spp. The macrolides are commonlyadministered for respiratory, skin, tissue, and genitourinary infections caused bythese pathogens.Examples: erythromycin, oleandomycin, clarithromycin, flurithromycin,dirithomycin, azithromycin.

Mode of action:

Macrolide antibiotics are bacteriostatic agents that inhibit protein synthesis bybinding irreversibly to a site on the 50S subunits of the bacterial ribosome. Thus,inhibiting the translocation steps of protein synthesis at varying stages of peptidechain elongation (hinder the translocation of elongated peptide chain back from 'A'site to 'P' site). The macrolides inhibit ribosomal peptidyl transferase activity. Somemacrolides also inhibit the translocation of the ribosome along with the mRNAtemplate.

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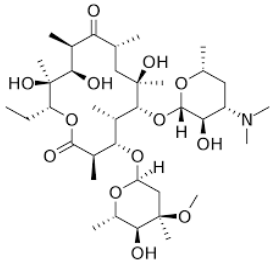
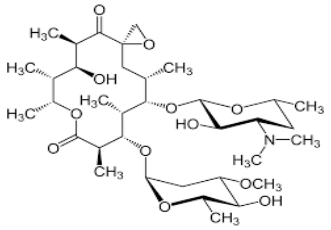
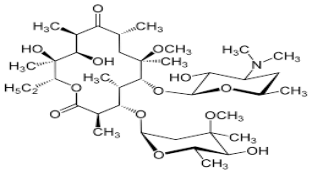
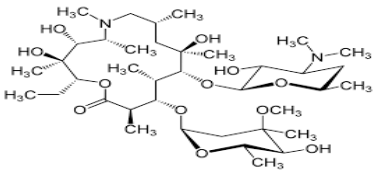
1.Erythromycin 		It is used to respiratory tract infections, skin infections, diphtheria, intestinal amebiasis,acutepelvic inflammatory disease, Legionnaire's disease, pertussis, and syphilis.
2.Oleandomycin 		Oleandomycin inhibits the bacteria responsible for upper respiratory tract infections.
3.Clarithromycin 		It is also used in combination with other medicines to treat duodenal ulcers caused by H. pylori. This medicine is also used to prevent and treat Mycobacterium avium complex (MAC) infection.
5.Azithromycin 		such as bronchitis; pneumonia; sexually transmitted diseases (STD); and infections of the ears, lungs, sinuses, skin, throat, and reproductive.

7. OTHER ANTIBIOTICS

Examples of other antibiotics are chloramphenicol, rifampicin and mupirocin.Chloramphenicol or chloromycetin Chloramphenicol has a spectrum of activityresembling that of the tetracyclines except that it exhibits a bit less activity againstsome gram-positive bacteria. It is isolated from Salmonella venezuelae by Ehrlich etal in 1947. It contains chlorine and is obtained from an actinomycete, and thus,named aschloromycetin. It is specifically recommended for the treatment of seriousinfections caused by H. influnzae, S. typhi (typhoid), S. pneumoniae, and N.meningitides. Its ability to penetrate into the CNS presents an alternative therapy formeningitis and exhibits antirickettsial activity.

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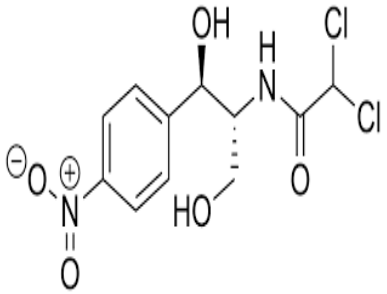
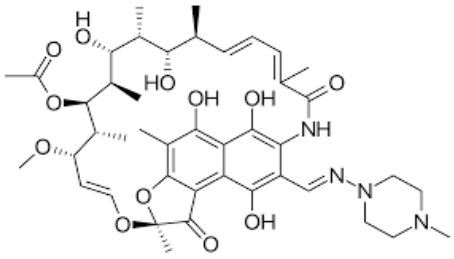
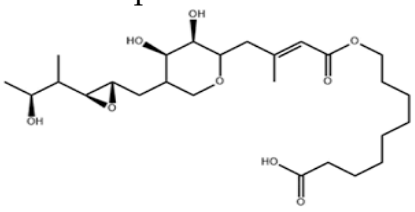
1.Chloramphenicol 		medication used in the management and treatment of superficial eye infections such as bacterial conjunctivitis, and otitis externa.
2.Rifampicin 		treatment several types of mycobacterial infections including Mycobacterium avium complex, leprosy.
3.Mupirocin 		Mupirocin, an antibiotic, is used to treat impetigo as well as other skin infections caused by bacteria.

CONCLUSION

A recent analysis showed that there are five new antibacterial drugs in development by large pharmaceutical companies. This analysis does not evaluate drugs under development by biotechnology firms. At the present time, it is the experience at the FDA that more biotechnology firms are becoming involved in drug development of antimicrobials. This would seem to be the nature of capitalism; as one group decides to exit a given area, another group perceives an opportunity. At recent FDA meetings, members of biotechnology firms emphasised that the smaller markets of serious and life-threatening diseases are more attractive to smaller firms who can survive with smaller profits.Some have questioned whether these firms will have the necessary resources to perform clinical trials to develop new drugs.

REFERENCES

Maekawa T, Tamura H, Domon H, Hiyoshi T, Isono T, Yonezawa D, Hayashi N, Takahashi N, Tabeta K, Maeda T, Oda M, Ziogas A, Alexaki VI, Chavakis T, Terao Y, Hajishengallis G. Erythromycin inhibits neutrophilic inflammation and mucosal disease by upregulating DEL-1. JCI Insight. ;5(15), 2020

1. Hutchings, MI., Antibiotics: past, present and future. Current Opinion in Microbiology, 2019.

ISBN : 978-81-966183-3-9

2. Marchant JM, Petsky HL, Morris PS, Chang AB. Antibiotics for prolonged wet cough in children. Cochrane Database Syst Rev. ;7(7), 2018.
3. Wakabayashi H, Ogawa Y, Machida A, Endo M, Tamai T, Sakurai S, Hibino S, Mikawa T, Watanabe Y, *et al*., Validation of cefazolin as initial antibiotic for first upper urinary tract infection in children. Global Ped Health 3: 1–7, 2016
4. Guo MT, Yuan QB, Yang J. Insights into the amplification of bacterial resistance to erythromycin in activated sludge. Chemosphere.;136:79-85, 2015
5. Kaushik D, Mohan M, Borade DM, Swami OC. Ampicillin: rise fall and resurgence. J Clin Diagn Res. (5):ME01-3, 2014

6. Camilleri M, Parkman HP, Shafi MA, Abell TL, Gerson L., American College of Gastroenterology. Clinical guideline: management of gastroparesis. Am J Gastroenterol, 108(1):18-37, 2013
7. Liang JH, Han X. Structure-activity relationships and mechanism of action of macrolides derived from erythromycin as antibacterial agents. Curr Top Med Chem. ;13(24):3131-64, 2013
8. Schmidt N, Gans EH. Tretinoin: A Review of Its Anti-inflammatory Properties in the Treatment of Acne. J Clin Aesthet Dermatol.;4(11):22-9, 2011
9. Aminov RI, A Brief History of the Antibiotic Era: Lessons Learned and Challenges for the Future. Frontiers in Microbiology 2010.
10. Varaldo PE, Montanari MP, Giovanetti E. Genetic elements responsible for erythromycin resistance in streptococci. Antimicrob Agents Chemother.;53(2):343-53, 2009
11. Babic M, Hujer AM, Bonomo RA., What's new in antibiotic resistance? Focus on β-lactamases. Drug Resist Updat 9: 142–156, 2006.
12. Tenson T, Lovmar M, Ehrenberg M. The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. J Mol Biol, ;330(5):1005-14, 2003
13. Amsden GW. Erythromycin, clarithromycin, and azithromycin: are the differences real? Clin Ther. ;18(1):56-72; 1996
14. Wilson WR, Karchmer AW, Dajani AS, Taubert KA, Bayer A, Kaye D, Bisno AL, Ferrieri P, Shulman ST, Durack DT. Antibiotic treatment of adults with infective endocarditis due to streptococci, enterococci, staphylococci, and HACEK microorganisms. American Heart Association. JAMA.;274(21):1706-13, 1995
15. Biggs BA, Kucers A. Penicillins and related drugs. Med J Aust. 1-15;145(11-12):607-11, 1986
16. Aronoff SC, Jacobs MR, Johenning S, Yamabe S., Comparative activities of the β-lactamase inhibitors YTR 830, sodium clavulanate, and sulbactam combined with amoxicillin or ampicillin. Antimicrob Agents Chemother 26: 580–582, 1984.
17. Ashford WA, Golash RG, Hemming VG., Penicillinase-producing Neisseria gonorrhoeae. Lancet 308: 657–658, 1976.

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SYNTHETIC DRUG DESIGNING APPROACH IN VACCINE PRODUCTION TECHNOLOGY

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ABSTRACT

The vaccines sector has struggled to stay afloat and has seen major corporations pull out of production due to the lack of significant technological advancements in decades. In the meanwhile, viral illness outbreaks have not decreased, even if the biopharmaceuticals sector is talking about cutting back. This is in line with the dispersed manufacturing approach, and the development of synthetic biology holds great potential for the creation of vaccines. Modern engineering is typified by the separation of design and manufacture through bio foundries. Unlike physically transferring cold-chain-dependent vaccines, digital code developed in a bio foundry may be transmitted to a small-scale production facility near the point of care. Digital biology and information systems will therefore be the foundation of a new age of bio manufacturing, which might be enabled via bio foundries and distributed manufacturing. This approach to managing pandemics and outbreaks in the future appears more sensible.

Keywords: Vaccines, synthetic biology, distributed manufacturing, bio foundry, point of care

INTRODUCTION

The COVID-19 pandemic has brought the vaccine business, which has been quietly struggling for decades, to light [1]. The amount of money spent on vaccinations in the United States "appears to be insignificant compared with that spent on other medical and social interventions that may have lesser social benefits," according to a 2003 National Academy of Sciences study [2]. Large vaccine-supplying companies have become fewer in number [3] five multinational corporations account for around 80% of all vaccine sales. Tension between the vaccine industry's comparatively low financial returns and the significant expenditures associated with production and research and development is at the root of the issue [4]. Capital-intensive centralized vaccination manufacturing facilities are not as common as small molecule medications [5]. In particular, fixed costs for novel vaccinations are considerable [6]. For instance, the requirement for eggs in production dates back at least 70 years, is costly and time-consuming, and is challenging to substitute [7]. High-income nations have historically paid more up until the fixed expenses are amortized. After covering the fixed costs, vaccinations have become more affordable, leading lower-income nations to embrace them. Vaccine sales generate more money in wealthier nations, although they also occur in lower- and middle-income nations in larger volumes. However, over half of the vaccines used in vaccination campaigns

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are currently supplied by vaccine makers in underdeveloped nations [8, 9], therefore this project is not in need of being launched from scratch. Final production could take place in a number of locations, provided they adhere to regulations, including modified university laboratories, laboratories at university teaching hospitals, and small company facilities at science parks and medical campuses. This is because a large number of doses can be produced from small volumes of product.

Instead of producing vaccinations in tonnes, nucleic acid vaccines would be generated in kilograms. Compared to a facility handling viral particles, an mRNA vaccine may offer a highly scalable, low-cost, cell-free, egg-free manufacturing method [10]. The actual manufacturing procedure can be quite straightforward, involving only a few standardized molecular biology stages, HPLC purification, and the final medication formulation [11]. The process is finished there when the ribosomes containing the immunogenic protein are delivered to the patient and come into contact with the host's immune system. Despite being oversimplified, contrast this with typical vaccination production procedures, which may entail hundreds of intricate stages. Production should be located as near to the end user as possible to maximize value from an economic standpoint, especially when it comes to emergency response and preparation [12]. It almost eliminates the requirement for a cold chain, which is vulnerable to logistical errors and temperature fluctuations [13]. Most significantly, a modest manufacturing plant near an outbreak location may be able to bridge the gap between regular production and an unexpected demand at the start of an outbreak, quickly building a stockpile [14]. Another important benefit of creating and producing mRNA vaccines is that they are a completely synthetic platform approach [15] for a variety of targets [11]. This will be a crucial component in developing an economically sustainable sector in the future. The process of optimising the product for the subsequent viral outbreak may then be quicker.

SYNTHETIC BIOLOGICAL APPROACH IN VACCINE PRODUCTION TECHNOLOGY

The technology used in vaccines has to be updated because it is decades old. One of the issues is that vaccine manufacture—particularly when it comes to whole cell synthesis—has shown to be less predictable and less standardized than the creation of many non-biological medications, which is accomplished using standardized chemical engineering techniques. Molecular design and strain engineering must be used to save costs in order to defy the economies-of-scale approach for vaccine production [16]. The burgeoning synthetic biology sector is well-suited to thrive here [17]. Synthetic biology's methodical workflow methodology would help with vaccine manufacturing [18]. It is possible to optimise mRNA technology by creating nearly infinite combinations of derivatives [11]. Similarly, the biofoundry provides a clear means of producing these mixtures. Biofoundries are becoming more and more involved in a systematic process. Biofoundries are highly automated establishments that utilise a large number of coordinated laboratory robots that are configured to carry out certain activities in accordance with a workflow [19]. Different platforms in biofoundries often carry out distinct activities, such as liquid handling, genetic assembly, and characterization. Because biofoundries are built on

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information infrastructures, their robots and other machinery may be programmed to follow intricate, multi-step processes [20].

A new branch of biology called "digital biology," which has the potential to completely transform the manufacture of vaccines and many other applications in biomedicine, is emerging quickly because to the combination of biofoundries and biodesign tools (BioCAD). This strategy aligns with dispersed manufacturing and supply networks, which might revolutionise global response to COVID-19 and other pandemics. Crone and colleagues [21] showcased the approach's potential by demonstrating the rapid deployment and scaling of an automated SARS-CoV-2 clinical diagnostics platform that was created in a biofoundry. Software enables the quick optimisation of industrial production design by enabling the simultaneous study of several experimental parameters or variables. Publicly financed biofoundries across North America, Europe, Asia, and Australia are part of the recently established Global Biofoundries Alliance [22] and might be utilised to help with the development of new vaccines as needed. Crone and colleagues [21] noted that members of the Global Biofoundry Alliance can swiftly incorporate and alter the procedures and workflows they created as needed.

DOWN STREAM OF SYNTHETIC BIOLOGY

Dispersed operations are well-suited for the vast number of doses of mRNA vaccines that may be produced from minuscule quantities. If a bioprocess is still needed, it may be made smaller and less intrusive by intensifying the cell culture process through feed batch [13] and, eventually, continuous manufacture [23]. Single-use disposable culture systems, which may be constructed faster than hard-pipe facilities and drastically decrease fixed costs, are a great option for smaller footprint operations [24]. Chemical engineers already have many of the instruments needed for bioprocess intensification, such as simplified machinery for quick process development and integration [25]. Having stated that, process robustness and standardisation are crucial for ensuring the product's quality and consistency. The interoperability of the hardware and software in various bio foundries and dispersed production units will be a necessary component of product standardization.

SUSTAINABILITY

Several nations might host final manufacture in reduced-scale production facilities, providing worldwide coverage and enabling manufacturing to be closer to the point of care. The Sustainable Development Goals (SDGs) of the United Nations specifically mention the need for accessible vaccinations. There is proof that the pharmaceutical sector produces a lot more pollutants than the car industry does in terms of its environmental impact [26]. The biofoundries, where the initial selection and design of vaccines are done, can be located anywhere, as long as they are kept apart from the production process. In this instance, the information is being delivered rather than the vaccine. This reduces costs and emissions, lessens the chance of cold chain breakdowns, and expedites the innovation process.

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CONCLUSION

We need a dramatic shift from the conventional methods of epidemic prediction, monitoring, prevention, and treatment both during and after COVID-19. Significant worldwide public and private money will be needed for it. Delivering radical deviations may seem costly and complicated, especially if global and democratic vaccine manufacturing is to be achieved, preventing vaccine nationalism [27, 28], and prioritising a shared plan above national self-interest [29]. But given the enormous human cost of COVID-19, the World Economic Forum's projection of $1 trillion in economic loss in 2020 alone, and the United Nations' estimate of 130 million additional people living in severe poverty, it won't seem pricey at all [30].

REFERENCES

1. Mullard, A. (2020) COVID-19 vaccine development pipeline gears up. Lancet 395, 1751–1752.

2. National Academy of Sciences (2003) Financing Vaccines in the 21st Century: Assuring Access and Availability, National Academies Press.

3. Offit, P.A. (2005) Why are pharmaceutical companies gradually abandoning vaccines? Health Aff. 24, 622–630.

4. Plotkin, S. *et al*. (2017) The complexity and cost of vaccine manufacturing – an overview. Vaccine 35, 4064–4071.

5. Nguyen, A. and Schwalbe, N. (2019) Apples and oranges? Can second generation vaccines become as low cost as generic medicines? Vaccine 37, 2910–2914.

6. Danzon, P.M. and Pereira, N.S. (2011) Vaccine supply: effects of regulation and competition. In NBER Work. Pap. Ser. No. 17205.

7. Trombetta, C.M. *et al*. (2019) Challenges in the development of egg-independent vaccines for influenza. Expert Rev. Vaccines. 18, 737–750.

8. Mao, H.H. and Chao, S. (2020) Advances in vaccines. Adv. Biochem. Eng. Biotechnol. 171, 155–188.

9. Munira, S.L. *et al*. (2019) A cost analysis of producing vaccines in developing countries. Vaccine 37, 1245–1251.

10. Maruggi, G. *et al*. (2019) mRNA as a transformative technology for vaccine development to control infectious diseases. Mol. Ther. 27, 757–772.

11. Jackson, N.A.C. *et al*. (2020) The promise of mRNA vaccines: a biotech and industrial perspective. npj Vaccines 5, 11.

12. Harrison, R.P. *et al*. (2018) Decentralised manufacturing of cell and gene therapy products: Learning from other healthcare sectors. Biotechnol. Adv. 36, 345–357.

ISBN : 978-81-966183-3-9

13. Xu, J. *et al*. (2020) Development of an intensified fed-batch production platform with doubled titers using N-1 perfusion seed for cell culture manufacturing. Bioresour. Bioprocess. 7, 17.

14. Zhang, C. *et al*. (2019) Advances in mRNA vaccines for infectious diseases. Front. Immunol. 10, 594.

15. Rauch, S. *et al*. (2018) New vaccine technologies to combat outbreak situations. Front. Immunol. 9, 1963.

16. National Academies of Sciences, Engineering, and Medicine (2019) Continuous Manufacturing for the Modernization of Pharmaceutical Production: Proceedings of a Workshop, National Academies Press.

17. Pedrolli, D.B. *et al*. (2019) Engineering microbial living therapeutics: the synthetic biology toolbox. Trends Biotechnol. 37, 100–115.

18. National Academies of Sciences, Engineering, and Medicine (2016) Deriving Drug Discovery Value from Large-Scale Genetic Bioresources: Proceedings of a Workshop, National Academies Press.

19. Kitney, R. *et al*. (2019) Enabling the advanced bioeconomy with engineering biology. Trends Biotechnol. 37, 917–920.

20. Chao, R. *et al*. (2017) Engineering biological systems using automated biofoundries. Metab. Eng. 42, 98–108.

21. Crone, M.A. *et al*. (2020) A role for biofoundries in rapid development and validation of automated SARS-CoV-2 clinical diagnostics. Nat. Commun. 11, 4464.

22. Hillson, N. *et al*. (2019) Building a global alliance of biofoundries. Nat. Commun. 10, 2040.

23. Jungbauer, A. and Hammerschmidt, N. (2017) Integrated continuous manufacturing of biopharmaceuticals. In Continuous Manufacturing of Pharmaceuticals (Kleinebudde, P. *et al*., eds.), Wiley Online Library.

24. Black, S. (2020) Need for Efficiency, COVID-19 Vaccine Spurs Biomanufacturing in 2020, The Science Advisory Board.

25. Strube, J. *et al*. (2018) Process intensification in biologics manufacturing. Chem. Eng. Process. Process Intensif. 133, 278–293.

26. Belkhir, L. and Elmeligi, A. (2019) Carbon footprint of the global pharmaceutical industry and relative impact of its major players. J. Clean. Prod. 214, 185–194.

27. Kupferschmidt, K. (2020) 'Vaccine nationalism' threatens global plan to distribute COVID-19 shots fairly. Science Published online July 28, 2020. https://doi.org/10.1126/science.abe0601.

ISBN : 978-81-966183-3-9

28. Weintraub, R. *et al*. (2020) The danger of vaccine nationalism. Harvard Bus. Rev. May 22, 2020.

29. National Academies of Sciences, Engineering, and Medicine (2020) Discussion Draft of the Preliminary Framework for Equitable Allocation of COVID-19 Vaccine, National Academies Press.

30. United Nations Conference on Trade and Development (2020) Impact of the COVID-19 Pandemic on Trade and Development. Transitioning to a New Normal. UNCTAD/OSG/2020/1, United Nations Publications.

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ROLE OF MICROBIAL SYMBIONTS IN AGRICULTURE

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The major goal of agricultural microbiology is a comprehensive analysis of beneficial microorganisms. Fundamental knowledge of the ecology and evolution of interactions could enable the development of microbe-based sustainable agriculture. Plant growth-promoting bacteria (PGPB) have gained worldwide importance and acceptance for their agricultural benefits. This is due to the emerging demand to reduce dependence on synthetic chemical products within a holistic vision of developing and focalizing environmental protection. Beneficial microorganisms also help to solubilize mineral phosphates and other nutrients, enhance resistance to stress, stabilize soil aggregates, improve soil structure and organic matter content, and inhibit phytopathogens. Several efforts have been made in research to clarify definitions as well as develop commercial inoculants using these organisms, with a special emphasis on formulations that interact synergistically and are currently being devised. In addition, numerous recent studies indicate increased crop performance with the use of these commercial inoculants. In this chapter, the progress to date in the use of beneficial microbes for agricultural applications is summarized and discussed.

Plant growth-stimulating rhizobacteria (PGPR) are the symbiotic soil-dwelling bacteria existed at the outer part of the plant root and participate for growth and improvement of the crops. Various regulatory substances are secreted by these bacteria in the circumstances of rhizospheric regions. Normally, PGPR mechanisms simplify the growth of a plant by fixing the nitrogen from atmospheric regions, dissolved the phosphorus and other raw materials, siderophores assembly which liquefy the appropriated iron, or controlling the phytohormones levels at numerous phases of growth (Badel *et al* (2011). When unplanned development of plant growth takes place, the activities of PGPR diminish or avoid the disastrous effect of one or more plant pathogens microbes in the form of biocontrol agents. Various researchers have been recognized to improve the fitness and proficiency of aquanaut's species of plants by using the growth-supporting rhizospheric bacteria under systematic and harassed circumstances. The advantageous rhizobacteria of the plant may reduce the comprehensive dependency on hazardous agronomic compounds which disrupt the agro-biota. This chapter emphasizes on the insight of the rhizospheric microbe which supports the growth of plant under the existing viewpoints. Conclusively, these favorable rhizospheric bacteria in various agro-biota have been offered scientifically under normal and stress circumstances to focus on current developments with the objectives to improve forthcoming visions (Yadav *et al* 2013).

Microorganisms colonize not only roots but also other plant tissues and organs including stems, leaves, flowers, seeds, and fruits. The aerial part of plants colonized by microbes is called the phyllosphere, whereas the rhizosphere is the soil adjacent to the root. In contrast to the rhizosphere, the above-ground parts of plants are scarce in water and nutrients. Only a small number of microorganisms that reach the surface of the plant will land on beneficial spots and

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will have conditions to survive. As a consequence, the number of microorganisms living in the rhizosphere is much higher than in the phyllosphere. Microbes are present at every stage of plant development, from seed to fully developed plant producing a new generation of seeds. Some microorganisms live on the surfaces of plant organs, i.e., epiphytes whereas others are able to colonize the internal tissues of plants, i.e., endophytes.

Plant growth-promoting microorganisms stimulate plant growth and development through various direct and indirect mechanisms. Production of phytohormones, nitrogen assimilation, solubilization, and mineralization of macro- and micro-elements, and modulation of the endogenous level of ethylene in plants tissues are examples of direct mechanisms. Examples of indirect mechanisms are inhibition of pathogens growth through antibiosis, secretion of lytic enzymes], and competition, e.g., via siderophores production, induction/inhibition of plant genes expression, induction of plant immune response, and manipulation of plant microbiome composition (Kohler *et al* 2007). This work aims to summarize the current knowledge about the interactions of plants with beneficial microbes, and how those interactions affect the overall health of the plant. For further development of environmental-friendly methods of plant cultivation, is it crucial to deeply understand the molecular mechanisms underneath (i) the recruitment of useful microbes by plants, (ii) the interactions among microorganisms, and (iii) the plant-microorganism interplay. The interactions between plants and microorganisms can be divided into three types, i.e., interactions are either neutral, negative, or positive in their effects on the host plant. This review focuses exclusively on positive interactions and mechanisms underneath those interactions. In this work, we also discuss the role of stringent responses in interactions between plants and microorganisms.

Biofertilizers

Meeting the projected demand for healthy and sustainable food production is a crucial challenge. Increasing crop productivity by mitigating climate change and preserving agroecosystems is one of the significant goals of sustainable agriculture. Sustainable agriculture has been defined as an alternative integrated approach that could be used to solve fundamental and applied issues ecologically related to food production. It integrates biological, physical, chemical, and ecological principles to develop new practices that are not harmful to the environment. Successful application of microbes helps in maintaining soil health, improving water holding capacity, carbon storage, root growth, availability and cycling of essential nutrients, filtering pollutants, and also in conservation of biodiversity (Pindi, 2012). Microbes in soil and overall soil health can be depleted by common agricultural practices; nevertheless, but this can be prevented by various ways of improving soil quality.

Integrated pest management (IPM), as one of the effective methods used in modern agriculture, takes into account all plant protection methods available in the application (Kloepper *et al* 2004). IPM defines the management of pests by reducing pest numbers to acceptable levels, taking into consideration protecting the environment, non-target organisms, and human health. IPM implies the integration of appropriate measures that minimize the risks for human health and the environment by preventing the development of pest populations and by ensuring the use of plant protection products and other forms of intervention at economic and ecologically

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justified and reduced levels. In insect pest management, several plant products derived from neem, custard apple, tobacco, pyrethrum, etc., have been used as safer insecticides. Compounds, such as limonene, pyrethrum / pyrethrins, rotenone, sabadilla, and ryania, are widely used across the globe to control fleas, aphids, mites, ants, roaches, ticks, beetles, caterpillars and thrips, squash bugs, harlequin bugs, thrips, etc.

Rhizosphere and Root Exudates

The rhizosphere is defined as "the field of action or influence of a root", i.e., it is soil adjacent to the roots which are influenced by root exudates that are the mixture of several compounds produced and secreted by roots. The main components of root exudates are water, enzymes, amino acids, nucleotides, vitamins, organic acids, fatty acids, sugars, phenolic compounds, anions, volatile organic compounds (VOCs), polysaccharides, and proteins

The rhizosphere is the hotspot of plant–microorganism interactions. Interactions between those organisms have a direct influence on the availability of soil nutrients for plants and on plant tolerance toward biotic and abiotic stresses. Exudates are a rich source of carbon and other nutrients and, therefore, the abundance of microorganisms in the rhizosphere can be up to a hundred times greater than in the bulk soil. Moreover, root exudates allow plants to communicate with rhizosphere microorganisms and affect their behavior through the secretion of various signaling molecules

Root exudates serve as a chemo attractant, by which plants "recruit" microorganisms. Several studies showed that the composition of root exudates has an enormous effect on the composition of plant microbiome (Sandhya *et al* 2009). The composition of root exudates is specific for each plant species, which enables plants to attract a particular set of microbes. Moreover, plants can secrete substrates that are available only for selected microbial groups or compounds that are toxic for certain groups of microorganisms in order to inhibit their growth

Symbiotic nitrogen fixation (SNF)

Symbiotic nitrogen fixation (SNF) is a mutualistic association between plants and microbes. The symbiotic nitrogen fixing microbes have the capability of fixing atmospheric nitrogen symbiotically and provided access to all types of plants. Mutualistic relationships begin once the plant starts to secrete flavonoids and iso-flavonoids in its rhizosphere, where it is identified by *Rhizobium*. *Rhizobium, Sinorhizobium, Bradyrhizobium,* and *Mesorhizobium* are a few examples of bacteria living symbiotically with leguminous plants, *Frankia* with non-leguminous plants and shrubs. Out of these symbiotic nitrogen fixing bacteria, *Rhizobium* is the leading cause of legume crops' symbiotic nitrogen fixation. Besides bacteria, some small fern is also working as symbiotic nitrogen fixers. For example, *Azolla* is a small, free-floating aquatic fern that collaborates with cyanobacteria (*Anabaena*) to fix atmospheric nitrogen. The appropriate environment, phytohormones, and nutrients are provided by Azolla to Anabaena in the interchange of fixed nitrogen. In *Anabaena,* the phenomenon of nitrogen fixation happens in heterocyst cell (Fernández *et al* 2012). *Azolla* contributes primarily to rice cultivation by fertilizing the soil with nitrogen and incorporating biomass. Actinomycetes, for example, Frankia can produce root nodules for the actinorhizal plants. *Frankia* can be nodulated by certain other genera, such as *Allocasuarina, Myrica, Eleagnus, Coriaria and Casuarina*. They are

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monocot plants with a promising future in agricultural and land reclamation. N is fixed by *Azotobacter* and *Bacillus* species, and they also help in the growth and development of maize plants and forest crops. Inoculation of *Bradyrhizobium japonicum* enhanced plant growth, nodulation, and N fixation in soybean (Kilian *et al* 2000).

Non - symbiotic or free living nitrogen fixation

Free-living nitrogen fixers are found in the root zone of plants and obtain food and nutrient from plants, and in favor of return fixed nitrogen under a free-living state. Non-symbiotic nitrogen fixation is also accomplished by diazotrophs that stimulate the development of non-leguminous plants such as rice and radish. Certain other rhizopheric bacteria that fall under the genus *Azotobacter, Burkholderia, Azoarcus, Azospirillum, Gluconacetobacter, Diazotrophicus, Pseudomonas, Enterobacter and Cyanobacteria* (*Anabaena, Nostoc*) that also act as non-symbiotic nitrogen fixers. Hungria *et al*. 2013, suggested that *Azotobacter chroococcum* can be utilized as a biofertilizer because of its capacity to fix 10 mg N/g of in-vitro-supplied carbon source. According to Galindo *et al*. *A. brasilense* lowers N fertilization, enhances plant nutrition, and boosts plant biomass and wheat grain yield.

Associative nitrogen fixing bacteria are *Herbaspirillum, Acetobacter, Azospirillum and diazotrophicus* which are accompanied by plant root cells of the gramineae family. *Azospirillum* is aerobic, non-nodulating, gram-negative, associative nitrogen-fixing bacteria living with C4 plants, such as maize, bajra, sugarcane, sorghum, and cereals like rice, barley, wheat. The inoculation of *Azospirillum* showed marked results in maize, sorghum, wheat, and other grass seedlings. According to Montanez *et al*., bacteria can provide up to 25% of rice and corn's overall nitrogen needs.

Plant Immune System in Plant–PGPM Interactions

The plant immune system plays a key role in plant–microorganism interactions. It is crucial not only for controlling pathogenic microorganisms but also for balancing the homeostasis of the microbiome and for overseeing commensal microbes. The prominent role in plant–PGPM interactions play patterns recognizing receptors (PRRs), which recognize conserved microorganisms-specific molecules referred to as pathogen-/microbe-associated molecular patterns (P/MAMPs), such as flagellin, lipopolysaccharides, antibiotics, and VOCs. PRRs are transmembrane multimeric protein complexes located at the plasma membranes present in all plant organs and tissues.

Biocontrol

Pathogenesis Related (PR) Enzymes production

PGPR act as effective biocontrol agents through the production of various hydrolytic enzymes that affect the growth of pathogens. These enzymes include chitinases, proteases, glucanases, cellulases, urease and catalase; most of which are effective against fungal pathogens. Most of the biocontrol microbial agents use to produce pathogenesis related enzymes i.e chitinase and b 1,3 glucanase for controlling the encounter microbes in order to inactivate and kill the harmful one. Since the fungal cell wall contains chitin as for cell structure integrity, once the chitinase enzyme is abundant, there will be high possible to disintegrate the chitin structure by which the harmful pathogenic fungal cell wall got collapsed.

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Siderophores production

Siderophores are small organic molecules that carry out antibiosis by supplying iron (Fe) to crops, consequently making pathogens impoverished of iron. Iron is a necessary mineral nutrient for plant development and growth and is needed as a protein cofactor used in metabolic phenomena like respiration and photosynthesis. Mathiyazhagan *et al*. reported that iron deficiency suppresses pathogen growth by obstructing main processes including sporulation and nucleic acid synthesis. PGPRs have evolved numerous iron absorption approaches to remain alive and adapted to their environment to solve this challenge and supply iron to the plant (Xavier 2009). The generation of siderophores is one of these strategies. Bacterial species such as *Pseudomonas* use the siderophores formed by other rhizosphere microbes to complete their iron requirements. Gouda *et al*. reported that *Pseudomonas putida* has the ability to utilize heterologous siderophores made by other microbes present in the root area to increase the iron level existent in the natural environment. Sarwar *et al*. reported that application of siderophore-producing Bacillus sp. enhances the plant growth of groundnut. The production of siderophore and antioxidant enzymes by *Pseudomonas koreensis* in maize plants prevented the development of plant pathogens. The available literature has confirmed that fluorescent Pseudomonas sp. generates two major types of siderophores viz pseudobactins and pyochellins. According to Battu and Reddy, siderophores are considered growth promoters of plants and biocontrolling agents of fungal diseases cognate with other crops. Therefore, it is crucial to clarify the role of siderophores produced by Pseudomonas strain B324 in preventing the pathogen *Pythium* which causes root rot disease in wheat.

Starter (Decomposer Microbes)

Microorganisms i.e.. Bacteria Actinomycetes and Fungi they play the major role in the composting process. They use the organic matter in the compost bin or heap as a source of food resulting in its decomposition to the composted material with easily available nutrients called as compost. We can speed up the natural decomposition process by providing optimum conditions for the soil microorganisms to breakdown more quickly than would occur without our intervention. Many of these bacteria, fungi, and actinomycetes break down the organic material chemically, in contrast to the physical action of the macroorganisms (Hermosa *et al* 2012). The actual species of composting microorganism in any given heap will vary, depending on the climate, moisture content, Compost pH, temperature and the conditions within the particular part of the heap at the time of collecting the samples for identification and counting. These bacteria, actinomycetes and fungi will be present in massive numbers

Drought tolerance (antitranspirants)

Plants defend themselves against ecological stresses including drought. Therefore, they adopt various strategies to cope with stress, such as seepage and drought tolerance mechanisms, which allow plant development under drought conditions. There is evidence that microbes play a role in plant drought tolerance. Pink pigmented facultative methylotrophic (PPFM) bacteria are ecologically distributed microorganisms. A review of the literature describing the initiation of drought tolerance mediated by plant inoculation with fungi, bacteria, viruses, and several

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bacterial elements, as well as the plant transduction pathways identified via archetypal functional or morphological annotations and contemporary "omics" technologies. Overall, microbial associations play a potential role in mediating plant protection responses to drought, which is an important factor for agricultural manufacturing systems that are affected by fluctuating climate.

Microbes against Plant stress

Global climate records in the last decades have revealed a rise in global temperature alongside changes in rainfalls, resulting in various serious implications on environmental and agricultural aspects (Füssel *et al*., 2012). Crop plants are more frequently exposed to abiotic stresses caused by climate change because, aside from direct implications of abiotic stresses on plants, climate change could increase the number of pests and diseases, as well as increase the severity and frequency of the outbreak of diseases (De Wolf and Isard, 2007; Garrett *et al*., 2016). According to recent estimates, abiotic stresses are anticipated to cause up to 50% losses, or higher, in worldwide agricultural productivity, depending on the region (Kumar and Verma, 2018). These losses, coupled with the continual rise in the human population, revealed that about 60% boosting of agricultural production is needed to meet the world food needs (Wild, 2003), with a concrete risk of dramatic deforestation increment and loss of natural ecosystems (Byerlee *et al*., 2014). Increased plant resilience to mitigate climate change-associated stresses is a sustainable method for ensuring food security with a restricted increase in agricultural surface, and the use of microbial biostimulants is one of the best options to achieve this goal (Calvo *et al*., 2014; Yakhin *et al*., 2017).

Plants are associated with a diverse group of microorganisms (the microbiome) in their endosphere (internal compartments), rhizosphere (attached soil to roots), and phyllosphere (aboveground parts), making microbial biostimulants particularly fascinating (Compant *et al*., 2019; Babalola *et al*., 2020). Crop plants coexist with microbial symbionts, which play key roles in plant production, performance, nutrition, and tolerance to abiotic stress (Vandenkoornhuyse *et al*., 2015; Enebe and Babalola, 2018; Ojuederie *et al*., 2019). For instance, geological evidence indicates that the relationship between microbes and plants predates the emergence from the water, suggesting that symbiosis involving arbuscular mycorrhizal was important in the process of terrestrialization (Selosse and Le Tacon, 1998). Moreover, microorganisms are involved in multiple biogeochemical cycles, such as nitrogen and carbon cycling, nitrogen fixation, soil formation and plant nutrition acquisition in the ecosystems (Wagg *et al*., 2014; Igiehon *et al*., 2019). As a result, many microbial symbionts can be used as a biofertilizer, releasing additional nutrients to the plant through synergistic mechanisms, which include nitrogen fixation (e.g., *Mesorhizobium loti, Rhizobium etli, Azotobactervinelandii, and Azospirillumbrasilense*), phosphate solubilization (e.g., Arbuscular mycorrhizal fungi, *Azospirillum* spp., *Bacillus* spp., and *Pseudomonas* spp.), cellulolytic activity (*Aspergillus* spp., *Trichoderma* spp., *Bacillus* spp., and *Penicillium* spp.), soil acidification (*Bacillus* spp. and *Arthrobacter* spp.), and production of siderophores (e.g., *Pseudomonas s*pp.) (Bhattacharyya and Jha, 2012; Orozco-Mosqueda *et al*., 2021). Also, thanks to its abilities to boost plant development, defenses, antibacterial compounds, combat pathogen infections and feed

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on nematodes, Trichoderma spp. is a well-studied symbiotic fungal genus (Adnan *et al*., 2019; Szczałba *et al*., 2019). Despite the beneficial effects exhibited on their hosts, some of which include increased protection from abiotic stresses and nutritional efficiency, some weaknesses may limit the use of *Trichoderma* spp. as commercial biostimulant products, such as difficulties of in vitro cultivation and escalating of bioproduction, the lack of understanding on host specificity and population dynamics in the agroecosystem (Du Jardin, 2015). Other types of fungi can form part of the beneficial microbiome associated with plants, such as yeasts belonging to *Brettanomyces naardensis, Candida oleophila, Aureobasidium pullulans, Metschnikowia fructicola, Cryptococcus albidus, and Saccharomyces cerevisiae* (Freimoser *et al*., 2019; Nafady *et al*., 2019). Foliar infections can be controlled by yeasts that could colonize the leaf of a plant using direct antagonism (Preininger *et al*., 2018) or through the induction of systemic resistance (Lee *et al*., 2017). Likewise, yeast inhabiting the soil can enhance the growth of the plant through phosphate solubilization, digestion of organic materials, soil aggregation and stimulation of root development, and suppressing root infections (Sarabia *et al*., 2018). Plant growth-promoting bacteria (PGPB), which includes rhizobacteria or bacterial endophytes, are known to majorly populate the plant rhizosphere and the most studies genera are *Azospirillum, Azotobacter, Arthrobacter, Burkholderia, Gluconacetobacter, Pseudomonas, Bacillus, Streptomyces, and Serratia* (Kour *et al*., 2020a). Additional genera are more recently proposed as possible bioinoculants with biocontrol and/or plant growth-promoting activities, such as Rouxiellabadensis and Rahnella spp. (Morales-Cedeńo *et al*., 2021). Physiological, molecular, and biochemical investigations of the interactions that exist between plant and beneficial microorganisms have shown that the presence of microbe-induced plant stress responses (Farrar *et al*., 2014; Igiehon and Babalola, 2021), which may trigger induced systemic tolerance (IST) against abiotic stressors (Yang *et al*., 2009; Vacheron *et al*., 2015).

Microbial biostimulants are a viable alternative for supporting plants exposed to abiotic stresses in the current context of fast-developing climate change (Santoyo *et al*., 2021b). While recent advancements and laboratory studies have revealed the positive activities of plant-associated microorganisms, the efficacy of microbial biostimulants is yet to be successfully validated in field experiments. As a result, microbial biostimulants are often used as supplemental therapies rather than being used to their full potential in crop management. The goal of this paper is to summarize current information about microbial biostimulants, especially the current commercially available products, examine their applications in enhancing plant tolerance to abiotic stresses caused by climate changes, and forecast the creation of novel products that may be used in adverse conditions.

Application Technologies

BCAs can be delivered to the plants through several means (typically by inoculation of seeds, soil or aerial plant parts) depending on the mode of action of the BCA, the plant growth stage and the type of formulation. Hence, formulations for the control of soil-borne pathogens require a different application approach to that required for the control of above-ground pathogens (Ulloa-Muñoz *et al*., 2020). Likewise, the method of application of wettable powders, water-dispersible granules, and liquid formulations differs from dry powders and granules.

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Seed Application

Seed application is a common and practical application technique for the protection of seed and seedlings from seed-borne and soil-borne pathogens. Powders and granules for seed coatings are mostly applied using the slurry method. This method involves mixing the solid with a strong adhesive solution, to form a slurry, which is then applied to the seeds. Liquid inoculants (generally supplemented with adhesives) are typically sprayed onto the seeds. Adhesives commonly used for seed coating include arabic gum, CMC, hydroxyl propyl methyl cellulose, starch, wheat flour, sucrose, vegetable oils, and non-toxic commercial preparations (Igiehon *et al*., 2021). Sometimes a superfine powder of limestone (CaCO3) is added immediately after coating. Seed coatings can be performed either by hand, rotating drums, large dough cement mixers, mechanical tumbling machines or automated seed coaters. Subsequent drying is performed by forced air using seed drying equipment. Coating and drying can also be performed simultaneously in fluidized beds and seed coaters with integrated dryers by dispersing the seeds on a cushion of pressurized air while applying the formulation.

A major constraint of this application technique is that seeds can be coated only with a limited amount of inoculant, which can be a limiting factor since a threshold of a BCA may be needed for successful biological control. Moreover, if the inoculant is not well attached to the seed, some may be lost during sowing. Factors influencing microbial survival on seed are the release of toxic exudates from seed coat or incompatibility between the inoculant strains and seed-applied chemicals.

Soil Application

Soil application is an alternative technique for the application of BCAs against soil-borne pathogens and involves the direct application of the formulation into soil, usually in the seeding furrow. Soil application is generally done using granular inoculants, wettable powders, water-dispersible granules or liquid inoculants. They are often applied under, above or alongside the seed by using granular applicators. Powder inoculants are also suitable for soil application. However, they are dustier than granular inoculants and therefore less user-friendly. Instead, wettable powders, water-dispersible granules, and liquid formulations can be delivered to the soil by hand or by mechanical spraying equipment, which allows for an even distribution of the formulation over the crop area (Meier *et al* 2012). Furthermore, they can be delivered directly to the root zone of individual plants by drip irrigation, hydroponic systems or by drenching furrows. Soil application is particularly used when large populations of BCAs need to be introduced to the soil. It avoids damage to fragile seeds and overcomes the adverse effects of seed-applied chemicals.

Aerial Application

Aerial application directs BCAs to the above-ground plant parts, particularly to the leaves (foliar application), and it is especially convenient to treat above-ground pathogens. This technique allows for multiple applications of the microbial formulation during crop cultivation and for the control of the location and application rate. This is of great interest as the dosage and frequency of application can be standardized based on each pathosystem. The most effective means of aerial application is the use of spray equipment, which can range from an aerosol can

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to hand or mechanical equipment, including aircraft. The main disadvantage of aerial application is that its use is mostly limited to the early morning or the late evening, when the temperature is lowest, relative humidity is highest and leaves are turgid, especially in warmer regions. The major hurdle in foliar applications is conserving the viability and threshold inoculum potential of the BCA for effective pathogen/pest control. This still appears to be a major hurdle for the development of effective BCAs for pre-harvest application.

Agroforestry

Agroforestry is multiple land-use systems in which crops and woody perennials are grown on the same land management unit. Agroforestry system is practiced all over the world, and it has major importance in reducing the impact of climate change. Nowadays, climate change is the problem of developed and developing countries, thus, meeting to find out a solution to reduce the impact of climate change on agriculture, biodiversity, and food security. Thus, agroforestry is now receiving increasing attention as a sustainable land management option the world over because of its ecological, economic, and social attributes. Agroforestry, an ecologically and environmentally sustainable land use, offers great promise toward mitigating the_rising atmospheric CO2 levels through carbon sequestration. Synergies between climate change adaptation and mitigation actions are particularly likely in situations involving income diversification with tree and forest products. These options also reduce the susceptibility of land-use systems to extreme weather events, enhance soil fertility, and favor the conservation and restoration of forest and riparian corridors. Sequestration of carbon in soils and in the biota, along with payments to resource-poor farmers for the ecosystem services rendered, would be a timely win–win strategy in the fight against food insecurity and global warming.

The importance of agroforestry cannot be overemphasized, as it has several advantages in the provision of food and other basic needs (i.e., fuel wood, staking materials, fibers, timber, medicinal concentrates, oils, fruits, and fodder for animals) for a large proportion of the rural population as well as its role in soil fertility restoration and the control of weeds in addition to amelioration of environmental degradation. Advocates have contended that soil conservation is one of its primary benefits (Hungria *et al* 2013). The presence of woody perennials in agroforestry systems may affect several biophysical and biochemical processes that determine the health of the soil substrate. The less disputed of the effects of trees on soil include amelioration of erosion, primarily through surface litter cover and understory vegetation; maintenance or increase in organic matter and diversity, through continuous degeneration of roots and decomposition of litter; nitrogen fixation; enhancement of physical properties, such as soil structure, porosity, and moisture retention through the extensive root system and canopy cover; and enhanced efficiency of nutrient use because the tree–root system can intercept, absorb and recycle nutrients in the soil that would otherwise be lost through leaching.

Soil Beneficial Microorganisms as a Link to Sustainable Agriculture

Soil is a natural medium for plant growth and development. Soil is the most diverse and complex habitat that consists of the smallest organisms in the soil and includes bacteria, actinomycetes, fungi, algae, etc. The plant microbiome is a key determinant of plant health and productivity and has received substantial attention in recent years. Microbes in the rhizosphere

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can establish beneficial, neutral, or detrimental associations of varying intimacies with their host plants. Specific interactions between microbes and model plants, such as in *Rhizobium*–legume symbioses, are well understood. A significant amount, 5–20%, of the products of photosynthesis (the photosynthate) is released, mainly into the rhizosphere (the soil–root interface) through roots. These photosynthates include rhizodeposit exudates, mucilage, and sloughed cells. Root exudates contain a variety of compounds, predominately organic acids and sugars, and also amino acids, fatty acids, vitamins, growth factors, hormones, and antimicrobial compounds . Root exudates are key determinants of rhizosphere microbiome structure. The composition of root exudates can vary between plant species and cultivars and with plant age and developmental stage

Among the various advantages of the microbiome for plants, symbiotic associations between nitrogen-fixing bacteria, mainly rhizobia, arbuscular mycorrhizal fungi, and phosphate-solubilizing bacteria, are typical examples of the microbiome for how plants obtain nitrogen and phosphorus, respectively. The interaction between nitrogen-fixing bacteria and legume crops, and arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria increases the availability of nitrogen and phosphorus for plants since the bacteria fix the nitrogen and solubilize phosphorus ions, while the fungi translocate them to the plant (Meier *et al* 2012). Bacillus, *Azotobacter, Microbacterium, Erwinia, Beijerinckia, Enterobacter, Flavobacterium, Pseudomonas, and Rhizobium* bacteria are known as phosphate solubilizers. The microbiome containing rhizobia, the legume nodule microbiome, consists of other endophytic bacteria, both of which are responsible for direct and indirect growth promotion mechanisms in plants. Some nitrogen-fixing endophytes are cyanobacteria (*Anabaena, Nostoc, Calothrix*), *Azotobacter, Azospirillum, and Gluconacetobacter* . However, fixators can also be free living and establish themselves in non-leguminous plants, as is the case, for example, with the genera Beijerinckia, Klebsiella, and Bacillus.

Another method is phytostimulation or biostimulation intrinsically linked to plant growth and made up of the production of phytohormones by the microbiome, such as indole-3-acetic acid (IAA), auxin, gibberellins, cytokinins, and salicylic acid (SA). Besides, some bacteria can secrete an enzyme, 1-carboxylic acid-1-aminocyclopropane (ACC) deaminase, which reduces the level of ethylene in the plant. Aguiar-Pulido *et al*. Bacteria from the tomato rhizospheric microbiome produced the hormone IAA, and promoted plant growth. Duran *et al*. In few cases, in the roots of cereals (wheat) and oilseed (soybeans) crops, rhizospheric bacteria producing IAA and ACC deaminase, which were Pseudomonas spp. The microbiome also plays an essential role in plant tolerance to extreme conditions, such as salinity, drought, and exposure to heavy metals. Soil salinity has hindered the growth rates of plants and reduced their yield. However, the negative impact of high levels of salt in the soil can be minimized through the production of phytohormones by the microbiome, with a consequent increase in plant resistance to these extreme environments. the rhizosphere microbiome was able to promote germination and growth of Hibiscus hamabo under salinity conditions (Finore *et al* 2014). Recently, a model was proposed to explain the establishment and maintenance of the beneficial and degrading microbiome in the rhizosphere of contaminated soil plants. Four strategies were identified,

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including plant selection based on the microbiome, interference from root exudates, disturbance, and feeding of supply lines, to ensure that the microbial community is kept under control in polluted environments. Plants that live in oil-contaminated soils depend on their microbiome for survival, growth optimization, and biomass production. At the same time, as the contamination of these areas increases, there are changes in the composition of the microbiome, favoring hydrocarbon-degrading microorganisms associated with plant growth. The plant–microbiome interaction will not always be efficient for phytoremediation; therefore, human interventions are necessary to optimize this interaction and promote the degradation of pollutants. Understanding the potential of the microbiome for agriculture can lead to its use as an inoculant or its manipulation, to select more efficient microbial groups for plant development. Besides that, reducing the use of pesticides and chemical fertilizers based on an understanding of the potential of the plant microbiome is of paramount importance in advancing sustainable agricultural practices

Conclusion

This present review article examined the role of the microbes in sustainable agriculture and human health. In sustainable agriculture, soil health is mainly determined by the presence and diversity of microbes present in the soil rhizosphere. The diversity and abundance of soil and rhizosphere microorganisms influence plant composition, productivity, and sustainability. Deploying microbes to improve agriculture productivity is an extremely attractive approach that is non-transgenic and can be viewed collectively as the extended plant genome. Because these same microbes can contribute to restoring soil health and productivity, they have a bright future in low-input, sustainable agriculture. Improved assessment of soil health indicators is necessary to further enhance our understanding of how production strategies and environmental factors affect the physical, biological, and chemical stability and dynamics of the soil–rhizosphere–plant systems and their impact on short- or long-term sustainability.

Future Prospects

One of the challenges for future research work includes the protection and conservation of rhizosphere biodiversity and its potential application in agricultural soils. The current use of microbial inoculants has proven useful to address some agronomic challenges; however, large-scale adoption remains low mainly owing to inconsistency in the efficacy under different environmental conditions. The increasing demand for safe food and better nutrition, advancing research technologies, and interest in sustainable agriculture have further renewed global interest in the microbiome. More knowledge and deeper understanding are needed on how agronomic practices under changing climatic conditions affect the composition, abundance, and biofunctionality of microbes in delivering multiple agroecosystem services. Sustainable agriculture is important for today's agricultural practices because policymakers, conservationists, ecologists, scientists, farmers, and biologists are all interested in debating the topic of sustainable agriculture. Presently, the agricultural soil has been polluted due to extensive use of mineral fertilizers and agrochemicals. The use of the microbiome offers an assured distinct advantage over many other control agents and methods. The use of microbial technology would be fruitful for the development of ecological and sustainable agriculture.

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REFERENCES:

1. Agrawal PK, Agrawal S, Kundan R, Bhatt M (2014). Application and perspective of plant growth promoting rhizobacteria in the development of sustainable agriculture. Int J Curr Res 6:9044–9051

2. Ahemad M (2015). Phosphate-solubilizing bacteria-assisted phytoremediation of metalliferous soils: a review. Biotech 5:111–121

3. Ahemad M, Khan MS (2013) Pesticides as antagonists of rhizobia and the legume-Rhizobium symbiosis: a paradigmatic and mechanistic outlook. Biochem Mol Biol 1:63–75

4. Adesemoye AO, Kloepper JW (2009) Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl Microbiol Biotechnol 85(1):1–12

5. Albareda M, Rodríguez-Navarro DN, Camacho M, Temprano FJ (2008) Alternatives to peat as a carrier for rhizobia inoculants: solid and liquid formulations. Soil Biol Biochem 40:2771–2779

6. Ali S, Charles TC, Glick BR (2014) Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. Plant PhysiolBiochem 80:160–167

7. Ana Bejarano & Gerardo Puopolo, 2021. Bioformulation of Microbial Biocontrol Agents for a Sustainable Agriculture.

8. Ansari SA, Matricardi P, Di Meo C, Alhaique F, Coviello T (2012) Evaluation of rheological properties and swelling behavior of sonicated scleroglucan samples. Molecules 17:2283–2297

9. Araujo FF (2008) Seed inoculation with Bacillus subtilis, formulated with oyster meal and growth of corn, soybean and cotton. CiêncAgrotecnol 32(2):456–462

10. Araujo FF, Henning AA, Hungria M (2005) Phytohormones and antibiotics produced by *Bacillus subtilis* and their effects on seed pathogenic fungi and on soybean root development. World J Microbiol Biotechnol 21:1639–1645

11. Arzanesh MH, Alikhani HA, Khavazi K, Rahimian HA, Miransari M (2011) Wheat (*Triticum aestivum* L.) growth enhancement by Azospirillum sp. under drought stress. World J Microbiol Biotechnol 27(2):197–205

12. Avis TJ, Gravel V, Antoun H, Tweddell RJ (2008) Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity. Soil Biol Biochem 40:1733–1740

13. Badel S, Bernardi T, Michaud P (2011) New perspectives for Lactobacilli exopolysaccharides. Biotechnol Adv 29:54–66

14. Badri DV, Weir TL, van der Lelie D, Vivanco JM (2009) Rhizosphere chemical dialogues: plant-microbe interactions. Curr Opin Biotechnol 20:642–650

15. Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 16:729–770

16. Bashan Y, De-Bashan LE, Prabhu SR, Hernandez JP (2014) Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). Plant Soil 378:1–33

17. Berger LRR, Stamford NP, Santos CERS, Freitas ADS, Franco LO, Stamford TCM (2013) Plant and soil characteristics affected by biofertilizers from rocks and organic matter

ISBN : 978-81-966183-3-9

inoculated with diazotrophic bacteria and fungi that produce chitosan. J Soil Sci Plant Nutr 13:592–603

18. Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28(4):1327–1350

19. Bogino PC, Oliva MM, Sorroche SG, Giordano W (2013) The role of bacterial biofilms and surface components in plant-bacterial associations. Int J Mol Sci 14:15838–15859

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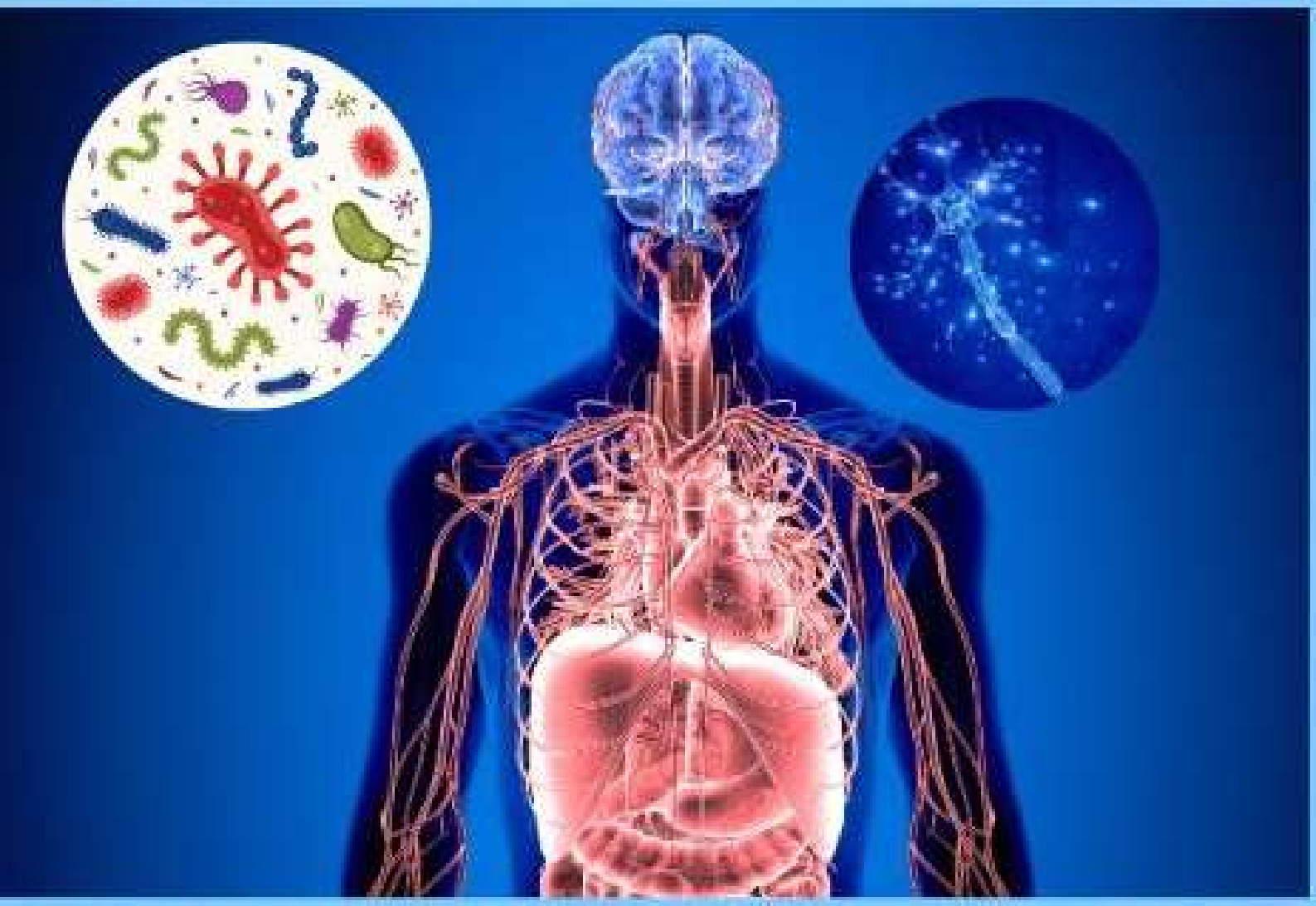

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