FRONTIERS IN ANTI-INFECTIVE AGENTS

VOLUME 6

Editors:

Parvesh Singh
Vipan Kumar
Rajshekhar Karpoormath

Bentham Books

Frontiers in Anti-Infective Agents

(Volume 6)

Edited by

Parvesh Singh
School of Chemistry and Physics
University of Kwa-Zulu Natal (UKZN)
Westville Campus, Durban
South Africa

Vipan Kumar
Department of Chemistry
Guru Nanak Dev University
Amritsar
India

&

Rajshekhar Karpoormath
Department of Pharmaceutical Chemistry
University of Kwa-Zulu Natal (UKZN)
Westville Campus, Durban
South Africa

Frontiers in Anti-Infective Agents

Volume # 6

Editors: Parvesh Singh, Vipan Kumar and Rajshekhar Karpoormath

ISSN (Online): 2705-1080

ISSN (Print): 2705-1072

ISBN (Online): 978-981-4998-42-0

ISBN (Print): 978-981-4998-43-7

ISBN (Paperback): 978-981-4998-44-4

need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.

3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

Bentham Science Publishers Pte. Ltd.
80 Robinson Road #02-00
Singapore 068898
Singapore
Email: subscriptions@benthamscience.net

CONTENTS

PREFACE

Pathogens have historically affected human populations worldwide, resulting in epidemics and pandemics of different origins and epidemiology, as well as high mortality rates. Despite advancements in detection mechanisms and treatment of many known diseases through the development of novel drugs, the increase in the pace of evolution of drug resistance remains the greatest obstacle in drug design and discovery. The most recent threat to mankind is the SARS-coronavirus-2 (COVID-19), a viral zoonosis, which is difficult to diagnose due to many symptomatic similarities to influenza. Approaching the virus *via* a standardised treatment protocol has been inefficacious due to its rapid mutation, either being more virulent or becoming drug-resistant. The viral infection has engulfed the world in a fight response, in search of an appropriate vaccine or treatment to reduce the risk of infection and loss of human life. Biomolecular engineering and molecular bio-computing have been our greatest tools in drug discovery and development. It has enabled the repurposing of existing drugs by understanding the structure-activity relationship and pharmacokinetic properties against new targets or biological systems.

The book offers an insightful perspective on the most up-to-date developments and research engaged in the combat against pathogens and COVID-19. The contributions from distinguished researchers and leaders in their field are a critical analysis of vaccine development strategies, novel heterocyclic drug scaffolds, the history and biology of infection, and natural products as quorum sensors.

The chapter by Arindam Mitra emphasises reverse vaccinology approaches in vaccine design, intuitively targeting multiple pathogens, including the novel coronavirus, to combat the current pandemic of COVID-19. It also highlights the major advantages of reverse vaccinology for the discovery of novel vaccines with reduced time and cost in development. The second chapter by N. Ramalakshmi *et al*. is a critical review of leptospirosis and its treatment. The current remedies for milder cases of leptospirosis involve antibiotic administration *viz* penicillin, ampicillin, cefmetazole, oxalactam, ceftizoxime, and cefotaxime. This review summarizes the most recent literature on synthetic lead molecules, natural product chemotherapies, and drug targets. The third chapter by Nachimuthu Ramesh *et al*. sheds light on phage therapy and its evolution from lab to bedside endpoints in treating patients. The history and fundamentals of phage biology and its significance in treating infectious diseases have been provided, with commercialization strategies undertaken by the pharmaceutical industry. The fourth chapter by Debaprasad Parai *et al*. focuses on quorum sensing inhibitors (QSIs) from natural products. Quorum sensing is a signalling process, which regulates the expression of several virulence factors in both gram-negative and gram-positive bacteria *via* an autoinducing loop. When a critical bacterial cell density is reached, a complex of regulatory proteins and specific signalling molecules enable the autoinduction of the quorum sensor and the expression of the target genes. This chapter provides a literature review describing the various QSIs obtained from natural sources and their role as anti-infective agents. The fifth chapter by Shaik Baji Baba *et al*. reveals the importance of nitrogen and oxygen-based heterocycles as potential anti-infective agents. It details the development of 1,2,4-triazoles, isatin, and coumarin-based anti-infective agents. Structure-activity relationship studies provide scope for future researchers to develop the most effective and least toxic anti-infective agents.

We would like to acknowledge the expert contributions of the authors mentioned in the review articles in accomplishing this book, which forms an updated base platform for novel drug discovery and development against infective agents. Each author has been recognised as

a dynamic leader in their field and we wish them well for their future research. We reserve a special recognition for the Bentham Science Publishing team, particularly Mrs. Fariya Zulfiqar (Manager Publications) and Mr. Mahmood Alam (Editorial Director), for the timely production of the 6th volume and promotion of this scientific collaboration.

Parvesh Singh
School of Chemistry and Physics
University of Kwa-Zulu Natal (UKZN)
Westville campus, Durban
South Africa

Vipan Kumar
Department of Chemistry
Guru Nanak Dev University
Amritsar
India

&

Rajshekhar Karpoormath
Department of Pharmaceutical Chemistry
University of Kwa-Zulu Natal (UKZN)
Westville Campus, Durban
South Africa

List of Contributors

A. Puratchikody	University College of Engineering, Bharathidasan Institute of Technology Campus, Anna University, Tiruchirappalli, India
Archana Loganathan	Antibiotic Resistance and Phage Therapy Laboratory, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore, India
Arindam Mitra	Department of Microbiology, School of Life Science and Biotechnology, Adamas University, Kolkata, India
Debaprasad Parai	Department of Microbiology, University of Kalyani, Kalyani, West Bengal, India
Kandasamy Eniyan	Antibiotic Resistance and Phage Therapy Laboratory, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore, India
N. Ramalakshmi	C.L Baid Metha College of Pharmacy, Chennai, India
Nachimuthu Ramesh	Antibiotic Resistance and Phage Therapy Laboratory, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore, India
Naresh Kumar Katari	School of Chemistry & Physics, College of Agriculture, Engineering & Science, Westville Campus, University of KwaZulu-Natal, P Bag X 54001, Durban-4000, South Africa Department of Chemistry, School of Science, GITAM Deemed to be University, Hyderabad, Telangana 502329,, India
Pia Dey	Department of Microbiology, University of Kalyani, WB, India- 741235
Prasanth Manohar	Zhejiang University-University of Edinburgh (ZJU-UoE) Institute, Zhejiang University, Haining, Zhejiang, P.R.China and The Second Affiliated Hospital Zhejiang University (SAHZU), School of Medicine, Hangzhou, P.R.China
Rambabu Gundla	School of Chemistry & Physics, College of Agriculture, Engineering & Science, Westville Campus, University of KwaZulu-Natal, P Bag X 54001, Durban-4000, South Africa
S. Arunkumar	SRM Modinagar College of Pharmacy, Modinagar, India
Samir Kumar Mukherjee	Department of Microbiology, University of Kalyani, Kalyani, West Bengal, India
Sebastian Leptihn	Antibiotic Resistance and Phage Therapy Laboratory, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore, India Department of Infectious Diseases, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, P.R. China Infection Medicine, Biomedical Sciences, Edinburgh Medical School, College of Medicine and Veterinary Medicine, The University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ, United Kingdom
Shaik Baji Baba	School of Chemistry & Physics, College of Agriculture, Engineering & Science, Westville Campus, University of KwaZulu-Natal, P Bag X 54001, Durban-4000, South Africa

CHAPTER 1

Reverse Vaccinology Approaches for Rapid Vaccine Design Against Emerging Infectious Diseases

Arindam Mitra[1,*]

[1] *Department of Microbiology, School of Life Science and Biotechnology, Adamas University, Kolkata, India*

Abstract: Reverse vaccinology uses computational approaches to identify potential vaccine candidates. With the increasing pace of genome sequencing, it is possible to identify all potential antigens from any sequenced pathogen. Reverse vaccinology uses computational data to identify potential antigens, express those potential antigens, and then screen them further for protective immune response. Thus, reverse vaccinology offers several advantages and enables identifying novel antigens even if the expression level is low or not abundant. Besides, reverse vaccinology approaches offer reduced time and reduced cost for the development of vaccines compared to conventional vaccination methods. Such a timely, speedy, and economical process for developing vaccines without compromising safety and immunogenicity is the urgent need of the hour to combat many emerging pathogens, including SARS-CoV-2. This chapter summarizes approaches and challenges in developing vaccines against many emerging pathogens, including SARS-CoV-2, by employing this innovative strategy.

Keywords: Bioinformatics, COVID-19, Pathogen, Reverse vaccinology, SARS-CoV-2, Vaccine design.

INTRODUCTION

Vaccines are one of the most successful and cost-effective prophylactic measures for improving the quality of health and saving lives from a wide range of infectious diseases worldwide [1, 2]. Eradication of smallpox and significant reduction of global polio cases are outstanding examples of successful vaccination programs where vaccines have significantly reduced mortality and the global burden of infectious diseases. Vaccines are weakened or attenuated from microorganisms or components, which, when introduced into an individual, stimulates the body's immune response and protects against an infectious disease

[*] **Corresponding author Arindam Mitra:** Department of Microbiology, School of Life Science and Biotechnology, Adamas University, Kolkata, India; Tel: +917305956643; E-mail: arindam.mitra@adamasuniversity.ac.in

Parvesh Singh, Vipan Kumar & Rajshekhar Karpoormath (Eds.)

caused by the pathogen or similar pathogens. Vaccinology focuses on vaccine development and the effect of vaccines on public health [3]. The classical steps of vaccine development include isolation, culture, and the weakening of a pathogen. Inoculation of a weakened or killed pathogen or a microbe component stimulates a protective immune response in the host. Purified components, such as capsules, recombinant proteins, or weakened toxins also confer protective immunity. Louis Pasteur designed this vaccinology approach of isolation, inactivation, and injection of the agent responsible for the disease. By reducing the pathogen's virulence or inhibiting the replication of a pathogen, the pathogen would be safer for hosts without compromising the immunogenicity of the pathogen. Targeting microbial components, such as capsules, toxins, and surface proteins, reduces the virulence of a given pathogen. This kind of vaccine strategy depends on the body's immune response to combat infectious diseases. It was successful against many infectious diseases such as smallpox, polio, mumps, measles, and rubella. Convalescent plasma therapy (CBT), also known as serum therapy or passive immune therapy, is another proven age-old therapeutic strategy. It depends on protective antibody responses from blood (sera and lymphocytes) from convalescent patients [4]. CBT treatment was successful against diphtheria, tetanus, pneumonia, anthrax, plague, tularemia, among many others [5]. In addition, this therapeutic strategy is employed against many emerging viral diseases, such as Ebola, SARS-CoV, and more recently against SARS-CoV-2 [6, 7]. Fig. (**1**) highlights classical approaches for vaccine development and convalescent plasma therapy against infectious diseases.

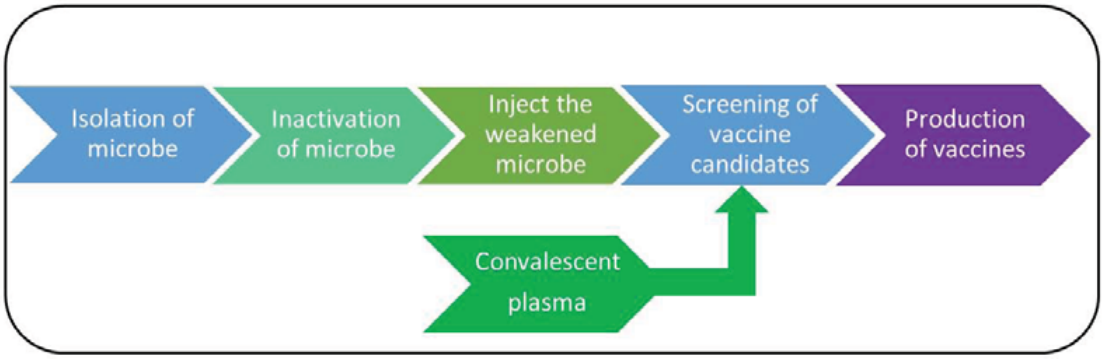


Fig. (1). Classical approaches for the development of vaccines and therapeutics.

However, there are certain limitations in the traditional approach for developing vaccines. Many infectious diseases such as tuberculosis, HIV, malaria, and others do not have an effective vaccine yet. In many cases, the development of a vaccine by the classical method is not feasible due to the lack of proper media for growing a given pathogen. It is difficult or not possible to express a given target antigen, as in meningitis and AIDS. The classical method is also not helpful in developing vaccines based on antigens that elicit strong autoimmune reactions or strains that are highly variable or where the mechanisms of pathogenesis are not well understood. Sometimes, the most expressed proteins may not be ideal vaccine candidates, or the antigens expressed *in vitro* are different from those expressed *in vivo*. Besides, the traditional method of developing vaccines can be time-consuming, and not all antigens can be made pure in adequate amounts for vaccine testing. Conventional vaccine development would not be effective against pathogens that do not induce immunity post-infection. The approach would not apply in cases of chronic diseases including AIDS, tuberculosis, gastritis and hepatitis [8].

Reverse Vaccinology

The sequencing of the first microbe, *Haemophilus influenzae,* in 1995 opened up new possibilities for vaccine design [9]. With the availability of whole-genome sequence of many pathogens at an incredible speed using next-generation sequencing platforms, it is now possible to identify all potential protein antigens *in silico* that may be antigenic or immunogenic without actually culturing the pathogen. Using specific criteria of predictive algorithms and combining tools of bioinformatics and biotechnology, it is also possible to screen the exhaustive list of all potential antigens down to few candidate antigens that can eventually move to safety and immunogenicity testing. In many cases, secreted or extracellular antigens are more likely to be exposed to antibodies than intracellular protein antigens and are likely to be potential vaccine candidates.

Reverse Vaccinology (RV), a term coined by Rino Rappuoli, uses genomics and bioinformatics tools to develop vaccine candidates [10]. This approach enables the identification of all potential protective antigens from sequenced genomes. It facilitates the development of a safe and effective vaccine against any infectious disease that requires a protein antigen to stimulate an immune response [11 - 19]. Choice of algorithms, appropriate criteria for proper selection of antigens, and critical evaluation of the information often determine the success of the RV strategies. Genome-based approaches facilitate identifying novel antigens or unique virulence factors in pathogens, thereby enabling a better understanding of pathogenesis and developing better vaccines. RV-based vaccines are typically targeted based on specific purified components or subunit vaccines and not based

CHAPTER 2

Leptospirosis - A Complete Review

N. Ramalakshmi[1,*], S. Arunkumar[2] and **A. Puratchikody[3]**

[1] *C.L Baid Metha College of Pharmacy, Chennai, India*

[2] *School of Pharmacy, Satyabama Institute of Science and Technology, Chennai, India*

[3] *University College of Engineering, Bharathidasan Institute of Technology Campus, Anna University, Tiruchirappalli, India*

Abstract: Leptospirosis is a rare but neglected bacterial infection that affects people and animals. It is caused by bacteria of the genus *Leptospira*. The disease was reported as early as 1886 by Adolf Weil. Leptospirosis may cause kidney damage, meningitis, liver failure, respiratory distress, and even death when it is not treated. Occupations at risk include surfers, slaughterhouse workers, farmers, sewer workers and, people working on derelict buildings. Amjad Islam *et al*. had reported that among Asian countries, its highest prevalence is found in India. In 2015, Federico Costa *et al*. estimated that leptospirosis causes 1.03 million cases worldwide each year. The pooled mortality rate is 25%. WHO has estimated that 0.1 to 1 per 100 000 people living in temperate climates are affected each year, with the number increasing to 10 or more per 100 000 people living in tropical climates. It is reported in all continents except Antarctica. Lesser availability of treatment resources is detrimental, and unfortunately, it is commonly reported in lower-middle-income group countries.

The current treatment modalities for milder cases of leptospirosis rely on antibiotic administration viz penicillin, ampicillin, cefmetazole, oxalactam, ceftizoxime, and cefotaxime.

Whereas, in severe cases, intravenous penicillin G has long been the drug of choice; the patients treated with penicillin for the management of this disease are to be monitored throughout the treatment to prevent the severe threat of potential Jarisch-Herxheimer reactions. In particular, this immune-mediated hypersensitivity reaction may occur within 4-5 h after administration of penicillins. Various kinds of human leptospirosis vaccines have been developed, including inactivated whole-cell, outer-envelope, and recombinant vaccines. Of these, only a multivalent inactivated leptospirosis vaccine (killed vaccine) is available in China, Japan, and Vietnam. However, human vaccines for leptospirosis are serovar-specific and require yearly boosters. So there is a need for the development of novel compounds which have leptospirocidal activity. Notably, drugs for treating leptospirosis are minimum; the present research for the development of novel lead compounds for this pathogen is very limited.

[*] **Correspondence author N. Ramalakshmi:** C.L Baid Metha College of Pharmacy, Chennai, India; Tel: +91 9840975248; E-mail: ramalakshmi.arunkumar@gmail.com

Parvesh Singh, Vipan Kumar & Rajshekhar Karpoormath (Eds.)

This review aims to summarize the most recent literature on synthetic lead molecules, natural products for its treatment, drug targets, *etc*., and provide recommendations to researchers who may encounter difficulties in finding details on the subject.

Keywords: Drug targets, Leptospirosis, Natural products, Synthetic analogs.

HISTORY OF LEPTOSPIROSIS

Leptospirosis is caused by the pathogenic bacteria of the genus Leptospira. This zoonotic disease is prevalent in every continent, except in the Polar regions. The tropical climate has highly favorable ecological conditions for transmission of leptospirosis than temperate climate. Even if leptospirosis is primarily considered a tropical disease, it is ubiquitously present in temperate climates due to changes in environmental conditions, and the migration of humans. As a consequence of ever-increasing awareness and high case incidence, this disease has been scheduled as an emerging global public health disease [1]. It is primarily transmitted through water, food, soil, and mud contaminated with the urine of infected animals to the human. When bacteria enter through skin abrasions and mucous membranes of the mouth, eyes, and nose, humans can acquire leptospirosis.

Leptospirosis was recognized as an occupational hazard of rice harvesters in ancient China. In Japan, leptospirosis was termed as *akiyami*, or autumn fever. Larrey, in 1812, identified leptospirosis as fièvrejaune among Napoleon's troops at the siege of Cairo in Egypt. Initially, the endemic disease was supposed to be related to the plague but not as contagious. About 100 years ago, workers in Japan and Europe contemporarily isolated Leptospira and identified it as a causative agent for the classical syndrome, the Weil’s disease. The nineteenth-century brought rational insights into the cause for the outbreak of icteric fever, which was also called by other names like Griesinger's bilious typhoid, bilious or hepatic fever, hepatic typhoid, icteric typhoid, catarrhal icterus, and febrile icterus. Later on, the icteric infection was known as Weil’s disease, and it occurs when humans are exposed to direct contact with water, marshy land, wet soil, or mud. In 1879, spirochete species of the Borrelia genus were the cause of relapsing fever of leptospirosis. Spirochaetes, later to be identified as leptospires, were discovered by Japanese investigators in field mice and rats in 1915 [2]. Leptospires were isolated from leptospirosis patients that occurred as mixed infections with yellow fever [3, 4]. In the beginning, it was thought that dengue, sandfly fever, and leptospirosis were caused by the same organism. But later, it was confirmed that dengue and sandfly fever were caused by flavivirus and phlebovirus, respectively, and not by leptospirosis [5].

In Western Europe, *Leptospirainterrogans* serovars icterohaemorrhagiae was introduced in the 18th century by the westward extension of the range of *Rattusnorvegicus* from Eurasia [6]. There was a report published in the landmark Institute of Medicine entitled "Emerging Infections: Microbial Threats to Health in the United States"; herein, leptospirosis was used as an example of an infection that had in the past caused significant morbidity in military personnel deployed in tropical areas [7]. A new infectious disease was identified from cattle in Georgia in 1935. This infection was associated with icterus, bloody urine, and skin necrosis. The mortality rate ranged between 10 and 60 percent. In 1938, it was reported convalescent serum from such infected cattle agglutinated Leptospira grippotyphosa in high titer. It was found that the disease was transmitted by mice. Convalescent sera served as an effective means of treatment [8, 9].

Veterinarians, farmers, sewer workers are at high risk of contracting the disease. Recreational activities involving freshwater, foreign travel, or a combination of both spike the chances of being infected with leptospirosis infection [10]. During World War I, outbreaks were confirmed in French, German, and British troops [11]. This is due to favorable conditions conferred by trench warfare which include water and rat manifestation. "Fort Bragg Fever", a form of leptospirosis, led to hospitalization of 40 soldiers at Ft. Bragg in North Carolina in the summer of 1942 [12]. In 1961, US military troops were also infected with the pathogen. In 1987, Japanese troops were infected during a water-borne outbreak in Okinawa [13].

Recently, a surge has been witnessed in the number of leptospirosis cases partly due to incessant rain, environmental factors such as polluted water, poor waste management, *etc*. in Ernakulam district, Kerala India. Despite the availability of a drug with proven efficacy as prophylactic against leptospirosis, the refusal of people to take the drug, given at free of cost continues to baffle the district health department [14].

EPIDEMIOLOGY

Epidemiological studies on this subject have been conducted worldwide with the purpose to understand the types of risk factors associated with this disease, and what action needs to be taken to prevent further spread out. However, no answers have been provided as to what causes this illness to a specific individual.

The higher incidence of leptospirosis is located in tropical countries like Latin America, India, Southeast Asia [15, 16]. The division of WHO known as Leptospirosis Burden Epidemiology Reference Group (LERG) conducted a systematic literature review that estimated global annual incidence of endemic

CHAPTER 3

Phage Therapy as an Alternative Antibacterial Therapy

Nachimuthu Ramesh[1,*], Prasanth Manohar[2], Archana Loganathan[1], Kandasamy Eniyan[1] and **Sebastian Leptihn[2,3,4]**

[1] Antibiotic Resistance and Phage Therapy Laboratory, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore, India

[2] Zhejiang University-University of Edinburgh (ZJU-UoE) Institute, Zhejiang University, Haining, Zhejiang, P.R.China and The Second Affiliated Hospital Zhejiang University (SAHZU), School of Medicine, Hangzhou, P.R.China

[3] Department of Infectious Diseases, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, P.R. China

[4] Infection Medicine, Biomedical Sciences, Edinburgh Medical School, College of Medicine and Veterinary Medicine, The University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ, United Kingdom

Abstract: Antibiotic resistance is one of the growing concerns in healthcare settings. Most of the clinical and community (bacterial) strains have grown immune to almost all the available antibiotics. The discovery of new antibiotics or resurging of available antibiotics has failed to outcompete the growing resistance within the bacterial community. Thus, finding an alternative antibacterial modality to treat infectious diseases has become a significant objective among the scientific community around the globe. Phage therapy is one such an antibacterial therapy for the treatment of severe bacterial infections. The bacteriophages (or phage) are viruses that prey on bacteria for their multiplication and survival. Discovery of bacteriophage dates back to the early 1910s when Frederick W. Twort and Felix d'Herelle observed bacteriolytic activity. Before the discovery of antibiotics, phages were the choice of treatment against bacterial infections, but with the inconsistent research, phage therapy lost its importance in the therapeutics. With the emergence of antibiotic resistance, phage therapy and phage research has got a shape to revolutionize the growing bacterial infections. Phage therapy has shown promising results against severe bacterial infections in the circumstances where antibiotic treatment is ineffectual. This emergency has shed light on this forgotten therapy. This chapter will elucidate the history and fundamentals of phage biology and its significance in treating infectious diseases. With the special focus on advancements in phage research and their clinical outcomes which supports the use of phage therapy in humans. It also deals with the

[*] **Corresponding Author Nachimuthu Ramesh:** Antibiotic Resistance and Phage Therapy Laboratory, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore, India; Tel: 9842660673; E-mail: ramesh.n@vit.ac.in

Parvesh Singh, Vipan Kumar & Rajshekhar Karpoormath (Eds.)

regulatory inputs required for phage therapy and the commercialization strategies undertaken by pharmaceuticals in the globalization of phage medicine. Besides, the authors would like to brief on the personalized phage therapy and their evolution from lab to bedside endpoints for treating the patients and other future perspectives that hold promise.

Keywords: Antibacterials, Antibiotic resistance, Bacteriophages, Bacterial pathogens, *Caudovirales*, Phage resistance, Phage therapy.

INTRODUCTION

Phage therapy is the use of live bacteriophages (or simply phages) to treat infections caused by pathogenic bacteria. Bacteriophages are known to infect and replicate inside the host bacterium. Bacteriophages are thought to be the widespread, abundant and diverse microorganisms on Earth. The use of bacteriophages for the treatment of bacterial infections can be dated back to 1917; when the bacteriophages were found to kill pathogenic bacteria that were causing diarrhoea and cholera. Though there were improvements in bacteriophage exploration in the 1920s, the discovery of penicillin in 1928 and the subsequent introduction of antibiotics in the 1940s had reduced the applications of bacteriophages in therapy [1]. Antibiotics are antibacterial compounds that are used to kill or inhibit bacteria. Since its introduction as an antibacterial agent, antibiotics have saved millions of lives. As the need for antibiotics started increasing, many pharmaceutical companies were involved in large scale drug discovery from the 1950s to early 1990s. Over half a century antibiotic ruled the medicinal world, until, in the late 1990s the problem of antibiotic resistance has shaken the healthcare sector [2]. Unfortunately, the easy availability of antibiotics paved the way for its misuse and overuse, which eventually leads to resistance among bacteria. As the problem of resistance started increasing, the antibiotic developmental pipeline also becomes dry causing a major crisis to treat bacterial infections. The majority of the infections caused by multi-drug resistant (MDR) bacteria are becoming untreatable and the resistance towards last-resort of antibiotics such as carbapenem, colistin and tigecycline are posing serious threats [3].

Untreatable bacterial infections are causing high mortalities throughout the globe and it was estimated that about 10 million deaths per year by 2050 due to antibiotic-resistant infections [1]. The bacteria that cause hospital-acquired infections (HAI), tuberculosis, co-infections and secondary bacterial infections are becoming difficult to treat. This growing antibiotic crisis is causing a huge burden to the patients both economically and health-related. Though few of the major contributing companies have already stopped their search for new antibiotics, there is still a huge sum of funding available for antibiotics research [4]. The

problem with discovering new antibiotics is that there is no guaranty that the bacteria will not develop resistance again. Therefore, there is a need for alternative therapy or non-antibiotic therapy that could be safe, cost-effective, reliable and advantageous. The renewed interest in phage therapy is mainly due to the increasing antibiotic-resistant bacteria and to treat the infections caused by multi-drug resistant bacteria.

History: From the Vault

The historical evidence for the discovery bacteriophages can be dated back to 1880's, even though the scientific literature explaining these bacteria-eaters are very minimal. Accordingly, in 1896 Hankin reported the antiseptic properties in the water samples collected from Jumna and Ganges Rivers in India [5]. Hankin showed that the antiseptic properties in the water could kill the bacteria, *Vibrio cholerae*. In 1901, Emmerich and Löw described a substance that was causing lysis in the autolysed cultures and it might cure experimental infections but no further experiments were reported [6]. Though there were literature evidences about the reports on bacterial autolysis by Scientists, Gamaleya, Malfitano, Kruse, and Pansini in 1900's, there was no concluding evidence about the action of bacteriophages [7]. Successful improvements in bacteriology, especially bacterial cultures, made significant improvements in bacteriophage research as well, where localized bacteriolysis (plaques) was observed in later years.

The year 2015 marks the century into the 'discovery of bacteriophages' because Frederick W.Twort published his bacteriophage research in 1915 which was considered as the beginning of 'modern phage research'. It was Félix d'Herelle who started working with Twort's phenomenon of 'glassy transformation' and it was later described as bacteriophages. In 1917, d'Herelle described about 'microbes' that were able to lysis the bacteria in the liquid cultures and formed patches on the agar surface, which was termed as 'plaques' [8]. d'Herelle considered them as ultraviruses and later named as 'bacteriophages'. At the Pasteur Institute in Paris, d'Herelle started working with dysentery samples (enteric pathogens) where he identified the lysis of pathogenic bacteria and the formation of clear spots (called as virgin spots) by the invisible microbe. His concept of bacteriophages as the bacterial parasite was found logical. He also hypothesised that bacteriophages require living cells to multiply and interpreted the life cycle as, infection, multiplication, release and reinfection. d'Herelle's research was focused on, (i) the biology of bacteriophages- to understand the biological nature of bacteriophages, and (ii) the therapeutic use of bacteriophages- to cure infectious diseases caused by bacteria. Though the biological nature of bacteriophages was not completely understood, the use of bacteriophages in therapy had overshadowed other biological studies. As soon as d'Herelle observed

CHAPTER 4

Quorum Sensing Inhibitors from Natural Products: A New Anti-infective Approach

Debaprasad Parai[1,*], Pia Dey[2] and **Samir Kumar Mukherjee[2]**

[1] *ICMR-Regional Medical Research Centre, Bhubaneswar, India*

[2] *Department of Microbiology, University of Kalyani, Kalyani, West Bengal, India*

Abstract: Currently, the World Health Organization (WHO) considers antibiotic resistance a serious threat to the treatment of infectious diseases. Moreover, the WHO has promoted a complex action plan, based on the slogan "no action today, no cure tomorrow" to control the occurrence and spread of resistant strains that include strategic actions for mitigation, prevention and control. In the last 50 years, only three new classes of antibiotics have been approved by WHO and US Food and Drug Administration (FDA), among which the third one was only approved this year and found to be effective against the Gram-negative Enterobacteriaceae group for the first time after 1962. Keeping all the above facts of continuous emergence of antibiotic resistance in planktonic bacteria and in their biofilm counterpart, this book chapter has tried to add some more alternative drug resources by means of natural products, which could ultimately lead to the betterment of humankind. Several recent studies have shown that natural products (mainly phytochemicals) exhibit their antibacterial activity through different mechanisms of action like bacterial membrane damage, inhibition of virulence factors, quorum sensing signalling, inhibition of enzymes and toxins. Quorum sensing is a signalling process to regulate the expression of several virulence factors in both Gram-negative and Gram-positive bacteria via an autoinducing loop. When a critical bacterial cell density is reached, a complex of the regulatory proteins and specific signalling molecules enable the autoinduction of the quorum sensor and the expression of the target genes. Quorum sensing inhibitors (QSIs) interfering with this regulatory network have either been derived from natural sources *viz.* phytochemicals and fungi, or they have been chemically synthesized. In this chapter, we will summarize the updates from the available literature describing the various QSIs obtained from natural sources and their role as anti-infective agents. We will also discuss the feasibility of these sources towards the development of future drugs to cure systematic infections in the human body.

Keywords: Alternative drug, Antibiotic, Antimicrobial resistance, Anti-infective agent, Infectious disease, Natural product, Pathogen, Quorum sensing, Quorum sensing inhibitor, Virulence factor.

[*] **Corresponding author Debaprasad Parai:** ICMR-Regional Medical Research Centre, Bhubaneswar, 751023, India; E-mail: debaprasad.bio@gmail.com

Parvesh Singh, Vipan Kumar & Rajshekhar Karpoormath (Eds.)

ANTIMICROBIAL RESISTANCE AND ITS MECHANISMS

Antimicrobial resistance (AMR) is defined as the inability of the existing drugs to completely eliminate the target microorganisms. After the discovery of the first commercialized antibiotic, penicillin, by Sir Alexander Fleming in 1928, there was no looking back. It was succeeded by some overwhelming discoveries of a wide variety of antibiotics. But eventually, pathogens started developing resistance against existing drugs and later acquired resistance against subsequent newly discovered drugs. The scenario even worsened by the advent of multi-drug resistant (MDR) pathogens which resisted the action of many antibiotics and chemotherapeutic agents. Multi-drug resistance in bacteria occurs by the accumulation of resistance (R) plasmids or transposons, with each gene coding for resistance to a specific agent, and/or by the presence of multidrug efflux pumps which can pump out one or more than one type of drug [1 - 3].

AMR threatens the effective prevention and treatment of an ever-increasing range of infectious microorganisms like bacteria, viruses, parasites and fungi. In nature, antimicrobial resistant organisms are naturally developed with the course of time due to genetic mutations following the natural process of evolution, and then disseminated among humans, animals, food, plants and environment. Among various grounds, some of the common reasons that can lead to the development of AMR are nature's selection pressure, mutation, and horizontal gene transfer (HGT) along with inappropriate use of broad-spectrum antibiotics. It is only in the past few years that gene exchange has been established as a universal property of bacteria that has occurred throughout eons of microbial evolution [4]. Moreover, the importance of HGT in genomic evolution was strengthened by the presence of putative bacterial gene sequences in eukaryotic genomes. Other notable factors contributing to unrestrained occurrences of resistance are identification and distribution of genomic islands carrying genes for pathogenicity along with plasmid-mediated transfer of antibiotic resistance [5]. Most of the bacterial pathogens have evolved into MDR forms with enhanced morbidity and mortality due to high levels of mutation rate [4]. Many MDR strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Clostridium difficile*, *Salmonella enterica*, *Mycobacterium tuberculosis*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Neisseria gonorrhoeae* and *Candida auris* have been observed over the past half-century to cause a variety of diseases in humans [6, 7].

AMR is a major threat to public health and leads to economic losses. A global collaborative approach to fight against AMR is required from all concerned sectors that are affected directly or indirectly. The inability to treat common infections is directly rooted with antimicrobial resistance, which, in turn is caused

by the emergence and spread of drug-resistant pathogens that have acquired new resistance mechanisms. Unfortunately, the spread of antibiotic resistance is a complex web where the path is interconnected, including the food chain, healthcare facilities and the environment. The rapid spread of multi- and pan-drug resistant bacteria (also known as "superbugs") that cause infections, which are not cured by existing antimicrobials like common antibiotics, is a matter of grave concern. Another outstanding factor that influences and enhances AMR is the occurrence of microbial biofilms [8 - 10]. According to World Health Organization (WHO), with the global rise in AMR, the recalcitrant nature of biofilms is becoming a major concern affecting the healthcare setup, threatening medical procedures, endangering sustainable pharmacological progress and ultimately causing an economical burden. The situation further deteriorates due to the lack of new antimicrobials in the clinical pipeline, lack of access to quality antimicrobials and serious antibiotic shortages affecting entire health-care systems. In 2019, WHO identified 32 antibiotics in clinical development that address the WHO list of priority pathogens, out of which only six were designated to be innovative [11]. The world might be on the inception of a "post antibiotic era," as antibiotics are becoming increasingly ineffective, accompanied by global spread of drug-resistance, which finally ends up producing a more difficult situation in treating infections and causing enormous loss of life. The fundamental approach in treating AMR across the globe primarily lies in the outlook of people towards responsible use of antibiotics [9, 12].

Antimicrobial resistance is reported from all regions of the world. Modern travel of people, animals, and goods accelerates the easy spread of antimicrobial resistance across borders and continents. For example, the rate of resistance towards ciprofloxacin, an antibiotic commonly used to treat urinary tract infections, increased from 8.4% to 92.9% for *E. coli* and from 4.1% to 79.4% for *K. pneumonia*, in countries reporting to the Global Antimicrobial Resistance Surveillance System (GLASS), a program launched to support the global action plan on antimicrobial resistance in collaboration with the WHO AMR Surveillance and Quality Assessment Collaborating Centres Network [13].

CURRENT UPDATE ON ANTIMICROBIAL RESISTANCE IN CHRONIC BACTERIAL INFECTIONS

The presence of large gaps in the existing surveillance system has restricted the prediction of the exact scenario of antibiotic resistance across the globe. The Centres for Disease Control and Prevention (CDC) and WHO are two global surveillance agencies that work tirelessly to control the introduction and spread of infectious diseases along with providing consultation and assistance to other nations and international agencies to assist in improving their disease prevention

CHAPTER 5

Nitrogen and Oxygen-based Heterocycles as Potential Anti-Infective Agents

Shaik Baji Baba[2], Naresh Kumar Katari[1,2,*] and **Rambabu Gundla[1]**

[1] *Department of Chemistry, School of Science, GITAM Deemed to be University, Hyderabad, Telangana-502329, India*

[2] *School of Chemistry & Physics, College of Agriculture, Engineering & Science, Westville Campus, University of KwaZulu-Natal, P Bag X 54001, Durban-4000, South Africa*

Abstract: The success of Anti-infective agents (AIAs) is determined by the interaction between a drug and the binding sites. Significant contributions have been made to the synthetic and dynamic relationship between drugs and pharmacodynamics for the past few years. In general, AIAs include antibacterial, antiviral, antifungal, and antimicrobial agents, *etc*. The clinical benefit of using anti-infecting agents significantly impacts bloodstream infections with central venous catheters (CVCs), pregnancy, and lactation. Recent reports suggest income from AIGs formed 30.1% of the total income of hospitality management. However, many more technical difficulties remain, such as acquiring biologically relevant chemical diversity and achieving activity across diverse pathogens, including highly challenging Gram-negative pathogens with safe drugs. This chapter reviewed 1,2,4-triazoles, isatin, and coumarin-based anti-infective agent developments from the past five years and their biological studies against the various bacterial strains based on new challenges on viral and bacterial infections and viruses. SAR studies also discussed the importance of hybrids and substitutions in biology. We believe that this chapter helps future researchers to develop the most effective and less toxic anti-infective agents.

Keywords: Anti-infective agents, Coumarin, Isatin, Toxicity, Triazole.

INTRODUCTION

Viruses include a large group of pathogens, which are the main ones responsible for producing different infectious diseases. More than 40% of the deaths worldwide are associated with various infectious diseases. From the past few dec-

[*] **Corresponding author Naresh Kumar Katari:** Department of Chemistry, School of Science, GITAM Deemed to be University, Hyderabad, Telangana, INDIA; & School of Chemistry & Physics, College of Agriculture, Engineering & Science, Westville Campus, University of KwaZulu-Natal, Durban, South Africa;
E-mail: dr.n.k.katari@gmail.com

Parvesh Singh, Vipan Kumar & Rajshekhar Karpoormath (Eds.)

ades, the development of antiviral agents has played a pivotal role in the research. In the past 50 years, antiviral drugs directly targeted viral proteins and inhibited viral activity, showing better activity [1]. The world is facing different dangerous viruses such as Ebola, Spanish flu, cholera, *etc*. In 2019-2020, we fought with the most destructive virus, COVID-19 [2]. While developed nations were locked down and quarantined, they have also been struggling to establish a new class of antiviral drugs to kill the new type of Coronavirus. For the past few years, infectious diseases have been the leading cause of death worldwide [3]. In the 1940s, penicillin was introduced as an antibacterial agent from natural products. Natural and synthetic antibiotics have significant effects on human health. However, the treatment of viral and bacterial infections is a challenge because of a growing number of viruses and bacteria, which are multidrug-resistant microbial pathogens [4]. Many AIAs have been available in the market for the past few decades, but the need to develop a new class of drugs is required to minimise the side effects and toxicity. Reports from WHO indicate that diseases like pneumonia, an acquired immunodeficiency syndrome (AIDS), chronic liver disease, chronic obstructive lung disease, and neoplastic diseases like stomach cancers, cervical cancers, and liver cancers are the primary cause of human death worldwide. The severity of infectious diseases causes deaths every year due to AIDS (2.5 million), tuberculosis (1.8 million), and other diseases (more than one million due to malaria, dengue, and chikungunya) as per WHO reports [5]. The *Staphylococci, Pseudomonas aeruginosa*, and *Enterococci* are significant contributors of nosocomial type infections, which have also increased over the past few years. This infection is also known as hospital-acquired infection and is mainly transmitted *via* healthcare workers, hospital devices, and patients [6].

Antibiotics are the second most needed category of medication used in medical treatment. The mechanism of antibiotics includes bactericidal (bacteria killers) or bacteriostatic (inhibits microorganism multiplication). Most commonly known active bactericidal agents including penicillins, cephalosporins, and aminoglycosides such as neomycin, kanamycin, gentamicin, tobramycin, streptomycin, amikacin, and a few other bactericides are colistin, vancomycin, bacitracin, and polymyxin B. Bacteriostatic agents are clindamycin, sulphonamides, lincomycin, chloramphenicol, trimethoprim, erythromycin, and tetracyclines [7]. At present, inappropriate antibiotic usage and antibiotic resistance are major global issues because of a direct relation between antibiotics and antibiotic resistance developments. More than 85% of *Staphylococcus aureus* strains are resistant to penicillin and β-lactams in USA hospitals. Antimicrobial agents destroy the microorganism's structural parts or performed by interfering in microbial biosynthetic functions. Infective agents like chloramphenicol, tetracyclines, lincomycin, streptomycin, *etc*., damage the protein synthesis and affect ribosomal subunits.

In 1963, Idoxuridine was the first antiviral drug introduced on the market. After that, nearly 95 antiviral drugs were approved in the past 50 years. Several types of antiviral drugs are developed for medical use to save human beings from viruses.

From 1993 to 2019, more than 110 antiviral drugs have been approved to treat nine different human viral infectious diseases. Few are under clinical trial phases, and thousands of antiviral agents and inhibitors are reported by multiple researchers globally [8]. The approved antiviral drugs are mainly characterised into thirteen functional groups to treat nine human viral infection diseases, shown in Table **1**.

Table 1. Characterization of approving antiviral functional groups for nine human infectious viral diseases [8].

S. No	Viral Infection	Antiviral Agents/Class
1	Human immunodeficiency virus (HIV)	Protease inhibitors, integrase inhibitors, nucleoside reverse transcriptase inhibitors, acyclic nucleoside phosphonate analogues
2	Hepatitis B virus	Lamivudine, interferons, nucleoside analogues, *etc*.
3	Hepatitis C virus	Ribavirin, NS3/4A protease inhibitors, NS5A inhibitors, NS5B polymerase inhibitors
4	Herpesvirus infections	5-substituted 2'-deoxyuridine analogues, pyrophosphate analogues, acyclic guanosine analogues
5	Influenza virus	Ribavirin, RNA polymerase inhibitors, and neuraminidase inhibitors, matrix two protein inhibitors
6	Human cytomegalovirus infections	Acyclic guanosine analogues, pyrophosphate analogues, and oligonucleotides
7	Varicella-zoster virus infections	Acyclic guanosine analogues, 5-substituted 2'-deoxyuridine analogues, and antibodies
8	Respiratory syncytial virus infections	Ribavirin and antibodies
9	Human papillomavirus	Imiquimod, podofilox and sinecatechins

More effective antiviral drugs/vaccines to treat against emerging viruses such as the Ebola virus, Coronavirus, *etc*., are not available in the present market. In the antiviral drug development journey, most of the approved antiviral agents are chemically synthesised hybrids. However, natural products play a prominent role in developing antivirals by providing insights into chemical compound synthesis for antiviral drugs [9]. Based on their broad spectrum, antiviral agents kill and inhibit the reproduction of viruses.

This chapter highlights the recent developments of 1,2,4-triazoles, isatin, and coumarin-based anti-infective agents such as antiviral and antibacterial agents based on their applications in medicinal chemistry against viral and bacterial strains. Many research studies have been reported from the past few years. All

SUBJECT INDEX

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

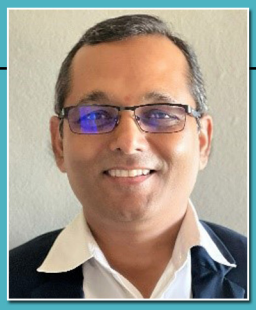
Z

PARVESH SINGH

Dr. Parvesh Singh is an NRF-rated researcher in the School of Chemistry and Physics (SCP) at the University of KwaZulu-Natal (UKZN), Durban (South Africa). After completing his Ph.D. degree (Organic Chemistry), in 2006, from the Guru Nanak Dev University (India), Dr. Singh joined a pharmaceutical company (Jubilant Organosys) as a Research Scientist and then moved to South Africa to pursue his Post-doctoral studies at the Durban University of Technology. In 2014, Dr. Singh was appointed as a Senior Lecturer at UKZN and then promoted to Associate Professor in the same institution. Dr. Singh's research primarily focuses on the synthesis of heterocyclic scaffolds and their biological testing and molecular modelling studies. Dr. Singh is a recipient of a prestigious DST-NRF Free-standing fellowship and has been featured as one of the emerging researchers in South Africa by the National Research Foundation (NRF). He has also been a recipient of top University and College research awards on several occasions. He has co-authored more than 100 research papers in top tier international journals and serving as an editorial board member and ambassador for several reputed publishers.

VIPAN KUMAR

Dr. Vipan Kumar Ph.D. has been working as an Assistant Professor in the Department of Chemistry, Guru Nanak Dev University, Amritsar, since 2009. He obtained his Ph.D. with Prof. Mohinder P Mahajan in the Department of Applied Chemistry, GNDU. In 2007, he moved to the University of Cape Town (UCT), South Africa, to pursue his post-doctoral studies with Prof. Kelly Chibale. In 2015, he was awarded a research fellowship from Carl Tryggers research foundation, Sweden to carry out research at University of Umea, Sweden. His research interests include the development of diverse synthetic protocols for the synthesis of novel molecular frameworks targeting tropical infections. He has also been engaged in the utilization of b-lactam synthon protocols for the synthesis of functionally decorated and biologically relevant heterocycles with medicinal potential.



RAJSHEKHAR KARPOORMATH

Prof. Rajshekhar Karpoormath graduated with his Ph.D. (2013) from the School of Chemistry and Physics, University of KwaZulu-Natal (UKZN), Durban, South Africa. He is currently an Associate Professor and Academic Leader of Research in the College of Health Sciences, UKZN. He is also a nationally recognized rated researcher by National Research Foundation-South Africa (NRF-SA). Prof Karpoormath started his independent research carrier in 2014 and established a research group, Synthetic and Medicinal Chemistry Research Group (SMCRG), at UKZN. Over the years, he has successfully secured several national and institutional grants and has established a well-equipped Drug Discovery Laboratory at UKZN. His research work is focused on target-based drug design and synthesis, Methodology development, and development of nanomaterial-based electrochemical biosensors. Since 2014, his research group has published more than 100 peer-reviewed articles in international journals, four book chapters, and two patents. He has successfully graduated 23 postgraduate students (12 Ph.Ds. and 11 Masters) since 2014. He is one of the top researchers at UKZN and was the recipient of several awards in recognition of his achievements.