Resistance to Antibiotics
Are We Prepared to Handle This Growing Ghost?

Edited by
Ashima Kushwaha Bhardwaj
Preface

Drug resistance is a problem that was forecast in the noble award lecture of Dr. Alexander Fleming, is witnessed by doctors but somehow neglected or not acknowledged amply by the governments and the society. This has led to a serious predicament in the recent times where mankind is afflicted with deadly superbugs and the magic of antibiotics is dwindling fast. The situation demands immediate attention and requires absolute commitment to eradicate this problem worldwide. While giving the final touches to this book, came the news from USA, of a woman who died of septic shock and was untreatable by any of the known 26 antibiotics. Extremely drug resistant and totally drug resistant tuberculosis reported from Hinduja Hospital, Mumbai, India and the spread of NDM-carrying superbugs, are very few examples of the problem of MDR rampant in these times. The WHO has sounded an alarm bell. Appearance and spread of resistance to last resort drugs like carbapenem and colistin is turning into a nightmare. A very recent visualization of the evolution of drug resistant bacteria in a matter of 10 days by Harvard Medical School is a clear indication of the problem at hand.

The governments as well as entire communities of scientists, clinicians, social workers and industry have started realizing the serious consequences of this affliction. A lot has been written about the burgeoning problem of multi drug resistance by stalwarts in this field. This book is yet another attempt to showcase the various aspects of this problem. Coming from the fields of antibody engineering, phage display, diagnostics and malaria vaccine in the early stages of my career, when I first embarked on my journey with drug resistant microbes, my initial reaction was of being engaged in some medieval science. On the contrary, in the last ten years of this journey, each day has been full of surprises with these microbes changing themselves so fast for their survival, the mind boggling diversity of genes and mechanisms that they utilize for this purpose and also how DNA sequences tell so much about these organisms and their evolution. The main aim behind the book was to compile the views about this problem of MDR from various sections of the society. It contains perspectives from scientists/academicians, clinicians, industry representative, policy maker and naturopathist. Therefore, this book has been intended for a wider readership where students, researchers, medical practitioners, industrial entrepreneurs and every individual of the society can explore various aspects of MDR. It is sincerely hoped that the readers would enjoy and benefit from these views and join this drive of mankind to live with these microbes, understand them, benefit from them and vanquish them whenever required.

Dr. Ashima Kushwaha Bhardwaj
Dr. Ashima Kushwaha Bhardwaj has been working at the Indian Institute of Advanced Research (IIAR), Gandhinagar, Gujarat, India, since 2006. At IIAR, she directs the Department of Human Health and Diseases with a major focus on the molecular epidemiology of antibiotic resistance. Dr. Bhardwaj’s current research is centered around unraveling the mechanisms of spread of antibiotic resistance amongst clinical isolates of microbes causing major diarrheal diseases in South East Asian peninsular regions. As an Associate Professor, she also teaches undergraduate and graduate courses at the University of IAR, Gandhinagar. She has directed several post-graduate and Ph.D scholars’ theses at IIAR. Her research has been published in leading peer-reviewed journals and has been internationally acclaimed. Dr. Bhardwaj completed her education from the Delhi University including doctoral work on the nuances of antibody engineering using phage display and development of novel recombinant fusion proteins for use as diagnostic reagents. Later, she worked at International Centre for Genetic Engineering and Biotechnology at New Delhi for understanding the biochemical role of key proteins and enzymes of the malarial parasite. Another aspect of her work at ICGEB revolved around identification and development of recombinant vaccine candidates from the liver and asexual blood stages of the parasite. Over the years, Dr. Bhardwaj has attracted grants from the research funding agencies such as Departments of Biotechnology of both Government of Gujarat and Government of India and the Indian Council of Medical Research. She is academically associated with leading Universities of the country and has active research collaborations at other research Institutions. Dr. Bhardwaj is a recipient of many prestigious awards throughout her research career such as Prof. Umakant Sinha Memorial award of the Indian Science Congress Association, the Young Scientists Award of the International Union of Biochemistry and Molecular Biology and the Best Research Group Award of IIAR.
Acknowledgements

Editing this book has turned out to be an unexpectedly smooth journey. This has been made possible because of many of my colleagues, collaborators and family whom I wish to thank here. First and the foremost, The Almighty God, who gave me strength to accept this challenge in the year when a number of my doctoral students had submissions of their theses, and I was deep down in my academic obligations. Each assignment is a team work of many people on and off stage. Though myself and the authors would be the face of this book, I am particularly grateful to Ms. Sara Williams, Omics group, for her help, editorial assistance and advice that made the sailing smooth. Each of the authors have been highly supportive and co-operative. I gratefully acknowledge their patience and valuable contributions, and admire their faith in me. I wish to thank my colleagues Mr. Priyabrata Mohanty, Dr. K. VinothKumar and Mrs. Neha Rajpara, for being their always, whenever I needed them. A very special thanks to Vinoth for his support and the immense help that he provided during the preparation of this book with his occasional coffees. I thankfully acknowledge the support from the Puri Foundation for Education in India and especially Mr. Ashwani Puri, for allowing me independence in my scientific and literary pursuits. The financial support provided by the Gujarat State Biotechnology Mission, Department of Science and Technology, Government of Gujarat (No. GSBTM/MD/PROJECTS/SSA/1535/2013-14) for this e. book is gratefully acknowledged. This endeavour would not have been possible without the unending and unconditional support from my family- I sincerely appreciate their faith in me and their patience while I was handling such projects. Finally, I wish to thank all my readers in anticipation and urge them to share their opinion about this book and its chapters.

Ashima Kushwaha Bhardwaj
This book is dedicated to my daughter Prerita and my soulmate Devesh, who have always brought me immense joy and zest for life and inspired me to excel in all my endeavours.
Multi drug resistance (MDR) observed in a wide range of pathogens of bacterial, viral, fungal or parasitic origin and cancer cells has emerged as a global public health concern. Diminishing glory of the antibiotics has led to the search for newer strategies for future therapies. According to the WHO reports released in 2014, we are fast heading towards post-antibiotic era. The global dissemination of antibiotic resistance and the genes encoding such resistance phenotypes is due to horizontal gene transfer that utilizes diverse processes and diverse genetic elements such as mobile genetic elements. Swift and wide dissemination of the drug resistance genes crosses the species and genera barriers. Therefore, to search for the newer targets for antibiotic production, newer drugs and newer mechanisms, it is of paramount importance that the mechanisms that lead to the origin, evolution and dissemination of MDR be understood in greater details. A large body of work has been carried out across the globe to decipher the structures, mechanisms and functional aspects of mobile genetic elements such as integrons, plasmids and transposons and chromosome-borne resistance factors, to appreciate their diversity and their role in dispersal of MDR genes. The present eBook is aimed at understanding the overall philosophy of drug resistance.

The problem of MDR affects everyone in the society and therefore, each individual is a stakeholder here. There are few important features of this book. it has been attempted to include people from different walks of life, that is, scientists, clinicians, pharmaceutical industry, policy makers and naturopathist on a single platform. I feel this is a unique feature of this book and I hope that readers appreciate this. Pharmaceutical industries have a vital role to play in the drug discovery programmes. The current status of the antimicrobial drug development, alternatives to conventional antimicrobials and various bottlenecks for drug discoveries, commercialization and marketing have been reviewed in this book with special focus on Indian industries. Another important feature of this book is that a believer of naturopathy has made a contribution in this forum. I believe that most of the problems can be solved with extreme caution and simple strategies that are based on the mother nature. Mrs. Raghavan’s chapter would support this belief as she describes natural plant based products used in every kitchen as alternatives for antibiotics that may have side effects. A special section (Is the scare of MDR real?) has been included at the end of this book to have views from the scientists and clinicians about the gravity of this problem in the form of a commentary. Very importantly, this book has a chapter where all the national and international policies/steps taken by various governments have been concisely presented by Dr. Abdul Ghafur of “Chennai Declaration” fame.

Chapter 1 is intended to be an introductory chapter to prepare the readers with the overall knowledge of the MDR problem. In Chapter 2, Vibrio spp and Shigella spp. have been taken as examples to explain the problem of MDR and its
consequences. Chapter 3 presents details on ICU-related pathogens and a vivid description of antibiotics as treatment options for these deadly MDR pathogens, with advantages and disadvantages of using each drug. Chapter 4 is a perspective from pharmaceutical industries about the drug discovery and various roadblocks. It describes with clarity and examples, how can the wheel of antibiotic discovery be kept rolling. Chapter 5 is an exhaustive review of the gravity of the problem of MDR and the newer strategies as alternatives to conventional antimicrobials. It also describes the simple animal models that are utilized for the evaluation of new drugs and various in silico tools utilized for drug discovery. Chapter 6 outlines the policies made at national and international level to tackle the problem of MDR. Chapter 7 is one of the unique chapters in this book where nutritious simple diet and life style choices have been advocated as the order of the day for the holistic health and a simple alternative to MDR. The writing style and the tone for this chapter is different from the other chapters of this book that are filled with complicated technology-derived solutions to the MDR and replete with scientific jargon. The last section in this book “Is the scare of MDR real?” presents the views from four eminent scientists and clinicians about the problem of MDR. Dr. Abdul Ghafur and Dr. Neelam Taneja have very vividly painted a real life scenario of how MDR translates into high mortality of patients with minor problems such as cholera which is very touching, especially in the case of children. Dr. Amit Ghosh and Dr. Thandavarayan Ramamarthy share their experiences as researchers about how the antibiotics are used inappropriately in some hospitals leading to an imminent disaster.

Happy reading...

Ashima Kushwaha Bhardwaj
# Table of Contents

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Chapters</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Problem of MDR: An Overview</td>
<td>1-8</td>
</tr>
<tr>
<td>2</td>
<td>Multidrug Resistance in <em>Vibrio</em> Spp. And <em>Shigella</em> Spp.: Emergence of</td>
<td>9-22</td>
</tr>
<tr>
<td></td>
<td>Untreatable Pathogens</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Multidrug Resistant (MDR) Organisms in Clinical Practices</td>
<td>23-27</td>
</tr>
<tr>
<td>4</td>
<td>New Antibiotic Discovery or the Lack of it: Role of Pharmaceutical</td>
<td>28-39</td>
</tr>
<tr>
<td></td>
<td>Industry</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Alternatives to Conventional Antimicrobials: Exploring New Strategies</td>
<td>40-62</td>
</tr>
<tr>
<td>6</td>
<td>National and International Initiatives to Tackle the Challenge of</td>
<td>63-68</td>
</tr>
<tr>
<td></td>
<td>Antimicrobial Resistance (AMR)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Naturopathy and Multiple Drug Resistance</td>
<td>69-77</td>
</tr>
<tr>
<td>8</td>
<td>Is the scare of MDR real?</td>
<td>78-80</td>
</tr>
</tbody>
</table>
Abstract

Antibiotics, the magic bullets first used in 1940s, have vanquished many disease-causing bugs. However, the bacterial resilience to these wonder drugs demands newer strategies and molecules to control the formidable pathogens that have become a bane for hospitals and communities. Multidrug Resistance (MDR) encompasses all the bacterial species (pathogenic/non-pathogenic) and covers all the existing antibiotics and therefore impedes social, political and economic growth of any region or country. Antibiotics kill bacteria by interfering with the vital housekeeping functions in a bacterial cell such as cell wall formation, protein synthesis, DNA and RNA metabolism. As a survival strategy, the bacteria have evolved various mechanisms to outwit the drugs designed to kill them. This battle between mankind to design new drugs and the superbugs to combat these drugs appears interminable. In this chapter, various aspects of MDR including the reasons for this problem, consequences and possible solutions have been discussed. It is amply clear that the solution would involve a multifaceted approach on a global scale from various sections of society including the government, citizens, medical and research community and pharmaceutical companies.

Keywords: Antibiotics, Multidrug resistance, Pathogens, Pharmaceutical companies, Governments

Introduction

Antibiotic resistance is the phenomenon due to which a microorganism originally sensitive to a drug, acquires the ability to withstand this drug that was designed to kill it. When an organism becomes refractory to many of these drugs, the phenomenon is known as Multi Drug Resistance (MDR). Mankind has derived remarkable benefits from the antibiotics which are now at the brink of losing their charm owing to the problem of MDR. In spite of many years since discovery of antibiotics and their use in treating human infections, there is much to learn about these wonder drugs and the arsenal of genes in bacteria that are rapidly making these drugs obsolete. MDR is a global problem felt more strongly in under developed or developing countries. Therefore, the solutions for this problem need to be exercised at global level because bacteria can travel across the globe with their battery
of drug resistance genes and disseminate them across their fellow bacteria crossing the genera and species barrier [1]. It is feared that if steps are not taken to tackle this problem soon, by 2050, 10 million lives a year would be at risk amounting to the economic losses of 100 trillion US Dollars [2]. Though the term MDR could apply to many organisms such as bacteria, fungi, parasites, viruses and also cancer cells, this chapter would discuss only the antibacterials. The chapter presents an overview of the problem of MDR, briefly discussing the reasons for it and possible solutions to mitigate it. As the readers go through the book, most of the topics viewed in this chapter would be discussed with their details in subsequent chapters by seasoned authors with expertise in their respective fields related to antibiotic resistance.

Origin of Antibiotic Resistance Genes

The presence of drug resistance genes predates the antibiotic era. Their provenance in the antibiotic biosynthesis pathways in the antibiotic-producing strains or as part of normal metabolism in the bacterial cell explains this fact [3]. In Streptomyces, an antibiotic-producing bacterium, the cognate antibiotic resistance genes have been found clustered with the genes responsible for manufacturing antibiotics. It appears that these genes may have other important roles in cellular metabolism apart from protection [3]. The resistance genes possibly serve as bacterium’s own protection mechanism analogous to the restriction modification systems found in bacteria that evolved for the protection of host bacterium. For all cogent reasons, it would be a rule that any bacterium encodes and exercises multiple biochemical resistance mechanisms to ensure its survival from the antibiotics. Due to the ease and speed with which horizontal gene transfer takes place through the processes of transformation, conjugation and transduction, it is difficult to predict the immediate ancestors of any of the genes involved in MDR. Therefore, evolution of the myriad antibiotic resistance genes and mechanisms prevalent in nature is not easy to assess [4,5]. In the bacteria that do not produce antibiotics or that do not produce a particular antibiotic; the resistance to any antibiotic could evolve due to random mutations followed by the process of natural selection. Additionally, the resistance could be engineered by putting the bacteria under antibiotic stress forcing them to acquire mutations or it could be transferred to the bacteria by the processes of horizontal gene transfer mentioned above.

Appearance and Dissemination of Superbugs

Though the antibiotic resistance genes could be as ancient as the microbes producing them, the profligate use of antibiotics post their discovery has led to the evolution and rapid dissemination of the drug resistance genes in the environment. With large amounts of antibiotics being released into the biosphere due to their indiscriminate use in humans, aquacultures, poultry, animal farming and agriculture, it should not be surprising that microbial world acquired multiple resistance determinants to avert the catastrophe due to antibiotics. MDR, XDR (extremely drug resistant) and TDR (totally drug resistant) versions of the pathogenic agents are common these days. As a result, there has been an increase in the pathogens in the nosocomial infections as well as in communities in the form of superbugs that are either difficult to treat or entirely untreatable. Some of them are: Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, Neisseria gonnorhoeae, Vibrio cholerae and Mycobacterium tuberculosis. Mankind is now afflicted with the superbugs such as MRSA (methicillin resistant Staphylococcus aureus), VRSA (vancomycin resistant Staphylococcus aureus), NDM (New Delhi metallo beta lactamase)-carrying Klebsiella pneumoniae and XDR Mycobacterium tuberculosis.

Staphylococcus aureus is one such interesting example of the superbugs, a bane of the hospitals. As a part of human flora associated with mucous membranes and skin of the humans, this pathogen has shown to be extremely recalcitrant to antibiotic pressures. In
the 1940s, it was the bacterium in which penicillin resistance was reported. Since then, the bug has been seen in multiple avatars of MDR requiring the use of newer drugs to control this superbug. MRSA, VRSA and recently CA-MRSA (Community-acquired MRSA) have afflicted hospitals and communities from the deadly infections of pneumonia, sepsis and necrotizing fasciitis caused by the pathogen [6,7].

Factors Responsible For the Problem of MDR

The problem of MDR arises due to multiple factors inextricably linked to each other. Some of these factors are discussed below.

Social Factors

Lack of awareness in the society regarding the proper usage of antibiotics and the consequences of rising problem of MDR is one of the major reasons for this problem assuming dangerous proportions. Overuse of broad-spectrum antibiotics, use of subinhibitory concentrations of antibiotics (underuse) and unnecessary prescriptions of antibiotics in case of viral infections (misuse) leads to the development of MDR bacteria. Use of antibiotics in animal farms, aquacultures and poultry for growth promotion of the animals should not be exercised. The apathy of the pharmaceutical industries over the antibiotic development arises due to the fact that the business of these drugs in not lucrative. Antimicrobials are not lifestyle drugs that are taken throughout the life of the individuals for conditions like hypertension and diabetes. Therefore, time taken for long processes and the high costs involved in antibiotic development are very difficult to recover in the regulatory and commercial climate of any country. As far as the research scenario goes, there is not much funding available to understand the problems and processes of antibiotic resistance.

Political Factors

Government policies have a major role in deciding the status of MDR affliction in any country. Rules are drafted by the government for proper prescription of the antibiotics and appropriate sale avoiding over-the-counter sale of antibiotics, active disease surveillance, and control of the disease, research fundings to understand the problem of MDR and very importantly provide incentives to the pharmaceutical industry to engage in the research and production of old as well as newer antibiotics [8-10].

Environmental factors

Due to indiscriminate use of antibiotics in the agriculture, animal farming, medicine, aquaculture, industries and research laboratories, large amounts of antibiotics are released into the environment contaminating water, soil and food [11]. This leads to accumulation of drug resistance genes in the bacteria (pathogenic as well as non-pathogenic) thriving in the biosphere replete with various antibiotics. This might also have toxic effects on natural flora and fauna of any geographical location. No rules have yet been formulated by The Environmental Protection Agency (EPA) and The Food and Drug Administration (FDA) on the possible contamination of antibiotics in drinking water.

Genetic Factors

Some of the genetic mechanisms responsible for MDR and their examples include drug inactivation (beta-lactamases), reduced drug accumulation (efflux pumps or porins), alteration of drug target sites (mutations in topoisomerases), drug modification (acetyl transferases) and protection of drug targets (quinolone resistance-conferring QNR proteins). All these mechanisms have been reviewed in detail [10]. These factors could be borne on the chromosomes or on various mobile genetic elements such as integrons, transposons or plasmids [10]. Often, a bacterium utilizes a combination of two or more factors to mount
an attack on the antibiotics. For example, resistance to tetracycline is a result of synergy between the efflux pumps specific for tetracycline as a substrate (encoded by tetA to tetG, tetK, tetL), oxidation of tetracycline and cytoplasmic proteins (encoded by tetM, tetO, tetQ) that confer protection to ribosomes from tetracycline [12].

The transferable resistance to antibiotics was first reported in the 1950s from Shigella strains of Japan, leaving the scientific and medical community in a mode of denial. So many decades later, the problem of transferable resistance is a well-recognized, well established and acknowledged problem throughout the world. The genes harboured by mobile genetic elements are swiftly dispersed in the environment leading to their rapid dissemination among bacterial isolates. The regulatory bodies at global, country, state and district level have understood the seriousness of this catastrophe and striving to mitigate it.

Consequences of MDR

If the antibiotics lose their effectiveness, key medical procedures such as surgeries, caesarean sections, joint replacements, and treatments that depress the immune system such as chemotherapy for cancer, could become too dangerous to perform. MDR has been an economy and security threat as pointed out by Baron Jim O Neil [2]. Since there are no newer drugs and old drugs are becoming ineffective, treatment failures are rampant. Prolonged hospital stays due to MDR pathogens directly leads to higher health care budgets, higher morbidity and mortality, and lesser manpower.

Possible Solutions to Avert This Catastrophe

A multipronged approach from all the sections of society including citizens, doctors, governments, pharmaceutical industries, researchers, academicians and non-government organizations, would be required to overcome the problem of MDR. Some of the obvious solutions are listed below.

- Increase in the awareness in the society about the nature of the problem, its causes and implications and the consequences- a global public awareness campaign is an absolute requirement in order to solve the problem on a global scale
- Discourage the indiscriminate use of antibiotics in humans, animals and agriculture
- Improvement in the disease surveillance programmes
- Development of new antibiotics
- Better diagnostics for all the infectious diseases
- Incentives to be provided to the pharmaceutical industry for drug development and marketing programmes
- The big pharmaceutical companies can engage in partnerships with small biotechnology companies. Such collaborations can bring the drug candidates developed by biotech companies to the market with the costs for clinical trials, launch and marketing covered by the bigger companies [8]. It is with the well-focused endeavours of the pharmaceutical companies, a ray of hope can penetrate this prevailing darkness in the field of medicine. Many such earlier efforts have yielded phenomenal success. For example, daptomycin (manufactured by Eli Lilly and marketed by Cubist) in 2003 was used for the treatment of complicated skin and soft tissue infections caused by Gram-positive bacteria. Similarly, gemifloxacin (in-licensed from GlaxoSmith Kline) was brought to market in 2004 by Oscent Pharmaceuticals for the treatment of pneumonia and chronic bronchitis. Both these companies, Cubist and Oscent Pharmaceuticals started on research platforms. Such mergers, partnering and acquisition of smaller companies have led to the unified approach for bringing many antibiotics to clinical trials followed by marketing [8,9].
- Infection prevention and control is one of the most powerful alternatives to combat MDR. Better hygiene, access to clean water and proper sanitation could control community-acquired infections. The use of simpler and cheap strategies such as washing of hands and maintenance of hygiene should be encouraged.
• Control in the spread of infections in clinical and hospital (ICU) settings where the majority of highly drug resistant bacteria thrive in concentrated doses in a smaller area.

• Governments, NGOs and corporate (Corporate Social Responsibility) should work towards advertising on prime time television, radio, newspapers, websites and different social media to spread awareness regarding MDR.

• FDA from each country should be able to draft policies so that the same drugs are not used for human and animal treatments. Cows, chicken, fish are used as human food and the drugs used to treat such animals would affect their products like meat, milk and eggs. The resistant bugs present in these animal products are likely to affect human health adversely. Various government agencies such as the Scandinavian and the European Union, American Society for Microbiology, American Public Health Association and American Medical Association are aware of this growing public health concern and have called for restricting antibiotics in food animals for growth promotion rather than treating infections.

• Studies should be performed on bacterial genomics to find new targets supplemented by the technology of synthetic combinatorial chemistries to search for new drugs for the novel targets.

• A global coalition should be made for the real action – via the G20, the UN and the WHO [2].

• Naturopathy could be used as an effective and safe alternative to treat superbugs. A lot of research done scientifically or traditional knowledge has shown the use of plant products with antimicrobial properties. Berberine from the herb Berberis vulgaris has been shown to block the efflux pumps responsible for MDR [13,14].

• Deciphering the genetic reasons for the acquisition and dissemination of MDR may aid in the design of novel molecules that can overcome the resistance mechanisms. Knowledge-based designs for new antimicrobials can be attempted for important players such as beta-lactamases, MDR efflux pumps, penicillin binding proteins and ribosomes [9].

• Development of alternatives such as vaccines, efflux pump inhibitors, quorum sensing inhibitors and phage therapy to overcome the MDR pathogens, could help resolve the problem [10,15,16].

• Not only should the new antibiotics be developed, efforts should also be directed towards using more effective forms of existing antibiotics as described in Table 1.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Drug name</th>
<th>Class of antibiotic</th>
<th>Resistance mechanism vanquished</th>
<th>Target pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ceftobiprole</td>
<td>Cephalosporin</td>
<td>Penicillin-binding protein</td>
<td>Streptococci and Staphylococci</td>
</tr>
<tr>
<td>2</td>
<td>WCK-771</td>
<td>Quinolone</td>
<td>mutation</td>
<td>Streptococci and Staphylococci</td>
</tr>
<tr>
<td>3</td>
<td>Tigecycline</td>
<td>Tetracycline</td>
<td>Efflux pumps and ribosome protection</td>
<td>Streptococci and Staphylococci</td>
</tr>
<tr>
<td>4</td>
<td>Oritavancin</td>
<td>Glycopeptide</td>
<td>Vancomycin resistance</td>
<td>Enterococci</td>
</tr>
</tbody>
</table>

Table 1: New drugs from established classes of antimicrobials that have been modified to overcome resistance

• Apart from drug development and marketing, another very important strategy for tackling the problem of MDR is the development of point of care rapid diagnostic systems for quick identification of infectious agents and the MDR displayed by them. If novel, faster and cheaper methods to detect and analyse MDR could be made accessible at the patient’s bedside especially the critically ill cases, it would be a great service aiding in the doctor’s decision.

• The numbers, pay and recognition of people working in infectious diseases should be improved.
Governments and Their Policies for MDR

From the discussions above, it could be concluded that the solutions to the problem of MDR are to be initiated at the level of governments from where they percolate down to the other sections of the society. Drafting relevant policies, their effective implementation, improvement of funding situations for research and innovation in the field of antibiotic resistance, providing incentives to pharmaceutical industries and measures to increase public awareness, all these measures require the involvement of governments and their sincere commitment. Additionally, governments from a large number of countries should unite to combat this problem assuming its mammoth proportions. In this direction, the efforts made by some of the governments have been discussed here. Though the greater details could be seen in the chapter by Dr. Abdul Ghafur in this book.

Recently, the UK government commissioned a review on the growing problem of antimicrobial resistance under the chairmanship of the macroeconomist Baron Jim O’ Neil and the sponsorship of the Department of Health and the Wellcome Trust [2]. The report was published in May 2016 and has made ten recommendations to tackle this problem [2]. The US Biomedical Advanced Research and Development Agency (BARDA), Broad Spectrum Antimicrobials programme, and the European Innovative Medicines Initiative (IMI), ‘New Drugs For Bad Bugs (ND4BB) programme’, together provide direct financial support to nearly 20 percent of all antibiotics currently under development globally, and half of those targeting Gram-negative bacteria.

In India, with the scourge of drug resistant infections such as cholera, extremely drug resistant tuberculosis, malaria and HIV going high, the government has taken measures to control the problem. Sadly, In India, 60,000 newborns die due to antibiotic-resistant neonatal infections every year. India has initiated a ‘Red Line Campaign’ for antibiotics packaging, launched earlier this year. This would sound the consumers about the nature of the medicine and their ‘responsible use’.

American Society for Microbiology (ASM) set up a task force on antimicrobial resistance in 1995. The CDC published their “Guidelines for the Evaluation of Surveillance Systems” in 1998 and CDC also issued a public health action plan to combat antimicrobial resistance in June 2000. The Infectious Diseases Society of America produced a shocking report in 2004 to draw the attention of the government towards the problem of MDR and to draw the attention of dwindling funds in research and development of new anti-infective agents by pharmaceutical industries. The report was titled “Bad Bugs, No Drugs: As Antibiotic Discovery Stagnates------- A Public Health Crisis Brews”. Similarly, the UK Department of Health issued an “Antimicrobial Resistance Strategy and Action Plan’ in 2000. “Keep antibiotics working committee” and “antibiotics are not automatic” 2002 campaign are some of the efforts made by the French government to curtail the unnecessary use of antibiotics. An efficient surveillance system has been set up in Europe by the name of European Antimicrobial Resistance Surveillance System (EARSS) (http://www.rivm.nl/earss). In India, national policy was made for containment of antimicrobial resistance by Directorate General of Health Services (2011) when the superbug carrying New Delhi Metallo-beta-lactamase was reported in a Swedish patient of Indian origin. A task force was constituted to work on various aspects related to national surveillance system for antibiotic resistance, enhancing regulatory provisions for use of antibiotics in human, veterinary and industrial use, to enhance the rational use of antibiotics and strengthen the diagnostic methods for antimicrobial resistance monitoring. Health ministry has also approved an antimicrobial resistance programme that will be monitored and reviewed by The National Centre for Disease Control (NCDC). Global Antibiotic Resistance Partnership (GARP) develops different policy proposals on antibiotic resistance for low-and-middle-income countries. Phase 1 of GARP has been initiated in India, Kenya, South Africa and Vietnam. In addition to this endeavour, in August 2012, the annual conference of Clinical Infectious Disease Society
was held at Chennai, India. In this meeting of medical societies of India which also had many national and international representatives, a roadmap was made to tackle the problem of antimicrobial resistance in India [17]. The document resulting from the discussions held at this meeting was named “Chennai Declaration”. The recommendations made in Chennai Declaration were to be considered by the Indian Ministry of Health for formulating a national antibiotic policy.

**Is Everything About the Drug Resistance Bad?**

For their own wrong doings, humans have started passing all the blame on to antibiotics. Carefully thought, not all is bad about antibiotics. It is up to us to use them judiciously to derive maximum benefit out of them. One can very well imagine the scenario if the resistance plasmids were not known. Probably the recombinant DNA technology and field of genetic engineering would not be thriving. Antibiotic resistance mechanisms have lent insights into the functioning of ribosomes. The role of rRNA in ribosome function was deciphered by the studies on the antibiotics such as aminoglycosides and macrolides as translational inhibitors.

**Conclusions**

Humankind is in a constant race with the huge diversity of the microbial world capable of accomplishing the magical jugglery of its genomes for any kind of challenge to its existence, antibiotics being just one of these challenges. It is rightly stated that microbes have the last word or never underestimate the power of bacteria [4,12]. As thieves can evolve strategies to open any kind of lock, bacteria can evolve multiple strategies to disarm the potential of any drug that has been designed to kill them. Mankind has witnessed the rapid appearance of very difficult invincible pathogens arising in hospitals and communities to outwit the newer antibiotics. From the above discussions, one can conclude that though the overwhelming problem of MDR is inevitable, it can be definitely controlled. Future would be bright if through the concerted efforts from each individual in the society, specific narrow spectrum antibiotics could be prescribed to the patients after accurate diagnosis and there would be a global action plan [18]. Simple public health measures can be really helpful for complicated problem of MDR. There definitely have to be rays of hope. Can we all simply wash our hands off from the problem of MDR?

**Acknowledgements**

The laboratory has been supported by the grants from the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India (No. BT/PR/11634/INF/22/104/2008), the Gujarat State Biotechnology Mission, Department of Science and Technology, Government of Gujarat (No. GSBTM/MD/PROJECTS/SSA/1535/2013-14) and the Indian Council of Medical Research, New Delhi, India (No. the grant AMR/49/11-ECDI). The author thankfully acknowledges The Puri Foundation for Education in India for providing infrastructure facilities.

**References**

4. Davies J (2007) Microbes have the last word. A drastic re-evaluation of antimicrobial treatment is needed to overcome the threat of antibiotic-resistant bacteria. EMBO Rep 8: 616-621.


Abstract

Gastroenteritis or infectious diarrhea, generally caused by viral or bacterial or parasitic infections is responsible for morbidity and mortality, especially in children. Bacterial gastroenteritis is caused by the agents such as *Vibrio* spp., *Salmonella* spp., *Shigella* spp., *Campylobacter jejuni*, *Yersinia enterocolitica*, enterohemorrhagic *Escherichia coli* (EHEC) and other diarrheagenic *E. coli*. Diarrheal diseases caused by multi-drug resistant bacteria such as *Vibrio* and *Shigella* spp. are a major health problem in the developing countries with poor hygiene and limited resources. Various mobile genetic elements such as integrons, plasmids and SXT elements are involved in drug resistance. Apart from mobile genetic elements, various inherent mechanisms such as mutations in drug target sites and efflux pumps are also involved in imparting drug resistance. This chapter describes the major diarrhea-causing pathogenic bacteria in India i.e. *Vibrio* spp. and *Shigella* spp., their prevalence, treatment complications and molecular epidemiology of antibiotic resistance.

Introduction

Gastroenteritis or infectious diarrhea is a common medical condition due to the inflammation of the stomach and small intestine. It is generally caused by viral or bacterial or parasitic infections and is manifested by diarrhea, abdominal cramps and vomiting [1]. The increase in morbidity and mortality rate due to acute gastroenteritis in the past decades has led to a worrisome situation. The diarrheal diseases exist as the second major cause of death in children worldwide (Figure 1) accounting for 1.6 to 2.5 million deaths annually and in developing countries every child encounters 3 episodes of diarrheal infections per year [2-4]. India ranks among the top countries with 336,600 cases and a very high number of annual child deaths due to diarrhea [3,4]. Though there is a decline in the mortality rate of...
diarrhea in the past few years, it still persists as one of the major causes of morbidity and mortality in children [2]. The gastroenteritis caused by protozoan parasites accounts for a relatively small proportion of cases in developing countries and is uncommon in developed countries. The parasitic agents such as *Giardia intestinalis*, *Cryptosporidium parvum*, *Entamoeba histolytica* and *Cyclospora cayetanensis* most commonly cause acute diarrheal illnesses in children [5]. The virus-mediated gastroenteritis is caused by the agents such as rotavirus, enteric adenovirus, norovirus, sapovirus, and astrovirus. Rotavirus remains as the most common etiological agent of diarrhea in children worldwide accounting for around 0.5 million deaths per year, followed by norovirus [6,7]. Bacterial gastroenteritis is caused by the bacterial genus such as *Vibrio*, *Salmonella*, *Shigella*, *Campylobacter jejuni*, *Yersinia enterocolitica*, enterohemorrhagic *Escherichia coli* (EHEC) and other diarrheagenic *E. coli*. In developing countries, gastroenteritis through bacteria is more prevalent whereas viruses are the predominant cause of acute diarrhea, especially during the winter season in industrialized countries [5]. The frequency of occurrence of intestinal infections due to *Vibrio* and *Shigella* species is more in developing countries like India [5,8,9]. Hence, the following sections would focus on the major diarrhea-causing pathogenic bacteria in India i.e. *Vibrio* spp. and *Shigella* spp. and the problems in their treatment.

**Figure 1:** Distribution of cause of death among children aged under five years. Diarrheal diseases cause 16% postneonatal and 2.6% neonatal deaths and thus exist as the second most common cause of child deaths worldwide. (Source: WHO, Global Burden of Disease estimates: 2004 update, 2008).

**The Enteric Pathogens: *Vibrio* Spp. and *Shigella* Spp.**

This section would describe briefly about *Vibrio* spp. and *Shigella* spp., the etiological bacterial agents of gastroenteritis in humans and their prevalence around the globe with a special focus on India.

**Vibrio spp. and their outbreaks**

The genus *Vibrio* consists of more than 200 species, but only about 13 of them were reported to cause diseases in humans [10]. Among different pathogenic *vibrios*, the focus here would be on three major diarrhea-causing vibrios, *V. cholerae*, *V. parahaemolyticus* and *V. fluvialis*, as the incidences of occurrence of outbreaks of these three *Vibrio* spp. is more in India [11-22].
The infection caused by the toxigenic *V. cholerae*, popularly known as cholera, is an ancient and heavily destructive disease that continues to pose a serious public health problem among developing world populations which have no access to adequate water and sanitation resources. The world has already witnessed seven pandemics in the past two hundred years (http://www.cdc.gov/cholera/index.html). The first six pandemics were caused by the infection of *V. cholerae* O1 classical biotype that seems to be originated from the Indian subcontinent. The seventh pandemic of cholera witnessed two new forms of *V. cholerae*, *V. cholerae* O1 El Tor biotype and *V. cholerae* O139 serogroup [23]. Globally, cholera outbreaks have increased continuously since 2005 and still affect several continents (http://www.cdc.gov/cholera/index.html).

African countries faced more cholera outbreaks than Asia and America. According to the weekly epidemiological record of WHO, in the year 2015, 55% of cases were reported from Africa, whereas, between 2001 and 2009, 93% to 98% of total cases worldwide were reported from that continent. In India, a total of 68 outbreaks were reported across 18 states and union territories in the country between 1997 to 2006 and the unreported cases may exceed even more [24]. The Kolkata region of West Bengal is considered as the endemic region for cholera in India and it witnessed several outbreaks in the past two decades [23]. Recent reports showed that cholera occurs over a wider geographical area in the country, such as Maharashtra, West Bengal, Tamil Nadu, Andhra Pradesh, Delhi, Goa, Orissa and Madhya Pradesh [21,24].

*V. parahaemolyticus*, the causative agent of food-borne gastroenteritis is indigenous to estuarine, marine and coastal environments throughout the world. This species of *Vibrio* was first isolated in the year 1950 [25], after which it has been recognized as the major cause of seafood-borne illness throughout the world. The species consist of more than 80 serotypes, based on the somatic (O) and capsular (K) antigens [26,27]. In India, the gastroenteritis caused by *V. parahaemolyticus* O3:K6 has increased at the beginning of 1996 in Kolkata. In the next few months, the gastroenteritis caused by the same serovar was reported in other neighboring countries such as Vietnam, Indonesia, Bangladesh, Japan, Korea and Thailand [26]. By the end of 2006, this serovar was isolated from Europe and the United States marking the beginning of the first pandemic of *V. parahaemolyticus* [26]. In Kolkata, the isolation rate of *V. parahaemolyticus* during 2001-2012 ranged from 0.5% to 4% of diarrheal cases. The overall isolation rate was 1.3%, which closely matched a report from Bangladesh [28]. In 2009, the isolation rate of *V. parahaemolyticus* increased to 4.2% compared to other years. The serovars predominantly isolated during 2009 were O1:K36, O1:K25 and O3:K6 [28].

*V. fluvialis* is an emerging foodborne pathogen all over the world which causes diarrhea in humans [10]. The organism was first isolated in 1975 from a hospitalized diarrhea patient in Bahrain and was categorized as group F *Vibrio* before it was named as *V. fluvialis* in 1981 [29]. *V. fluvialis* was found as the major causative agent of gastroenteritis in Tenri hospital, Japan, since 1979 [30]. In 1982 to 1988, about 10 cases of *V. fluvialis*-mediated gastroenteritis due to contaminated seafood were reported in Florida [31]. *V. fluvialis* was the second major cause of diarrhea in Zhejiang province of China during 1991 [32]. This pathogen was first reported in Brazil during 1990 [33] and subsequently in 1991 [34]. During 1993, in Gulf-coast, 6% of Vibrio-mediated infections were caused by *V. fluvialis* [35]. The prevalence of this species was 9.4% among the hospitalized patients in North Jakarta [36]. Bangladesh witnessed *V. fluvialis* outbreak after the 1998 flood [37]. In India, the first case of *V. fluvialis* was observed in Maharashtra during 1981 as a food-borne infection and it has been frequently reported from Kolkata since 1989 [10,13,16,18,19,22]. Oral rehydration therapy followed by administration of antibiotics is the standard acceptable form of treatment for *Vibrio* infections [9]. In the twentieth century, the introduction of antibiotics for treating diarrheal diseases led to an incredible reduction in the death rate of humans caused due to these diseases. But the emergence of resistance to antibiotics in bacteria rendered them an ability to rise up against these magic bullets.
Antibiotic Resistance and Its Mechanisms

Antibiotic resistance of a bacterium is its resistance to an antibiotic to which it was previously sensitive. Resistant microbes are able to withstand the effect of antibiotics so that standard treatments become ineffective and infections persist and spread to other organisms. Bacteria resist antibiotic action mainly in three ways: by reducing drug accumulation by efflux action or porin mutations, by altering or protecting drug targets and by enzymatic modification/inactivation of drugs [38]. Efflux pumps play a major role in conferring resistance to antibiotics by efficiently recognizing and throwing them out of the cells (Figure 2A). Efflux pumps confer only low-level resistance to the bacteria towards drugs but their over expression or co-operativity with other drug resistance mechanisms could result in moderate to high-level resistance [39].

Figure 2: Factors responsible for antibiotic resistance in bacteria. (A) Various mechanisms exhibited by bacteria to combat antibiotics (source: Encyclopedia Britannica, Inc. 2009). (B) Different processes of horizontal gene transfer responsible for acquired resistance traits of bacteria (source: https://www.studyblue.com/notes/note/n/medical-microbiology/deck/5418270).

Porins present in the cell membrane of bacteria are the passages which facilitate the entry and exit of antibiotics and other small organic molecules (Figure 2A). The decrease in the expression of porins results in reduced uptake of antibiotics. Mutations at the antibiotic target sites are the main mode of resistance to most of the antibiotics. Mutations occurring as a result of replication errors reduce the affinity of the antibiotics to their targets resulting in the resistant phenotype (Figure 2A). There are few enzymes that either degrade or chemically modify the antibiotics (Figure 2A) so that antibiotics cannot exert their action and few proteins are also known to protect the target from antibiotics [40]. Though antibiotic resistance is ancient, the indiscriminate use of antibiotics created an immense selective pressure on the bacteria facilitating the fast evolution of resistant bugs. This process was mediated through the pivotal role of various factors like efflux pumps/porins, mutations in the antibiotic target sites, and most importantly acquisition of resistance genes by Horizontal Gene Transfer (HGT). The process of HGT enables bacteria to exchange genetic material within themselves without the requirement of cell division (Figure 2B). Different kinds of Mobile Genetic Elements (MGEs) are transferred between bacteria through this process leading to the adaptation and evolution of bacteria/bacterial communities in tune with the changing environments. HGT is mediated by the processes of transformation, transduction
or conjugation and different types of MGEs such as integrons, transposons and plasmids could move through these processes of HGT [40].

**Prevalence of drug resistance in Vibrio spp.**

Antibiotics are widely used as a part of cholera treatment but their indiscriminate use led to the continuous emergence of drug-resistant bacteria. This section explains the problem of drug resistance which causes the difficulties while treating the infections of Vibrio species in humans. It was observed that *V. cholerae* O1 isolated between 1938 and 1993, from various regions of the world showed resistance for 1 to 3 antimicrobials, whereas the strains isolated from 1994 to 2005 carried 3 to 8 resistance markers including fluoroquinolones [41]. Due to the emergence of drug-resistant strains, an increase in the case fatality rate from 1% to 5.3% was observed in Guinea-Bissau, Africa, during the epidemic of cholera in 1996-97. Based on several published works (years 1970 to 2010) from National Institute of Cholera and Enteric Diseases, Kolkata, the changing profile of resistance in *V. cholerae* was observed [41]. In Bangladesh, most cases of cholera infections carried resistance to antibiotics such as tetracycline, trimethoprim, sulfamethoxazole, and erythromycin [42]. Antibiotic resistance has been largely reported in other clinical Vibrios also.

The recent studies suggested the high prevalence of antibiotic resistance in the clinical isolates of *V. parahaemolyticus*. A study on antibiotic susceptibility pattern in the isolates from diarrheal patients admitted in Infectious diseases hospital, Kolkata, India during 2001 to 2012 revealed a high rate of resistance to ampicillin (98%) followed by streptomycin (86%) [28]. However, resistance to nalidixic acid and chloramphenicol were 3.4% and 1.7% respectively [28]. A study in Guangdong, China on the *V. parahaemolyticus* isolates from outbreaks and sporadic cases showed MDR phenotypes in 83.33% and 37.21% of isolates respectively [43]. MDR phenotypes were also reported from 97.7% *V. parahaemolyticus* isolates from oysters in Brazil [44]. The recent antibiotic susceptibility studies on *V. parahaemolyticus* isolated from crustaceans, shellfish and shrimps displayed their resistance to various antibiotics such as ampicillin, rifampin, streptomycin, nalidixic acid, sulfisoxazole, tetracycline, trimethoprim and ciprofloxacin [45-47].

Like other vibrios, *V. fluvialis* has also exhibited MDR phenotype. These isolates were found resistant to ampicillin, carbenicillin, cefalotin, kanamycin and sulfadiazine-trimethoprim in Mediterranean fish farms [48]. In China, the majority of the *V. fluvialis* isolates were resistant to azithromycin, β-lactams and sulfamethoxazole [49]. In India, there are many reports on drug resistance in *V. fluvialis* isolates [13,16,18,22,50].

**The Enteric Pathogens: Vibrio Spp. And Shigella Spp.**

**Mechanisms/Factors Involved in Drug Resistance of Vibrio Spp.**

As mentioned in the earlier section, drug resistance is prevalent in Vibrios. Various factors such as mutations in the target sites, MGEs and efflux pumps play an important role in conferring antibiotic resistance. *Vibrio* species are known to resist quinolone action by exporting drugs through efflux pumps, chromosomal mutations and by acquiring the quinolone resistance gene-bearing plasmids or other MGEs [10,41,51,52]. Mutations in the quinolone-resistance-determining regions of topoisomerases, GyrA followed by mutations in the ParC is reported as the main mechanism of quinolone resistance in Vibrios [41]. *Vibrio* species utilize efflux pumps to throw the antibiotics and other foreign substances out of the cell. Vcam, one of the few ATP-Binding Cassette (ABC) superfamily efflux pumps in bacteria, was reported in *V. cholerae* to efflux out norfloxacin and ciprofloxacin along with other drugs such as tetracycline and doxorubicin [52]. The MATE pump NorM which effectively effluxes out hydrophilic fluoroquinolones were first reported from *V. parahaemolyticus* [53]. *V. cholerae* non-O1/non-O139 is known to use an array of MATE efflux pump systems, namely VcmB, VcmD, VcmH, VcmN, VcmA and VcrM [54,55]. Two MATE-type efflux pumps, namely VFH and VFD in *V. fluvialis* were found to be responsible for fluoroquinolone resistance [56].
The spread of drug resistance genes in *Vibrios* is facilitated by HGT through the Integrative Conjugative Elements (ICE), plasmids and integrons. One of the ICEs, SXT element was first described in *V. cholerae* O139 and it harbored various genes responsible for resistance to sulfamethoxazole, trimethoprim, chloramphenicol and streptomycin [57]. Later, many strains of *V. cholerae* O1, O139, non-O1/non-O139, *V. fluvialis* and other *Vibrio* species were reported which carried the SXT elements or different siblings of SXT elements [57-60]. Mutreja and his colleagues had shown the acquisition of the SXT family of antibiotic resistance elements that shaped the pandemic spread of *V. cholerae*. This study has also shown that this family was first acquired at least ten years before its discovery [61].

Apart from the SXT element, *Vibrios* have also harbored the integrons. Integrons are assembly platforms that incorporate many extraneous gene cassettes including antibiotic resistance genes through site-specific recombination in a site near to the promoter which drives its expression [62]. Based on the association of integrons with MGEs or chromosomes, they are classified into mobile integrons (MIs) and Chromosomal Integrons (CIs). MIs are of different types based on integrase sequences with ~40-58% identities and they are classified as class 1, 2, 3, 4 and 5. A typical class 1 integron consists of two conserved segments (CS) at their 5’- and 3’-ends, separated by a variable region that usually comprises of one or more extraneous gene cassettes. The 5’CS region contains the integrase gene, the integration site and a promoter region that allows expression of any number of gene cassettes inserted at the *attI1* site in a suitable orientation [62]. The 3’CS region usually comprises of *qacED1* encoding resistance to quaternary ammonium compounds and *sul1* encoding resistance to sulphonamides [62]. In *Vibrios*, class 1 integron has been very well characterized [13,16,52] and Class 2 integron was also reported [63-65]. Both classes carry multiple gene cassettes encoding resistance genes, for example, *dfr* for trimethoprim resistance. A superintegron was also first reported in *V. cholerae* N16961 that harbored various gene cassettes including the antibiotic resistance genes [66]. The evolutionary history of super-integron suggested that these elements helped in the adaptation of bacteria with the change in environment. It was the main source of mobile integron’s backbone and antibiotic resistance gene cassettes [67]. Many *dfr* cassettes in different environmental isolates of *V. splendidus* have been found while *catB9, carb7, carb9* (encoding carbenicillin resistance) and *qnr* (encoding resistance to quinolones) cassettes have been identified in *V. cholerae* superintegron.

Plasmids are also a key element for HGT of drug resistance genes in *Vibrios*. The genes conferring quinolone resistance such as *qnr, aac(6’)Ib-cr, oqxA/B* and *qepA*, residing on the plasmids, confer Plasmid-Mediated Quinolone Resistance (PMQR) and were frequently reported from *Vibrio* species. In *V. cholerae*, various alleles of *qnrVC* were found to contribute to quinolone resistance and those alleles were reported to be found in MGEs such as integrons, SXT and plasmids (Table 1) [68]. *V. fluvialis* strains were also known to harbor *qnr* genes such as *qnrVC5* [16,22,68] and *qnrA1* [18]. The *qnr* homologue VPA0095 was reported from *V. parahaemolyticus* [69,70] and *qnrVC5* and *qnrVC6* were found in the same species [68,71]. It was also hypothesized that the *Vibrionaceae* family could be a possible reservoir for Qnr-like quinolone resistance determinants [72]. The *aac(6’)* *Ib-cr* gene was reported from *V. fluvialis* [16,18] and *V. parahaemolyticus* [70].

<table>
<thead>
<tr>
<th>Allele</th>
<th>MGEs</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>qnrVC1</em></td>
<td>Class1 integron, SXT element</td>
<td><em>V. cholerae, P. aeruginosa</em></td>
</tr>
<tr>
<td><em>qnrVC2</em></td>
<td>Plasmid</td>
<td><em>V. cholerae</em></td>
</tr>
<tr>
<td><em>qnrVC3</em></td>
<td>Class1 integron</td>
<td><em>V. cholerae</em></td>
</tr>
<tr>
<td><em>qnrVC4</em></td>
<td>Class1 integron</td>
<td>*V. cholerae, E. coli, Aeromonas spp., Pseudomonas spp.</td>
</tr>
<tr>
<td><em>qnrVC5</em></td>
<td>Class1 integron, Plasmids</td>
<td><em>V. cholerae, V. parahaemolyticus, V. fluvialis</em></td>
</tr>
<tr>
<td><em>qnrVC6</em></td>
<td>Class1 integron</td>
<td>Acinetobacter baumannii, <em>V. parahaemolyticus</em></td>
</tr>
<tr>
<td><em>qnrVC7</em></td>
<td>Plasmid</td>
<td><em>V. cholerae</em></td>
</tr>
</tbody>
</table>

**Table 1:** *qnrVC* alleles and their association with MGEs
Apart from PMQR, plasmids also harbor the other resistance genes such as tetracycline resistance. A cholera outbreak in 1979, in Matlab, Bangladesh was caused by a highly drug resistant *V. cholerae* strain. This strain harboured a conjugative plasmid that conferred resistance to tetracycline, ampicillin, kanamycin, streptomycin, gentamicin and trimethoprim [73]. In another study from India, *V. fluvialis* was found to harbor conjugative as well as non-conjugative plasmids conferring the drug resistance for various drugs such as ampicillin, neomycin, kanamycin, cotrimoxazole, streptomycin, trimethoprim, sulphamethoxazole, chloramphenicol and ciprofloxacin [16,22]. Caccarelli and her colleagues reported a conjugative plasmid (p3iANG) in *V. cholerae* O1 isolate. This plasmid carried a set of three class 1 integrons harboring *dfra15*, *blaP1* and *qacH-aadA8* cassettes that coded resistance phenotypes for trimethoprim, beta-lactam, quaternary ammonium compounds and aminoglycoside respectively. The same plasmid also harbored chloramphenicol (*cat1*), sulphonamide (*sul2*) and tetracycline (*tetG*) resistance genes in between the spacer region of two integrons [74].

**Shigella**

Shigellosis is an intestinal infection caused by *Shigella* spp. that includes *Shigella dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei* [8]. Shigellosis is caused by direct contact with the bacteria or through contaminated water or food. Small volume of inoculum of bacteria (as few as 200 bacteria) is sufficient to cause the infection. Patients infected with the bacteria suffer from the frequent passage of small liquid stools that contain visible blood, with or without mucus, after one to four days of incubation period. Abdominal cramps, tenesmus, fever and anorexia are also common symptoms in shigellosis. Sometimes it causes some serious complications like hemolytic uremic syndrome, reactive arthritis, blood stream infections and seizures [75]. Young children, gay, bisexuals, HIV-infected persons and travellers have a high risk of shigellosis [75]. *Shigella* has the ability to invade and colonize the intestinal epithelium and cause its disruptions. *Shigella* can multiply within the intestinal epithelium cells and cause cell death. It also disseminates to infect and kill the adjacent cells, causing mucosal inflammation, ulceration and bleeding [8]. This section describes the outbreaks of *Shigella*, prevalence and mechanisms of drug resistance in shigellosis.

**Shigella outbreaks**

Shigellosis is estimated to cause 80-165 million cases of infections and 600,000 deaths worldwide [76]. This disease is more prevalent in children under the age of five years and more common in developing countries, where the problem of poor sanitation and unsafe water supply exists [76]. *Shigella* causes about 500,000 cases of dysentery in the United States annually while in Asia, around 125 million infections and 14000 deaths annually are caused due to *shigellosis* [77].

The geographical distribution of four species of *Shigella* is different and the reasons for this are still unclear [78]. *Shigella dysenteriae* type 1 was historically responsible for large epidemics in Central America, Asia and Africa [79] but recently there are very few reports on the occurrence of this species [80]. *S. boydii* is reported occasionally whereas *S. flexneri* is most frequently isolated around the globe especially in resource-poor countries [81]. *S. dysenteriae* has 17 and *S. boydii* has 20 serotypes. *S. flexneri* consist of 14 different serotypes that are distributed heterogeneously across different regions of the world, with predominant serotypes that include *S. flexneri* 2a, 3a, and 6 [80,82]. *S. sonnei* is also prevalent globally, most frequently detected in high-income regions and it has only one serotype [82,83]. In general, the disease caused by *S. sonnei* is less severe when compared to shigellosis caused by other spp [84].
**Drug resistance in *Shigella***

Administration of antibiotics comes as the first line of treatment for *Shigella* infections. Historically, shigellosis was treated with ampicillin, trimethoprim, sulfamethoxazole and ceftriaxone (http://www.mayoclinic.com/health/shigella/DS00719). Unfortunately, these bacteria have become resistant to one or more antibiotics. Center for Disease Control and Prevention has kept this organism in the category list of serious threat. They have also reported 27000 cases of drug-resistant *Shigella* infections per year in the United States [75]. A study from Oregon (Portland), with 369 isolates (during the year 1995 to 1998) showed that the isolates were resistant to ampicillin (63%) and co-trimoxazole (59%). Thirteen percent of the isolates were resistant to all the five drugs ampicillin, chloramphenicol, streptomycin, sulfisoxazole and tetracycline [85]. In another study from Foodborne Disease Active Surveillance Network sites, out of 1118 isolates from the year 2000 to 2010, 74% isolates were found resistant to ampicillin, 58% to streptomycin, 36% to co-trimoxazole and 28% to tetracycline [86]. Additionally, these antibiotic resistance patterns differed by race, ethnicity, age, travel, and species. For example, male individuals were more likely to be infected with multidrug-resistant *Shigella* than female individuals. Resistance to cephalosporins was markedly increased during 2010 to 2012 [87]. In a study of *Shigella* isolates from central Israel in 1998 to 2000, and comparison with the period 1991–1992, a significantly increased resistance to tetracycline (from 23% to 87%), high resistance to co-trimoxazole (94%) and ampicillin (85%) and developing resistance to quinolones (0.5–2%) was observed [88].

*Shigella* spp. was initially susceptible to co-trimoxazole but on the emergence of resistance to this antimicrobial, treatment recommendations were shifted to quinolone group of antibiotics and azithromycin [89]. But eventually, these bacteria also developed the mechanisms for quinolone resistance and recently, WHO reported fluoroquinolone resistance in *Shigella* isolates from all over the globe [89]. Resistance to nalidixic acid and ciprofloxacin in Asia-Africa (years 2007 to 2009) was progressively increased each year, reaching 64.5% and 29.1% respectively [90] as compared to Europe and America. There are also many reports on azithromycin resistance in *Shigella* [91-94]. This is a worrisome situation that *Shigella* are acquiring resistance to all the possible drugs which would complicate the treatment procedure.

**Mechanisms/Factors Involved in Drug Resistance of *Shigella***

As discussed in earlier sections, treatment of shigellosis has become difficult due to wide spread resistance to antibiotics. As described earlier, the drug resistance is mediated through various factors such as efflux pumps, porins, mutations in the antibiotic target sites and MGEs. HGT of integrons may be responsible for the dissemination of antibiotic resistance genes and the emergence of highly drug-resistant strains. Resistance to some of the antibiotics in *Shigella* has been attributed to class 1 and class 2 integrons. Dubois et al have analysed the *Shigella* isolates during the years 1990 to 2002 from Bordeaux, France [95]. From this study, class 1 integrons were found in *S. dysenteriae* and *S. flexneri* while class 2 integrons were present in *S. boydii* and *S. sonnei*. Additionally, it was reported that class 1 integrons harbored *aadA1* (aminoglycoside resistance) and *bla*$_{OXA30}$ (beta lactam resistance) gene cassettes while class 2 integrons harboured the *dfrA1* (trimethoprim resistance), *sat1* (streptothricin resistance) and *aadA1* (aminoglycoside resistance) [95]. Pan et al have found three kinds of gene cassette arrays, *aadA2*, *dfra17-aadA5* and *dfra1-aadA1* on class 1 integrons. Some of the isolates were devoid of 3’ conserved segment of class 1integron and therefore, the integron was named as *Shigella* atypical class 1 integron [96]. The atypical class 1 integron harbored *bla*$_{OXA30}$ and *aadA1* genes that conferred resistance to ampicillin and streptomycin respectively. This atypical class 1 integron was first found on a Pathogenicity Island (PAI) carrying *Shigella* Resistance Locus (SRL), on the chromosome
of \textit{S. flexneri} 2a strain YSH6000. This pathogenicity island carried various genes such as \textit{aadA1-bla}~_{	ext{oxa}}-\text{cat-tetA-tetC}, as resistance determinants for streptomycin, ampicillin, chloramphenicol and tetracycline [96-98]. The distribution and structural variations of SRL PAI were investigated in 71 \textit{Shigella} isolates and 28 other enteric pathogens [97]. The SRL PAI was found only in \textit{Shigella} isolates while it was absent in other enteric pathogens [97,98].

A study from Nanjing area of China revealed that class 1 and class 2 integrons were more prevalent in \textit{Enterobacteriaceae} including \textit{Shigella} [99]. All the class 2 integrons from \textit{Shigella} isolates contained the same cassette array \textit{dfrA1-sat1-aadA1}. A similar type of gene cassettes on class 2 integrons have also been reported by other researchers [96, 100-102]. Class 2 integrases are non-functional proteins due to an internal stop codon at 179\textsuperscript{th} position of the protein sequence [99]. Therefore, the majority of the cassette arrays on class 2 integrons are usually constant. There are also some reports which showed an unusual class 2 integron that harbored the \textit{dfrA1} or \textit{dfrA1-dfrA12} or \textit{dfrA1-sat1-aadA1-orfX} gene cassettes [103,104].

It has also been demonstrated that all the recent emergence of \textit{S. sonnei} infections are due to a small number of clones that dispersed globally from Europe within the last 500 years [105]. The four distinct lineages of \textit{S. sonnei} were also identified. Lineage III is globally most prevalent and becoming dominant in Asia, Africa and South America. \textit{S. sonnei} belonging to lineage III carried a distinct class 2 integron which conferred resistance to trimethoprim, streptothricin and streptomycin. Many of these lineages also harbored a genetic locus on a small plasmid that conferred resistance to tetracycline and sulphonamides. Similar studies were also carried out in \textit{S. flexneri}, but no correlation was found in the lineages and intercontinental spread of antibiotic resistance elements/loci [106].

During the late 1950s in Japan, Nakaya and his colleagues investigated \textit{S. flexneri} 2b isolated from human faeces and found it to be resistant to streptomycin, chloramphenicol, sulphonamide and tetracycline [107]. This resistance was transferable by a plasmid to another bacteria belonging to the family \textit{Enterobacteriaceae} through conjugation. This plasmid was further named as NR1 plasmid (National Institute of Health [Tokyo] R factor 1).

Quinolone resistance in \textit{Shigella} species is also a serious global problem especially in Asia and Africa compared to Europe and the US [51,90]. Mutations in DNA gyrase or topoisomerase IV subunits were found to be the major factor for quinolone resistance in \textit{Shigella} [51]. Phylogeographical data of \textit{S. sonnei} indicated marked differences in the global prevalence of \textit{gyrA} (DNA gyrase) mutations, which confer resistance to quinolones [105]. The involvement of efflux systems that pump out fluoroquinolones in \textit{Shigella} species has been demonstrated by various groups [108-110]. In the presence of ciprofloxacin, the induced expression of efflux pump genes, namely \textit{tolC}, \textit{mdfA} and \textit{ydhE} was documented [109]. PMQR has been reported frequently in \textit{Shigella} spp. [111,112]. The \textit{qnrS} was first reported in \textit{S. flexneri} [113] and subsequently, the same gene was reported from different geographic locations in the same genus [114-116]. The other \textit{qnr} genes such as \textit{qnrA} [115] and \textit{qnrB} [117] were also reported from \textit{Shigella} species. The \textit{aac (6') Ib-cr} gene was frequently reported in \textit{Shigella} species worldwide [114-118]. The plasmid-mediated and quinolone specific efflux pump QepA was also reported from this genus [118,119].

**Concluding Remarks**

The epidemiological and surveillance studies have indicated that \textit{Vibrio} and \textit{Shigella} spp. have been a major cause of diarrheal illness in humans throughout the globe, especially in the developing countries like India. Antibiotics play a major role in the treatment of infections caused by these bacteria but indiscriminate use of antibiotics has caused the emergence of
multi-drug resistant strains. The multi-drug resistant *Vibrio* and *Shigella* infections are very difficult to treat which increases the public health threat. Molecular epidemiology studies on antibiotic resistance explain the importance of mutations in the emergence of resistant bug and the role played by MGEs in disseminating the resistance traits among different bacterial groups. The MGEs such as SXT elements and SRL are unique for *Vibrio* and *Shigella* species respectively and have significant contribution in spreading the antibiotic resistance gene cassettes among their community. The measures such as the proper practice of handling foods, safe drinking water supply, improved sanitation and prudential use of antibiotics could overall reduce the occurrence of outbreaks and spread of resistant bugs. Continuous surveillance of antibiotic resistance in these bacterial isolates should be encouraged to understand and match these rapidly evolving pathogens pace to pace. There is also a need to develop alternative strategies and promising therapeutic approaches that could control multi-drug resistant *Vibrio* and *Shigella* species. Development of inexpensive and efficient oral vaccines will be a potential approach to control the infection of these species, especially in young children.

**Acknowledgements**

The laboratory is supported by the grants from Gujarat State Biotechnology Mission, Department of Science and Technology, Government of Gujarat (No.GSBTM/MD/PROJECTS/SSA/1535/2013-14) and Indian Council of Medical Research, New Delhi, India (No. the grant AMR/49/11-ECDI). K. Vinothkumar is the recipient of fellowship from ICMR grant (No.80/845/2013-ECD-I). The authors are grateful to Dr. Amit Ghosh, Dr. T. Ramamurthy and Dr. S. K. Niyogi, National Institute of Cholera and Enteric Diseases (NICED), Kolkata, India, for their support and advice.

**References**


75. CDC (2013) Antibiotics resistance threats in United States. Section 3; 75.


Multidrug Resistant (MDR) Organisms in Clinical Practices

M. N. Sumana* and Geethu Thomas
Department of Microbiology, JSS hospital, Mysore.
*Corresponding author: Dr. M. N. Sumana, Department of Microbiology, JSS hospital, Mysore, Phone: +919845128274; E-mail: mnsumana12@gmail.com

Introduction
Multidrug Resistant (MDR) organisms are predominantly bacteria that are resistant to one or more classes of antimicrobial agents. They are frequently resistant to all, leaving one or two antimicrobial agents, complicating treatment of illnesses they cause [1, 2]. MDR is defined as resistance to three or more first-line classes of antibiotics (β-lactams, aminoglycosides, fluoroquinolones) or resistance to carbapenems [3]. Intensive Care Unit (ICU) patients are frequently infected with MDR pathogens as these patients are predominantly vulnerable having undergone several procedures along with the use of invasive devices. Infection and related sepsis are the leading causes of death in noncardiac ICUs with mortality rates that reach 60% and account for approximately 40% of the total ICU expenditures [4]. This chapter presents details on Gram positive and Gram negative pathogens prevalent in clinical settings and a description of antibiotics as treatment options for these deadly MDR pathogens, with advantages and disadvantages of using each drug.

Most common MDR pathogens encountered are:

**Gram positive-**
- Methicillin resistant *Staphylococcus aureus* (MRSA)
- Vancomycin resistant *Enterococcus* (VRE)

**Gram negative-**
- Extended spectrum beta lactamase (ESBL) producing bacteria
- Carbapenemase producing Enterobacteriaceae and non-fermenters like *Pseudomonas* and *Acinetobacter*

Development of MDR in ICUs is because of the following reasons-

1. Selection pressure- Frequent antibiotic use over long periods of time puts selective pressure on bacteria resulting in mutational or transferable drug resistance.
2. Patient-to-patient transmission- Improper cleaning and disinfection of surfaces and equipment that may be contaminated with pathogens, which are in close proximity to the patient.
3. Improper hand hygiene.
4. Debilitation and co-morbid conditions in patients.

The following sections would describe the important MDR pathogens frequently found in clinical setups.

**MRSA**

MRSA is a major cause of healthcare-associated infections (bloodstream infections, surgical wound infections, Ventilator Associated Pneumonia (VAP), community acquired infections, wound infections and skin infections like boils, furuncles, carbuncles, etc.)

The most important risk factors for MRSA colonization and infection are previous stay in hospital or long-term care facilities, ICU stay, intravascular devices, prior or prolonged antibiotic therapy, chronic underlying conditions, surgical wounds, advanced age and cross-contamination.

Treatment options are-
- Vancomycin
- Linezolid
- Clindamycin
- Trimethoprim sulfamethoxazole
- Tetracycline or Doxycycline/Minocycline
- Daptomycin
- Quinupristin/Dalfopristin
- Tigecycline
- Dalbavancin/Telavancin
- Rifampicin

Although it appears that there are many treatment options, following are the limitations in treatment:
- Vancomycin is available only as injectable formulation and it is expensive. With rampant use of vancomycin, staphylococci are acquiring cross resistance from enterococci through plasmids [5].
- Linezolid is a good choice for MRSA treatment as it is also available as oral formulation. But it has to be used with caution as it needs to be reserved for treatment of MDR *Mycobacterium tuberculosis* [6] With the availability of linezolid as oral formulation, it is being misused and overused, resulting in the emergence of linezolid resistance (both mutational and transferable) [7,8]. Prolonged therapy with linezolid is associated with toxicity like thrombocytopenia, peripheral neuropathy, optic neuropathy and lactic acidosis [9].
- Clindamycin can be used only if the pathogen is found to be susceptible in vitro as inducible resistance due to macrolides is quite common [10].
- Trimethoprim-sulfamethoxazole can be used only if the pathogen is found to be susceptible in vitro.
- Tetracycline or Doxycycline/Minocycline cannot be used in children below 2 years of age.
- Daptomycin cannot be used in MRSA pneumonia as it gets inactivated by lung surfactant.
- Tigecycline has to be reserved for treatment of resistant gram negative bacteria.
- Quinupristin/Dalfopristin resistance is emerging now a days [11].
- Dalbavancin, Telavancin are new glycopeptides that have superior pharmacodynamic properties compared to vancomycin. They cause hemorrhagic side effects and elevation in the levels of liver enzymes [12].
- Rifampicin needs to be reserved for treatment of MDR *M. tuberculosis*.

With so many limitations, it becomes difficult to choose the right antibiotic for treatment of MRSA infection. In patients with hepatic and renal insufficiency, the choices are further limited.
VRE

VRE causes Urinary Tract Infection (UTI), abscesses, wound infections, pneumonia, endocarditis, meningitis and sepsis [13]. The risk factors for VRE infections include neutropenia, chronic diseases like diabetes or patients who have recently received antibiotics. It is also more common in patients with indwelling devices like intravenous lines or urinary catheters. The rate of vancomycin resistance varies among enterococcal species, and is highest in *E. faecium*, of which >80-85% are resistant to ampicillin and penicillin and >50% to high-level gentamicin [14].

Treatment options are:
- Linezolid should be used with caution because of the limitations described in the above section 2.
- Quinupristin/Dalfopristin has been found to be effective in 86% of the cases of VRE but resistance to Dalfopristin/Quinupristin has already emerged [11].
- Daptomycin cannot be used in lung infections.
- Tigecycline is to be reserved for treatment of resistant gram negative bacteria.
- Teicoplanin can be used only if vancomycin resistance is due to VanB.
- Dalbavancin, Telavancin and Oritavancin are new glycopeptides that have superior pharmacodynamic properties compared to vancomycin. They cause hemorrhagic side effects and elevation in liver enzymes.

ESBL Producers

ESBL producing gram negative bacteria liberate Beta-lactamases conferring resistance to the penicillins, first, second and third-generation cephalosporins and aztreonam. The most common ESBL-producing bacteria are *Escherichia coli* and *Klebsiella pneumoniae*. Other bacteria with ESBL production are *Enterobacter* spp., *Salmonella* spp., *Morganella morganii*, *Proteus mirabilis*, *Serratia marcescens* and *Pseudomonas aeruginosa*. ESBL producing bacteria cause skin and soft tissue infections [15], bone infections, gastrointestinal tract infections, UTIs, pneumonia, meningitis and sepsis.

Risk factors for ESBL-producing bacterial acquisition include [16]
- Diabetes Mellitus (DM)
- recurrent UTIs
- Fluoroquinolone administration in past 2 months
- Any hospital admission in the past year
- Patient receiving hemodialysis
- Intubation and mechanical ventilation
- Patient who previously had an antibiotic-resistant organism (e.g., MRSA, VRE)

ESBLs are spread via direct and indirect contact with colonized/infected patients and contaminated environmental surfaces. ESBL producers are most commonly spread via hands of health care providers.

Treatment options are:
- Carbapenems are regarded as the drugs of choice for treatment of infections caused by ESBL-producing organisms. Carbapenems are expensive. Unfortunately, use of carbapenems has been associated with the emergence of carbapenem-resistant bacterial species such as *Stenotrophomonas* spp. or *Pseudomonas* spp.
- Beta-lactam/beta-lactamase inhibitor combinations like amoxyclov, piperacillin-tazobactam, cefoperazone sulbactam etc. Although in *in vitro* tests ESBL producers are inhibited by beta-lactamase inhibitors, clinical efficacy is controversial particularly in more serious infections such as bacteremia [17,18].
• Fosfomycin achieves very high concentrations within urine and is therefore an excellent agent for cystitis, but should not be used for pyelonephritis or patients with bacteremia due to inadequate concentrations within the bloodstream [19].

**Carbapenem resistant Enterobacteriaceae (CRE)**

According to Centers for Disease Control and Prevention (CDC), CRE are defined as Enterobacteriaceae that are:

- Nonsusceptible to one of the following carbapenems: doripenem, meropenem, or imipenem and

- Resistant to all of the following third-generation cephalosporins: ceftriaxone, cefotaxime and ceftazidime.

Clinical infections are usually healthcare associated and are in most cases UTIs, surgical site infections, bacteremia and ventilator-associated pneumonia. Risk factors for CRE include advanced age, severity of the underlying illness, ICU stay, previous antibiotic exposure, invasive devices, organ or stem-cell transplantation, mechanical ventilation and prolonged hospital stays [20].

Treatment options though very limited are:

- Colistin is often the only agent active against CRE bacteria but nephrotoxicity and neurotoxicity are a primary concern.
- Since tigecycline achieves very low serum concentration, it cannot be used in sepsis. It is not approved for use in children below 2 years of age [18].
- Fosfomycin is developing increased resistance due to mutations [21].
- Gentamycin cannot be used as monotherapy [22].

**Concluding Remarks**

The battle between man and microbes was won when sulphonamides and penicillins were introduced in the early part of 20th century but the battle continues with emergence of drug resistance. The battle is likely to be lost when man is challenged with MDR pathogens. Although multiple antibiotics are available as treatment options, not many antibiotics are suitable for treatment of MDR pathogens. Added to this are the comorbid conditions like diabetes mellitus, ischemic heart diseases, chronic obstructive pulmonary diseases, immune incompetence (either use of immunosuppressive drugs or extremes of age) that pose greater challenge to the treating physicians. The problem gets compounded if the patient presents to the physician late with multi organ failure.

Therefore, if mankind has to win the battle against microbes, the following strategies should be employed:

- Newer antibiotics with greater potency should be introduced (for the past one and a half decades, no newer antibiotics have been introduced into the market).
- Salvage the antibiotics available in the market by preventing emergence of drug resistance.
- Stop irrational use of antibiotics- Antibiotic stewardship.
- Stop over the counter sales of antibiotics.
- Good control of comorbid conditions as mentioned above.
- Good hospital infection control practices, most important being good hand hygiene.
- Seek early medical help.
- Adequate prophylactic measures including vaccination.
References


Abstract

“The future of humanity and microbes will likely evolve … as episodes of our wits versus their genes” Dr Joshua Lederberg.

The drug-resistant bacteria are currently considered as a major public health problem. The emergence of multiple resistances among major pathogenic microbes has grown alarmingly over the past few years. The enormity of the problem is evident from the fact that the standard treatments readily available in most parts of the world have been rendered ineffective in almost all common infections, e.g. urinary tract infections, pneumonia and blood stream infections. Similarly, gonorrhea may soon become untreatable as resistance has been reported against last-resort third generation cephalosporins. The problem is compounded by the lack of new drug development to augment the fast-depleting war chest of available antibiotics. Diseases such as tuberculosis, malaria etc. are becoming difficult-to-treat with time. A scary scenario of return to pre-antibiotic era is not far from reality. Globally, governments and other stakeholders in the healthcare agencies have initiated intense programs to tackle the problem of antimicrobial drug resistance. At the same time, industry will be required to pitch in with renewed interest in new antibiotic drug discovery and development.

Introduction

Resistance to antibiotics has reached an alarming proportion with multi-drug resistant and extremely drug resistant to pan drug resistant pathogens being reported from all over the globe [1-2]. Most of the antibiotics discovered and developed during the so called golden age have become ineffective for control of many pathogens and this was recognised as a global problem by WHO way back in 2001 [3]. However, no substantial progress has been made till date to contain the issue; instead the situation has worsened since. The emergence of resistance is not a new phenomenon, earliest cases of resistant microbes were reported almost concomitant with the introduction of penicillin in the clinic [4]. Alexander Fleming even warned about the impending future then [5].

Natural antibiotics have existed for millions if not billions, of years [6]. They are almost as old as the microbes [7]. One of the postulated functions of the ancient antibiotics is elimination of other bacteria competing for resources and providing selective benefits for the
producing organisms [8-9] and cell-cell signalling [10]. Research has revealed the presence of tetracycline in the skeletal remains found in ancient Sudan dating back to 350-550 CE [11-12]. Tetracycline class of chemicals are strong chelators and get incorporated into the hydroxyapatite portion of the skeletal structure of bones and in the enamel of teeth. Another historical anecdote about the exposure of humans to antibiotics is given in the use of red soils in Jordan that were considered to have antibiotics-like properties. Modern research tools have indeed established the presence of antibiotic-producing bacteria in these soils [13].

Traditional Chinese and ancient Indian medicine systems have also reported use of many antimicrobial remedies for millennia. This also would have exposed large human population to antibiotics prior to their widespread use in clinical medicine. A good example is the discovery of anti-malarial drug artemisinin extracted from *Artemisia annua* plants. Chinese medicines have used the extract of this plant for thousands of years as a remedy for many illnesses [14]. The Indian system of medicine similarly has exploited the anti-bacterial activities in turmeric (*Curcuma longa*)-curcumin [15], neem (*Azadirachta indica*, [16-17] and others. Anti-microbial activity has been observed in several other herbs used in traditional Chinese and ancient Indian medicine systems [18]. Therefore, the antibiotics and microbes have been described to have co-evolved for millions of years.

Antibiotics were introduced in the clinical practice almost a century ago (Figure 1). Beginning of the 20th century saw quest for ‘magic bullet’ which led Paul Ehrlich and S Hata in 1910 to synthesise and screen series of organoarsenic compounds with anti- *Treponema pallidum* activity [19]. The resultant drug Salvarsan and Neosalvarsan remained the most prescribed pharmaceuticals for a long time.

With the introduction of sulphonamides in 1937, the antimicrobial discovery became a fulltime research activity, which got a tremendous fillip by the introduction of penicillin in the clinical practice in 1942. The antibiotic era had started with mass production and distribution of penicillin by 1945 [20]. Most of the antibiotics were discovered during 1950s and 1960s with more than 20 new classes of antibiotics being discovered during this period, christened as the Golden era of antibiotics discovery. Most of the natural scaffolds of the antibiotics had been discovered by the mid- to end-1960s and the golden era was coming to an end. The classical methods of discovering new antibiotics returned the same leads that had been found earlier. No new leads were being discovered. And then came the era of medicinal chemistry when improved versions of known antibiotics were synthesised on the existing natural scaffolds. There was a long period of lull before any new classes of antibiotics were added to the arsenal, before daptomycin (lipopeptides) was discovered in 1986 (approved for clinical use in 2004). By the 1990s it was clear that the microbes had outpaced antibiotic development – natural and synthetic both. The resistance spread far and wide with many extremely resistant and pan-resistant strains of pathogenic bacteria been identified in the environmental and clinical settings.

Given the long history of usage of the anti-microbial substances, it is quite plausible that the antibiotic resistance may have arisen much before the widespread use of antibiotics started after the onset of modern antibiotic era. The history of genetic evolution of the antibiotic-resistance genes can be explored by phylogenetic analysis of the microbial genomes. Indeed such studies have shown that the genes conferring resistance to many classes of antibiotics were present in the nature well before the widespread use of modern antibiotics [21-22]. For example, structural analysis of β-lactamases has established that these enzymes originated more than two billion years ago. Some of the β-lactamases were postulated to be present on plasmids for millions of years [23]. Interestingly many β-lactamase genes evolved over a long period, over 100 million years in Klebsiella, for example [24]. Other resistance genes could possibly have evolved from the pre-existing ancient genes in the resistome as was recently suggested for carbapenemase in *K. pneumoniae* [25-26].
Figure 1: Timeline of Antibiotic Development.
Pharmaceutical Industry: Discovery of New Antibiotics

Early growth of the pharmaceutical industry was almost parallel to the discovery and development of anti-infectives. The industry was at the forefront of discovery and development of antibiotic drugs during the golden era as well as during the medicinal chemistry era. Close to 300 different antibiotics and their derivatives have so far been developed for clinical use. However, 90% of those were developed in the 1970s and 1980s. In the last two decades, only two new classes of antibiotics have been discovered using the traditional screening methods. The known antibiotics scaffold was used to make better drugs for many years.

War on infectious organisms was considered over with the quick invention of close to twenty classes of antibiotics and multiple members/derivatives of each class. The resources and energy got slowly diverted towards finding solutions for more chronic problems such as cancer and heart disease. As the ghost of resistance started to grow, it was quickly realised that the arsenal to defeat it was highly inadequate. However, by then the rules of the game had changed drastically. The pharmaceutical industry which thrived on the development of anti-infectives had decidedly moved away from the development of new antibiotics. Most of the ‘big pharma’ players had significantly reduced their R&D allocations towards new antibiotics discovery by the onset of 1990s and investments in this area further dwindled in the following decades. This shift happened due to multiple reasons that were not purely profit-motivated. It would be realistic to believe that much of pharmaceutical advances in the chronic disease areas would not have happened had the attention and resources not been diverted away from the antibiotics research programs.

Antibiotics traditionally are used for a short period of time only during an infection. On the other hand, the drugs for other indications such as cancer, hypertension or diabetes are used for a much longer time, often for the rest of the life of the patient and hence have much bigger market – and profits. Increasingly stringent requirements enacted by the regulatory agencies escalated the cost of drug development to exorbitant levels. Especially the clinical phases of drug discovery and development have of late become so tedious and expensive that the developers are no longer motivated to conduct discovery programs for antibiotics. With ever increasing cost of development of new drugs, pharmaceutical industry with their limited research budgets developed criteria (for example calculation of ‘net present value’) for selecting and dropping projects based on economic considerations[27]. Over time, the largely profit-driven pharmaceutical industry invested less and less resources in antibiotics research and instead focused more on the development of other ‘blockbuster’ drugs, those that had potential of earning 1 B US$ or more in a year. This resulted in new antibiotic development projects getting dropped from the development programs of the major pharmaceutical companies leading to drying up of the product pipelines. Moreover, the industry focused more on developing analogues on the known scaffolds of known classes instead of doing a more risk-based discovery of new classes of antibiotics. The advantage of focusing more on analogues was greater probability of passing the toxicity and efficacy barriers as well as proven targets and clear regulatory paths. However, this approach crowded the shelf with numerous analogues against the same target as the parent scaffold and made each molecule individually less profitable. This approach was successful for a limited period of time before the microbes developed resistance even to the ‘tweaked’ antibiotics. And, that happened rather quickly in many cases[28].

General lack of interest in developing new antibiotics by the pharmaceutical industry witnessed redeployment of manpower skilled in the art and eventually getting retrained in discovering more profitable drugs and being rewarded for each blockbuster that they developed. This has resulted in the shortage of scientific staff trained in classical methods of microbial prospecting, isolation and improvement of strains for production of antibiotics. For example, the painstaking methods developed by Waksman are no longer known to the newer generation of microbiologists or biotechnologists who are trained on the High-
Throughput Screening (HTS), genomics, and bioinformatics and big-data analytics methods. Unfortunately, even such sophisticated screens and target validations have been a failure in terms of new antibiotic discovery[29].

The pharmaceutical industry had enthusiastically pinned its hopes on finding new targets and new molecules from the wealth of genomics data in the 1990s[30-31]. Experience gained while developing methods, algorithms and strategies for rational drug design, combinatorial chemistry and high-throughput screen combined with the genomics approaches was thought to be the answer to rapidly spreading antibiotic resistance. Metagenomics approaches were used for identification of ‘essential’ genes across bacterial families. Many targets identified thus were subjected to high throughput screening campaigns using vast chemical libraries. Dozens of high throughput campaigns later screening more than 150 putative targets, Glaxo Smithkline abandoned the search for novel chemicals as not a single lead was found with a reasonable spectrum of antibacterial activity [29]. Similar stories were reported by many other well-known names in the antibiotic drug development industry [32]. Eventually the interest in these approaches waned due to the economic reasons discussed earlier. Moreover, recently governments and drug administrations have clamped restrictions over the use of new antibiotics for common bacterial infections with a view to prevent resistance development in the population and environment. This step proves to be counterproductive as a company that invested huge resources and funds in developing new antibiotic drugs is prevented from selling the product and therefore from realizing the profits from the new product, further discouraging development [33].

Another factor leading to the slowing pace of new antibiotic development could be the limitations of the methods being deployed to look for them. For example, the soil that yielded the antibiotics producing Actinomycetes harbours many other species of bacteria which could produce antibacterial compounds. Actinomycetes were discovered due to ready ability to adapt and grow in the lab. However, the scientific community has faced a tremendous hurdle in making many other soil bacteria grow in the laboratory[34].Similarly, other environmental niche areas such as oceans, lakes, rivers, plants etc. may be rich sources of antibacterials that have not been adequately explored yet for the purpose of antibiotic discovery. Recently, a novel method of growing soil bacteria in the environment that is most conducive for their growth, the soil itself, was reported [34-35].iChip is a device that has perforated chambers which allow movement of nutrients and other growth promoting ingredients from the nearby habitats and support growth of soil microbes in their own niche. The soil is thought to harbour many more hitherto undescribed species of bacteria that might produce effective antimicrobial metabolites. Such an approach can help in discovering many new bacterial species with antibiotic or other compounds of importance by making it possible for the microbiologists to cultivate the ‘inhospitable’ bugs. iChip has already helped discover a new organism, a new class and a new antibiotic [36].Lewis and co-workers demonstrated that the new antibiotic, teixobactin did not have any demonstrable resistance in the mutant strains of Staphylococcus aureus and Mycobacterium tuberculosis. Much more exciting than the discovery of the new antibiotic was the use of iChip to cultivate inhospitable bacteria from the soil that led to the discovery of a new compound from a new source and validated a method that holds further promise. Clinical development and regulatory approvals of teixobactin may take a long time before this new antibiotic is approved for human consumption.

Encouragement to Pharmaceutical Industry for Antibiotics R&D

The pharmaceutical industry despite increasing disincentivization due to economic, regulatory and clinical hurdles, has invested in developing and bringing to market more than 20 antibiotic drugs since 2000 and more than 50 antibiotics are in various clinical phases of development [37]. These included new classes and subclasses of an existing drug with significant advantages in terms of the antibacterial spectrum. However, it is imperative
that all stakeholders must come together to ensure a consistent pipeline of effective antibacterials to be able to fight the microbial infections over any significant length of time now onwards. Therefore, national governments, drug regulatory agencies, philanthropic funding agencies, international health organizations (WHO), pharmaceutical and diagnostic industries, research funding agencies, patient advocacy groups, insurers etc. must ensure that enough emphasis is laid on an urgent basis to help boost R&D effort towards new antibiotic development [38-39].

Firstly, the pharmaceutical and biotechnology companies must be induced to restart the antibiotic development programs in the right earnest. This can be facilitated by taking slew of financial measures by the national governments by incentivising investments in the field of antibiotic development. Two types of mechanisms can be introduced to encourage more R&D activity on antibiotics in the industry. One of the mechanisms reduces the cost of development of new drugs for a company by making multiple parties to share the expenditures. Some of the components of this mechanism can include establishing effective public-private partnerships, sharing access to the cutting edge research being performed at the government funded institutions, offering tax incentives such as tax credits on the R&D expenditure and providing research grants to the private players. Second mechanism can include rewarding the successful development of a drug by ensuring and increasing the future revenue. Such rewards can be in the form of longer than usual market exclusivity to ensure higher revenue to offset the high cost of development, one time lumpsum cash awards, advanced market commitment, relaxed regulatory approvals which indirectly cuts down on the cost of development, rational regulatory framework and guidelines and favourable reimbursement prices to enable cost recovery. Alternatively, a hybrid model of the two mechanisms could be a win-win situation for all.

While it is agreed that the antibiotic drugs must be affordably priced, an equally compelling fact is that no pharmaceutical company would undertake a loss-making task of developing and producing an antibiotic product. The patients and their advocacy groups need to realize that the cost of development for a lifestyle drug such as a cholesterol-lowering statin or an antidiabetic gliptins is similar to an antibiotic drug. The other classes of medicines are used over a very long period of time, often for the entire life whereas the antibiotics are typically taken for a few days only. A patient willingly pays thousands of dollars for a cancer chemotherapy (at times even more) where the life expectancy increases by a few more weeks, but does not want to pay a few hundred dollars for a life-saving antibiotic that cures the infection. This complacency with regards to constant availability of the antibiotics forever needs to be shaken off and public and payers need to be educated about the dangers of the trend amongst pharmaceutical companies of not doing enough to generate a healthy pipeline of antibiotic products.

A different line of thought warrants attention too. In contrast to some of the suggestions for incentivisation outlined above, a different approach whereby the companies are punished for not doing enough for antibiotic development perhaps would also be effective. All the corporates, pharmaceuticals and non-pharmaceuticals, may be asked to contribute a fraction of their profits against tax credit to a global fund to support others who invest in antibiotic development initiatives, making them part of the corporate social responsibility programs. An additional punitive levy may be imposed on pharmaceutical companies that do not conduct research for new and novel antibiotic drugs development. The approvals for minimally tweaked or generic antibiotic drugs without significant improvement on either efficacy or spectrum may be denied or delayed. Non-healthcare use of antibiotics especially in agriculture, poultry and animal husbandry has significantly compounded the problem of resistance against antibiotics by making available huge resistome[40]. The ‘civil’ society can contribute towards curtailing the use of antibiotics in agriculture and allied activities
by denouncing the use of products that are not certified to be antibiotic-free. Major food chains may be forced in to pledging their support for not using meat from antibiotic-fed animals and may be coerced into contributing to the global fund for antibiotic research till they phase out the use of meat from antibiotic-fed animals.

**Social Awareness for Antibiotic Usage**

Patient awareness programs are also important. Numerous studies have noted pitfalls of self-medication with antibiotics and over-the-counter sales of these drugs without prescription and have established a relation between rampant and indiscriminate as well as incorrect use by ill-informed and ignorant patients[41-42]. This calls for social programs to educate and train patients and consumers in correct practices of antibiotic use. Societal awareness programs such as anti-smoking or seatbelt use among drivers have demonstrated usefulness of such initiatives. In the moderate to low income societies, effective programs such as ‘Eradicate Polio’ have been successfully implemented[43]. Similar social programs can be highly effective and useful in spreading awareness amongst masses and discourage incorrect use of antibiotics to curtail spread of resistance. Continuing Medical Education programs maybe devised to discourage the physicians from unnecessary prescriptions and use of antibiotics where it may not be required. All medical practitioners must be enrolled in to such training programs that provide information about the resistance to frontline antibiotics and the magnitude of the problem at hand. Similarly, the rapid and accurate tests must be developed to make early diagnosis and ensure the correct prescription. Nevertheless, industry and academia must devote time and resources for development of new antibiotic drugs to continue a healthy pipeline for the next 50-100 years. Some of the platforms of antibiotic development have been proposed for this.

**Platforms for Antibiotic Development**

Reliable antibiotic drug discovery platforms are needed to provide adequate pipeline of lead compounds that can be then converted into clinically useful antibiotics in order to be able to meet the demand of effective drugs in the face of the growing ghost of resistance. As such the ‘lead-to-product’ conversion ratio is very small, especially due to failures in the clinical phases of drug development. Lack of new leads in advanced clinical phases would mean that as soon as the existing drugs become ineffective – the pace of which is alarmingly high and increasing, humankind will not be able to fight infections as it has got used to during the past 6 to 7 decades. Therefore, unless there are reliable and efficient discovery platforms that churn out leads, development of new antibiotics at reasonably fast pace will remain a distant dream. Another and a good reason of developing platforms can be the probability of finding effective combinations of drugs to be used in the face of rising resistance. In this case, two or more antibiotic drugs that individually may not show very good therapeutic profiles may be combined together to achieve better efficacy against resistant pathogens. A well-known case in point is the development of combination of clavulanic acid (a natural β-lactamase inhibitor) with β-lactams to counter the resistance conferred upon the microbes by producing β-lactamases[44]. Famous combinations have also been developed for the treatment of tuberculosis.

A highly promising platform can be that of prodrugs. The prodrug gets activated only after it gets inside a bacterial cell and thus is harmless to the host. A bacteria-specific enzyme will then convert it into active drug that will then covalently bind to unrelated targets. Once inside the bacterial cell and covalently bound to one or more targets, the drug will not be pumped out by the MDR efflux pumps and will be a broad-spectrum pharmaceutical. The difficult task here is to redefine the principles of drug transport into the microbial cells because the rules so far used to conduct high-throughput screens were based on the Lipinski’s ‘rule of five’ [45] that were formulated on the basis of eukaryotic systems. The
prokaryotic membranes being of very different composition may need rewriting of these rules, especially for the permeability of drugs to cross the microbial cell membranes and cell walls with differences in lipid compositions. Many candidates failed to fulfil the criteria on the rules and were therefore dropped.

Several of the existing compounds, notably for tuberculosis are in fact prodrugs and are converted to active metabolites inside the bacterial cells where they covalently bind their targets. Similarly, metronidazole also is a prodrug activated by nitroreductases of the bacterial cells expressed by pathogens living under microaerophilic and/or anaerobic conditions such as *Helicobacter pylori*, *Clostridium difficile* etc. These prodrugs were discovered during the 1950s. The global compound libraries of those times had less than 10000 compounds. Compared to that, today there is over 1000 fold increase in the number of available compounds and therefore there is a very strong probability of finding new and more effective prodrugs against resistant organisms too. One has to realize the reasons for finding new compounds using better and bigger libraries after 1950s. The screens will have to be modified for acceptance and rejection criteria, such as the specificity test, for finding better/novel antibiotics. An effective revival of this platform can return next wonder drug(s).

The second platform approach can be of developing species-specific drugs. This platform can be put to good use with high throughput screens with pathogen-specific validated targets using synthetic compound libraries. One such screen using a *Mycobacterium* species resulted in selection of a compound that is currently the frontline anti-MDR TB agent, bedaquiline[46]. The drug binds specifically to mycobacterial ATP synthase owing to a minor difference in the C subunit of this and ATP syntheses from other bacteria. Mycolic acid synthesis pathway is unique to Mycobacteria and is a relevant target for many of the anti-TB compounds such as ethambutol[47](inhibits arabinosyl transferase) and isoniazid[48](inhibits enoyl-ACP reductase InhA). A single organism, validated target approach is relatively risk-free and can be adapted to HTS mode with commercial and private synthetic compound libraries. The same platform may be applied to other ‘difficult-to-treat’ species too with good probability of developing a useful antibiotic drug [49].

A species-specific drug discovery platform will have the advantage of being safer for humans as a microbial-specific target is unlikely to be present in mammalian host too. Big-data analytics and bioinformatics tools can be employed to identify novel and pathogen-specific gene products as targets for drug development. Another advantage of species specific antibiotic drug will be that automatic elimination will remove the compounds with undesirable properties in very initial stages. Being species-specific is less burdensome on the gut microbiota as well. Treatment with broad spectrum antibiotics are known to disturb and/or disrupt native microbiomes of the gut which then may or may not get restored further leading to various conditions such as adverse effects on immune system, diabetes and mental health apart from short term antibiotic-induced diarrhoea [50-53]. Species – specific drugs may have an additional advantage of being less susceptible to the development of resistance. One of the mechanisms of resistance development is lateral transfer of the mutations amongst communities of bacteria. For example if a symbiotic organism develops resistant phenotype to a drug, it can then horizontally transfer the same to a pathogen. This will get minimized to a large extent if narrow-spectrum species-specific compounds are only used.

Antibiotic discovery can get a booster shot, as discussed before with rewriting of the rules of penetration of microbial membranes, especially the gram-negative bacteria where the shortage of effective drugs is more acute. Lipinski’s ‘rule of five’ may be tweaked based on the analysis of properties of classes of compounds known to effectively penetrate the bacterial membranes as well as that do not. Armed with the new and more relevant rules, synthesis of large libraries of synthetic compounds and screening using high-tech platforms will be entirely fruitful. Compounds may be selected from the existing libraries.
with better than threshold penetration and retention to provide larger and deeper chemical space for molecule design. A classical approach of animal model based in situ screening of antibiotics is another approach that may be tried. A newer model of pathogen infected Caenorhabditiselegans has been tried to screen some compounds with some success. An automated approach based on such a screen can be employed to test a large number of molecules from the synthetic libraries [54-55]. Many as yet undiscovered antibiotics may be present in the various environments such as oceans and plants and soil. These resources can be very promising if methods to cultivate and screen the bacteria from these environments can be developed.

Rewiring and retraining of the bugs to produce antibiotics from the ‘silent’ operons in microbial genomes is another promising and interesting approach for novel antibiotic discovery using powerful techniques of genome engineering, genome editing etc. [30,56-58]. Model actinomycete, Streptomyces coelicolor genome has been found to harbour genes for expression of 20-odd secondary metabolites out of which only 3 antimicrobial compounds have been known [59]. Similarly genomes of other Streptomyces spp. also have multiple operons for expression of secondary metabolites that have not been identified in laboratory-grown bacteria. However, if the same bacteria can be grown in ‘close-to-natural’ environments, then many antimicrobial compounds and/or scaffolds of natural origin can be identified for drug development[60]. Stimulation of antibiotic production by varying growth conditions has been a standard approach to discovery. A simple technique of growing organisms in different growth media has been successfully used by industry leading to discovery of several antimicrobials [61]. Modern biotechnological tools of cloning a gene into heterologous expression systems to express antibiotics from silent operons of soil bacteria was also tried by several small and large biotechnology and pharmaceutical companies with mixed success rate[62] and eventually were abandoned, perhaps due to economic constraints.

**Developments in the Industry**

Biotechnology industry and small start-ups the world over are pitching in with innovative ideas and strategies for development of new antibiotic drugs. Certainly, the small companies cannot be expected to take the drug from discovery to clinic due to lack of financial and economic resources. However, with a proven concept and initial developmental data, large pharmaceutical corporations will be more than willing to support the small biotechnology companies in late-stage developments of these drugs rather happily.

Several Indian companies too are engaged in discovery of novel compounds with antimicrobial activities. Vitas Pharma of Hyderabad targeted gram negative and MRSA for new drug development with 6 projects. An approach involving in silico screening of virtual chemical libraries of inhibitors using models of essential bacterial proteins and iterative evolution of compounds followed up by in vitro and in vivo screening of selected compounds has been taken. Some leads have advanced into toxicology studies and others are in optimization phase. Similarly, Bugworks of Bengaluru is engaged in developing novel drugs against clinic-acquired infections and in developing new chemical entities based novel combinations against tuberculosis. Some New Chemical Entities (NCEs) have already reached the pre-clinical stage of toxicity studies. Then Cadila Pharmaceuticals of Ahmedabad in partnership with UK-based Helperby Therapeutics is working on an alternate strategy of developing a so called ‘antibiotic resistance breaker’ that can be used with an obsolete antibiotic drug. This approach, originally developed by Helperby’s Prof Antony Coates, if successful will make many antibiotics useful again. This would be a major breakthrough as it would be possible to extend life of many discontinued antibiotics due to inefficacious drug profiles.

Pharmaceutical companies such as Wockhardt and Cipla in India have active antimicrobial drug discovery and development programs. Wockhardt has so far two NCEs, WCK 771 and
WCK 2349 in the clinical phase and three compounds in different stages of pre-clinical phase, although all these molecules are based on already known scaffolds with structural tweaking. Some biotech and pharmaceutical companies are engaged in development of non-traditional therapeutic drugs including small molecules as well as monoclonal antibodies, vaccines and probiotics. Many antibody based products are in different stages of development targeting systemic infections. Over a dozen different monoclonal antibody based therapies and vaccines are under trial for treatment of infections caused by *P. aeruginosa*, *C. difficile*, *S. aureus* (Table 1). Another half a dozen products are under development in the form of vaccines against these pathogens. However, the success rate of antibiotics development leaves much to desire. A study compiled more than 15 anti-bacterial in various phases of clinical development almost a decade ago [63], although none belonged to a novel class. Of those, seven have been commercialised, some with restricted approval with another seven in various stages of clinical development [64]. A few compounds that entered clinical phase a decade ago failed to deliver on many counts and were withdrawn from the pipeline and a few have shown remarkably slow progress than anticipated through the clinic.

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>Phase</th>
<th>Target pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody-based therapeutics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerucin</td>
<td>Aridis Pharma</td>
<td>1</td>
<td>Broadspectrum</td>
</tr>
<tr>
<td>514G3</td>
<td>XBiotech</td>
<td>½</td>
<td>Bacteremia caused by <em>S. aureus</em></td>
</tr>
<tr>
<td>Tefibazumab</td>
<td>Bristol Myers Squibb</td>
<td>2</td>
<td>Bacteremia caused by <em>S. aureus</em></td>
</tr>
<tr>
<td>Medi4893</td>
<td>MedImmune</td>
<td>2</td>
<td>Pneumonia caused by <em>S. aureus</em></td>
</tr>
<tr>
<td>Pagibaximab</td>
<td>Biosynex</td>
<td>2/3</td>
<td>Bacteremia caused by <em>S. aureus</em> neonates</td>
</tr>
<tr>
<td>Salvecin</td>
<td>Aridis Pharma</td>
<td>2</td>
<td>Pneumonia caused by <em>S. aureus</em></td>
</tr>
<tr>
<td>Aerumab</td>
<td>Aridis Pharma</td>
<td>2</td>
<td>Ventilator associated pneumonia caused by <em>P. aeruginosa</em></td>
</tr>
<tr>
<td>MEDI3902</td>
<td>MedImmune</td>
<td>2</td>
<td>Ventilator associated pneumonia caused by <em>P. aeruginosa</em></td>
</tr>
<tr>
<td>Actoxumab, Bezlotoxumab</td>
<td>Merck</td>
<td>3</td>
<td>Recurrent <em>C. difficile</em> infection</td>
</tr>
<tr>
<td>Vaccines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDV-3</td>
<td>NovaDigm Therapeutics Inc.</td>
<td>1</td>
<td>Prevention of <em>S. aureus</em> infection</td>
</tr>
<tr>
<td>Group B <em>Streptococcus</em> vaccine</td>
<td>Novartis International AG</td>
<td>2</td>
<td>Prevention of Group B <em>Streptococcus</em> infection</td>
</tr>
<tr>
<td>PF-06425090</td>
<td>Pfizer Inc</td>
<td>2</td>
<td>Against recurrent <em>C difficile</em> infection</td>
</tr>
<tr>
<td>SA4Ag</td>
<td>Pfizer Inc</td>
<td>2</td>
<td>Prevention of <em>S. aureus</em> infection</td>
</tr>
<tr>
<td>IC43</td>
<td>Valneva SE</td>
<td>2/3</td>
<td>Prevention of ventilator associated bacterial pneumonia</td>
</tr>
<tr>
<td>IC84</td>
<td>Valneva SE</td>
<td>2</td>
<td>Against recurrent <em>C difficile</em> infection</td>
</tr>
<tr>
<td>Cdiffense</td>
<td>Sanofi Pasteur Inc.</td>
<td>3</td>
<td>Prevention of <em>C. difficile</em> infection</td>
</tr>
</tbody>
</table>

**Table 1:** Antibodies and vaccine based antibacterial therapeutics under development.

**Concluding Remarks**

Time is running out before concrete steps are taken to control the ever growing ghost of antibiotic resistance. The return to pre-antibiotic era should be avoided at all costs. It is important to expedite the growth of antibiotic portfolio against all kinds of microbes. But until that happens and the results of renewed interest in new and novel antibiotic development begin to show, it will be relevant to rationalise the use of antibiotics by altering the present widespread practice of empirical prescriptions. Only after confirmation of the pathogen, an appropriate antibiotic regimen should be prescribed. In this scenario, a very important role is envisaged for rapid (with a turnaround time of 20-30 minutes), simple to perform (with minimal sample preparation requirements), cheap and accurate Point of Care (PoC) diagnostic tests that can identify the infecting pathogen and its antibiotic susceptibility profile. The results should guide the physician to the best intervention strategy as well as the most effective drug to be used. The target pathogens to be considered for such PoC test
development can be all drug resistant microbes with very little susceptibility to the available drugs. Besides improving rational use of antibiotics, improved diagnostics will help in better surveillance of emergence of resistance to existing and new antibiotics. Finally, the wheel of antibiotic discovery must be oiled well to improve the pipeline and population must be sensitised towards the dangers of living in the world of unstoppable infections, even from the slightest of bruises.

**Acknowledgements:**

Support of the Mankind Pharma Ltd management is thankfully acknowledged.

**References**

lactamases showing high identity with KPC. J Antimicrob Chemother 71: 1493-1496.


Abstract

Antimicrobial Resistance (AMR) is among the forefront challenges staring at the modern human civilization. The high hopes of conquering infectious microbes, which arose with the discovery of first antibiotics, have now faded away to give way to the wiser understanding that our battle with the pathogenic microorganisms is a never-ending one. Currently available arsenal of therapeutic antimicrobials is rapidly becoming of limited utility owing to the development and spread of AMR among the pathogens. Discovery and development of new antibiotics, particularly those with novel structures and mode of action, though is an urgent call of time, many additional strategies also need to be worked upon. This chapter provides an overview of the gravity of the AMR problem, and current status of the new antimicrobial drug development in context of the declining interest among pharmaceutical companies for this type of research. Anti-virulence approach as a supplement to the conventional antibiotics and inducing Reactive Oxygen Species (ROS) production in target pathogens as an anti-pathogenic strategy of wide significance are also discussed. Suitability of the microbial siderophores, riboswitches, and quorum sensing circuits as new targets for the next generation antimicrobials is also highlighted, besides pointing the need for special attention to the microbial biofilms, and role of inhibitors of microbial efflux pumps and antibiotic degrading enzymes. Later part of the chapter describes utility of simpler life forms like Caenorhabditis elegans for preliminary in vivo efficacy assays of potential antimicrobials, and how in silico approaches can well be integrated with the traditional in vitro and in vivo approaches for improving the overall productivity of the drug discovery process.

Keywords: Antibiotic resistance; Biofilm; Caenorhabditis elegans; Horizontal Gene Transfer (HGT); Quorum sensing; Reactive Oxygen Species (ROS); Riboswitches; Siderophores

Introduction

Antibiotic-resistant microorganisms existed even before the clinical use of antibiotics was started. However, the real threat of the problem of drug-resistance was realized only after almost three decades of widespread clinical and non-clinical applications of antibiotics. Today antibiotic-resistance among pathogenic microorganisms has been very well recognized as one of the biggest challenges looming before our civilization. It has attracted the due attention from the scientific community as well as policy makers, and few initiatives to
tackle this issue have been started. For example, in July 2014, the UK Government has commissioned the Review on Antimicrobial Resistance in collaboration with the Wellcome Trust to understand and propose solutions to the problem of antimicrobial resistance, from an economic and social perspective [1]. Owing to increase in number of HIV infected patients, and those undergoing organ transplantations, there has been a considerable rise in the number of immune compromised people, which has resulted in a rise in nosocomial infections, as well as those caused by the opportunistic pathogens. Increased population of the people living with compromised immune system has also resulted in a rise in the number of fungal infections. Finding antibiotics fulfilling the criteria of ‘selective toxicity’ against fungi is much more difficult than to find such molecules against bacteria, as fungi being eukaryotic share many cellular components with their human host, which cannot be used as therapeutic targets. It has been one of the most pressing needs of the current times to find different possible ways for effectively handling the issue of drug-resistant infections. What is required besides efforts for finding new antimicrobials, is to identify novel molecular targets inside the pathogens, effective use of high-throughput screening platforms, finding alternatives to the currently prevalent antimicrobial approach (e.g. boosting the host immune system, focusing on the anti-virulence strategy), expanding the utility of currently available drugs, etc. A detailed dealing of these issues follows in the forthcoming text.

The Problem of Drug Resistance and Its Magnitude

Continuous and the indiscriminate use of antimicrobial drugs in the last few decades has led to the problem of drug resistance. Drug resistance has been an ever-increasing problem, applicable for all chemotherapeutic agents as it involves turning of a previously susceptible pathogen population into a tolerant/resistant one to the administered drug [2]. According to CDDEP (Center for Disease Dynamics, Economics & Policy, USA), resistant microorganisms are becoming stronger because the available drugs have been used for multiple purposes; e.g. for the treatment of bacterial infections in surgical patients, protecting cancer patients as well as in the treatment of immunocompromised patients and for the prevention of disease in livestock animals. Uses of antibiotics for purposes other than treating human infections have also contributed towards exacerbating the spread of resistance. Antibiotics have been applied in animal farms and poultries for the betterment of animal health and as a preventive measure to protect them against infections [3]. Some antimicrobials have also found applications in products for routine sanitization, hand-washes, etc. resulting in an unnecessary exposure of the microorganisms to the sub-effective concentrations of various antimicrobials, and thus enhancing the spread of resistance [4].

World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) have also recognized the gravity of the threat posed by increased occurrences of drug-resistant infections involving Extended Spectrum Beta-Lactamase (ESBLs)-producing Enterobacteriaceae, antibiotic resistant strains of Acinetobacter, Campylobacter, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella, Streptococcus pneumoniae, Mycobacterium, vancomycin-resistant Enterococci (VRE), methicillin/vancomycin-resistant Staphylococcus aureus (VRSA/MRSA) [5, 6]. Moreover, the CDC also studied the threat level of antibiotic-resistant bacteria by assessing the bacterial infections according to several factors like clinical impact, transmissibility, economic impact, etc. CDC estimated that antibiotic resistance is responsible for more than 23,000 deaths each year in the US; 25,000 deaths in Europe, and 58,000 neonatal sepsis deaths in India [7]. It is clear that the problem of Multidrug Resistance (MDR) is getting worse owing to the continuous emergence of new pathogens with the potential for rapid spread.

Survival of the bacterial strains in presence of antimicrobial agents and development of high-level resistance is not a transient process. ‘Primary resistance’ is the case when
the organism is found to be resistant to an antibiotic which has never been previously administered to it in a particular host. Secondary resistance arises following the exposure of the organism to a particular drug and is known as ‘acquired resistance’. Among the molecular mechanisms involved in the spread of antibiotic resistance, Horizontal Gene Transfer (HGT) has also a significant role in the evolution of pathogenicity among microbial populations. It actuates the spread of genes coding for virulence/pathogenicity from pathogenic to non-pathogenic microbes across large phylogenetic distance, whereas vertical transmission transfers the antibiotic resistance genes only to the daughter generations of the resistant population [8]. Genes encoding virulence factors, such as those constituting part of Pathogenicity Islands (PAIs), are acquired by non-pathogens from pathogens through the HGT. These PAIs contain genes having a role in events such as secretion of adhesins, toxins, invasins, altering host cell metabolism, iron and phosphate acquisition [9]. HGT is mediated by processes of conjugation, transformation, and transduction. After a particular span of time, microorganisms build up a capacity to battle the inhibitory impacts of no less than one or all of the effective antimicrobial medications. This is termed as Extensive Drug Resistance (XDR) [2]. In addition to that, problems like suppressed immune functions, poor drug bioavailability, and increased rate of drug metabolism are also helping MDR to provoke interference in disease control, which results in drug ineffectiveness and treatment failure. MDR is contributing to the global economic burden by causing considerable morbidity and mortality. Continuous development of newer drugs and improvement in the efficacy of existing drugs are the essential actions to be taken to control this problem of drug resistance [10].

**Current Status of New Antibiotics Discovery in Context of the Declining Interest among Pharmaceutical Companies**

Fleming discovered penicillin in 1928, and its use started during the early 1940s. Discovery of penicillin was followed by the discovery of multiple new antibiotics over the next two decades. Antibiotics became famous as ‘magic bullets’ due to their uniqueness among all the drugs used in medical applications. They have played a vital role in modern medicine and have saved millions of lives owing to their ‘selective toxicity’, towards the target pathogen without harming the infected host much. However soon after a golden period of antibiotic discovery, finding of more such magic bullets slowed down, and the effects of antibiotic misuse began to appear in the 1970s [11, 12].

The current scenario is that the effects of widespread antibiotic use and misuse, which has culminated into a large number of MDR infections across the globe, have reached the status of a worldwide emergency threat. The gravity of the situation can partly be attributed to the absence of major advancements in the area of development of novel antimicrobial agents by pharmaceutical companies in last few decades. According to data available on the website of Pew Charitable Trusts, as of March 2016, 37 antibiotics were under development; among them, 11 were in phase I clinical trials, 13 in phase II, and 13 in phase III [13]. Though the scenario is not all pessimistic, antibiotic research and development certainly is not as progressive as it should be.

There are several reasons why the process of antibiotic development is running slow. One of the major reasons is that drug discovery is a time consuming and expensive process, and developing new drugs to deal with resistant bacterial infections is a challenging task. Typically, it takes about 10-15 years for a drug to reach the market and requires a huge fund without surety of success of a drug. This issue is valid for all classes of medications,
but in the case of antibiotics, the problem is worsened by the fact that an antibiotic can become useless well before its patent expires owing to the ability of the pathogens to acquire and spread resistance at a rapid pace. Pfizer, one among the world’s leading pharmaceutical companies, during the 1940s, manufactured large amounts of penicillin, as it was a highly profitable antibiotic. However, after some time, this profit was reduced due to the emergence of penicillin-resistant strains. Finally, after facing economic and regulatory obstacles company closed its antibiotic research facility in 2011. Likewise, other major pharmaceutical companies have not taken any appreciable interest in research and development of new antibiotics since the 1990s [14-16]. This happens because antibiotics are typically not used for long periods by a particular patient, and old antibiotics can still be useful in the absence of resistance. As an interesting example of the latter scenario, despite two pharmaceutical firms reported to have already identified independently a new class of helicase-primase inhibitor claimed to be more potent than the current drug of choice (acyclovir), none of them has announced plans to go ahead with human clinical trials, for the simple fact that acyclovir is considered to be a safe and effective antiviral compound already in use for treatment of herpes simplex virus, and the cost of taking a new drug to market through clinical trials is enormous. The marketing strategy of any company is not likely to favour competing with a proven drug [17]. In addition, mechanisms of resistance can change unpredictably. Therefore, it becomes difficult to predict the health needs and estimate the future size of the market for a new antibiotic, whereas the pharma companies or manufacturers can predict the upcoming situation for drugs in the market for the lifestyle diseases like asthma, diabetes, cancer and heart diseases based on current and predicted future size of patient populations [12,18]. For this variety of reasons, pharmaceutical firms seem to find the development of novel antimicrobials, an economically not-so-attractive pursuit and are expressing declined interest to venture deep into this risk-prone investment of time and efforts. To overcome this problem GAIN Act (Generating Antibiotics Incentives Now Act) was introduced in 2012 by USFDA to help bring new antimicrobials to the market, resulting in approval of four antibiotics- Dalvance, Sivextro, Orbactiv and Zerbaxa in 2014, and one more antibiotic Avycaz in 2015 [19]. Initiatives like GAIN are welcome efforts to promote antibiotic development, but may not be enough to increase the interest of the pharma companies towards developing new antibiotics.

Another important issue is that of Intellectual Property Rights (IPR) and patents. Once patent on a particular drug expires, other companies can also start manufacturing that drug. This can result in the easy and increased availability of that drug in the market, at reduced price; and then perhaps more frequent use of the given antibiotic may increase the probability of rapid appearance of the resistant strains, making the handling of the problem of AMR bit more complicated. To tackle this problem, extension of the patent may be a reasonable strategy in certain cases, to keep the use of an effective drug restricted to the cases which require it the most [20, 21]. Whether off-patent or not, reserving the most effective antibiotics to be prescribed for the most serious infections is always logical.

The challenge of AMR needs to be entertained from all the possible angles with the goal of keeping emergence and spread of the resistance trait to the minimum possible, and speeding-up the development of novel antibiotics. Details of some of the antibiotics approved in recent past are summarized in Table 1.
### Drug (trade name) | Active ingredient (antibiotic) | Class | Year of Approval | Mechanism of action
--- | --- | --- | --- | ---
Doribax | Doripenem | Carbapenem | 2007 | Inhibition of cell wall synthesis
Vibativ | Telavancin | Glycopeptide | 2009 | Inhibition of peptidoglycan synthesis
Teflaro | Ceftaroline | 5th generation cephalosporin | 2010 | Inhibition of bacterial β-lactamases; Rapid bactericidal effect by binding to key Penicillin-Binding Proteins (PBPs)
Dificid | Fidaxomicin | Macrocyclic antibiotic | 2011 | Prevention of movement of the "switch regions" of bacterial RNA polymerase
Dalvance | Dalbavancin | 2nd generation lipoglycopeptide | 2014 | Disruption of cell wall synthesis
Sivextro | Tedizolid | Oxazolidone | 2014 | Inhibition of protein synthesis
Orbactiv | Oritavancin | Glycopeptide | 2014 | Disruption of cell wall synthesis
Zerbaxa | Ceftolozane-tazobactam | 5th generation cephalosporin | 2014 | Targets bacterial cell wall synthesis, while inhibiting bacterial β-lactamases
Avycaz | Ceftazidime-avibactam | 3rd generation cephalosporin | 2015 | Disruption of bacterial cell wall synthesis

| Expanding Utility of Available Antibiotics and Finding New Ones |

The AMR issue has received global attention, as it affects human populations in all parts of the globe. Numerous strategies to find out the answer to the AMR challenge are being discussed at different platforms. Two major directions these discussions point towards are: keep finding new antimicrobial agents and expand the utility of those already discovered. Most of the antibiotics discovered and introduced into market till now are products of microbial metabolism. These microbial metabolites belong to a limited number of structural classes, against which many of the pathogenic strains have already become resistant. Thus it seems logical to search for new classes of antimicrobial molecules not only among the microbial species but also from the animal and plant kingdom. A large number of crude extracts from terrestrial plants and marine life forms have been described in the literature to possess notable antimicrobial activity [22-25].

Exposing the pathogenic populations to new classes of antimicrobials will require them to develop resistance against new structures which they are not used to before. This may slow down the process of emergence of the resistant strains. Discovery of novel structures can also provide for new scaffolds for the synthesis of new antimicrobial agents in the lab. In addition to the high throughput screening of natural products for their potential antimicrobial activity, effective use of combinatorial chemistry and bioinformatics tools is required to give pace to the process of drug discovery. These tools along with the ‘omics’ approach (i.e. making use of data generated from genome sequencing of the pathogenic microbes) can put some novel antimicrobials of high efficacy in our hands. Finding new targets in the pathogen cell is need of the hour, if we want to develop novel antibiotics with new modes of action. The majority of the currently used antibiotics target any one of the limited targets of the pathogens known today e.g. cell wall synthesis, cell membrane, protein or nucleic acid synthesis. Looking at the limited number of targets available today, the importance of identifying new targets is easily understandable, as the organisms have evolved resistance mechanisms to protect themselves from the effect of existing antimicrobials having common targets.

Pathogens acquire resistance through mutations as well as HGT. Mutation is the driving force for evolution. Mutations leading to the acquisition of drug resistance occur
at increased frequency when microorganisms are put under a selection pressure in form of constant presence of the microbicidal antibiotics. Recently the possibility of developing anti-virulence (anti-pathogenicity) agents is being considered with some excitement, which may be used either alone or in combination with conventional antibiotics. These anti-virulence agents are expected to target the pathogenicity traits of the pathogen, rather than killing them outright. As retaining virulence is not necessary for survival, such anti-virulence agents are believed to exert lesser selection pressure on the target pathogen populations, and hence the development of resistance against them may be expected to occur at a slower pace.

Besides discovering new antimicrobials and new cellular targets among pathogens, increasing the utility of the already existing antibiotics is also a viable option. This can be done in different ways. For example, semi-synthetic or synthetic derivatives of existing antibiotics can be developed which may be effective against a broader range of pathogens, and also more stable under human physiological conditions. This has already been successfully achieved in the case of penicillins, where the derivatives are more stable under acidic conditions in stomach, and also against bacterial beta-lactamases. Another way of expanding utility of the existing antibiotics is to develop formulations wherein they are combined with an efflux-pump inhibitor or an inhibitor of the antibiotic-degrading enzyme so that the resistance mechanism of the pathogen can be rendered ineffective. Bacterial pathogens have evolved a multitude of mechanisms for survival in presence of drugs. Antibiotic efflux, target modification, and enzymatic degradation of the antibiotic are the well-known resistance mechanisms. Nevertheless, new resistance mechanisms are regularly being described, and new ways of their transmission are identified [26]. New compounds that may not necessarily possess intrinsic antimicrobial activity, but are able to sensitize the pathogen to a previously ineffective antibiotic or to reverse the effect of resistance, can be of great help in expanding the utility spectrum of existing antibiotics. Using such compounds in combination with conventional antimicrobials, or using two or more antibiotics as a combination is a sensible strategy to mitigate the AMR trouble. In recent times, this combination therapy or multidrug therapy has gained importance, as certain combinations have yielded better clinical outcomes. Such a combination approach can broaden the efficacy of antimicrobial compounds by exploiting the possible synergy between two compounds and prevent or delay the emergence of resistance during the course of antimicrobial therapy. Phytocompounds like polyphenols can modulate the susceptibility of *E. coli* to the antibiotics such as ampicillin, ciprofloxacin, and kanamycin [27]. One of the most suitable examples of the successful implementation of the concept of using resistance-modifying agents along with conventional antibiotics is that of clavulanic acid, which binds to many bacterial β-lactamases, and is commercially available in combination with amoxicillin (as Augmentin®), and ticarcillin (as Timentin®). Many plants have been assessed for their antimicrobial activity as well as resistance-modifying activity [28-30]. Such studies may be the starting points for finding few more effective resistance modifying natural molecules. In the following sub-sections, some of the strategies for expanding the utility spectrum of existing antimicrobials, as well as some relatively less explored drug targets are discussed. Additionally, few other anti-pathogenicity strategies are listed in Table 2.
### Anti-Virulence Agent

<table>
<thead>
<tr>
<th>Anti-Virulence Agent</th>
<th>Target Trait</th>
<th>Target Organism</th>
<th>Mode Of Action</th>
<th>Reference(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal antibodies</td>
<td>Toxin production</td>
<td>B. anthracis</td>
<td>Inhibit the binding subunit of anthrax toxin</td>
<td>[29]</td>
</tr>
<tr>
<td>Pilicides</td>
<td>Bacterial adhesion</td>
<td>Uropathogenic E. coli (UPEC)</td>
<td>Decrease the efficiency of colonization of UPEC isolates in the urinary tract</td>
<td>[30]</td>
</tr>
<tr>
<td>Acylated hydrazones</td>
<td>Specialized secretion systems</td>
<td>Chlamydia trachomatis, Yersinia spp.</td>
<td>Inhibit toxin secretion, interaction with host cells or functional assembly</td>
<td>[31]</td>
</tr>
<tr>
<td>SAM analogues L/D-S adenosylhomocysteine, Sinefungin</td>
<td>Quorum sensing</td>
<td>Multiple gram-positive and gram-negative bacteria</td>
<td>Block AHLs production</td>
<td>[33]</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Acylase, Lactonase, Oxidoreductases, Paraoxonases</td>
<td>Quorum sensing</td>
<td>Inactivation of AHLs</td>
<td>[29]</td>
</tr>
<tr>
<td>QS Antagonists 4-nitro-pyridine-N-oxide p-benzoquinone, 2,4,5-tri-bromo-imidazole, 3-nitro-benzen-sulfonamide</td>
<td></td>
<td></td>
<td>Inhibition of target transcriptional receptor</td>
<td>[31]</td>
</tr>
<tr>
<td>Apolipoprotein B, AIP analogues</td>
<td></td>
<td></td>
<td>Inactivate AIPs, Block membrane associated receptors</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Pathogenicity inhibition strategies.

#### β-Lactamase Inhibitors

β-lactamases, the microbial enzymes, inactivate the antibiotics having a β-lactam ring. β-lactamases have been detected in various gram-negative pathogens viz. Escherichia coli, P. aeruginosa [34], Vibrio cholerae, Serratia marcescens [35], Klebsiella pneumoniae, and Proteus mirabilis; as well as in gram-positive organisms viz. S. aureus [36]. Extended Spectrum β-lactamases (ESBLs) are frequently plasmid encoded and mediate resistance to all penicillins and third generation cephalosporins (e.g. ceftazidime, cefotaxime, ceftriaxone) and aztreonam. So, antibiotic options are limited for the infections caused by β-lactamase producing organisms [37].

The β-lactam antibiotics are extensively used clinically owing to their potential to inhibit the penicillin binding proteins, essential enzymes in bacterial cell wall synthesis [38]. β-lactamases are the primary mediators of bacterial resistance to β-lactam antibiotics. According to the Ambler scheme, β-lactamases are classified into four classes based on their protein homology. Beta-lactamases of class A, C, and D are serine β-lactamases, and class B enzymes are metallo-β-lactamases. The Bush–Jacoby–Medeiros functional scheme is based on functional properties of enzymes i.e., substrate and inhibitor profiles. β-lactamase inhibition is a good strategy for resistance modification to counter resistance [39]. Available β-lactamase inhibitors such as clavulanate, tazobactam, and sulbactam have been of significant clinical value in this regard, but they are not useful to inhibit the complete diversity of existing β-lactamases, as they are effective against class A enzymes only. These classical inhibitors also contain a β-lactam ring, and thus they are prone to resistance resulting from upregulation of β-lactamase expression, as well as
the acquisition of new β-lactamase or other mechanisms that would have evolved as a result of competition between susceptible bacteria and β-lactam-antibiotic producing microorganisms going over eons [40]. Moreover, the widespread existence of ESBLs and carbapenemases in multidrug resistant gram-negative pathogens, have impelled the search for new classes of β-lactamase inhibitors. [39]. The newer β-lactamase inhibitors such as avibactam and RPX700 can form a stable covalent bond with the catalytic serine in a wide range of β-lactamases. A list of β-lactamase inhibitors having broad-spectrum activity is provided in Table 3.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Active against</th>
<th>Target organism(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avibactam</td>
<td>Class A and C</td>
<td><em>P. aeruginosa</em></td>
<td>[41]</td>
</tr>
<tr>
<td>MK-7655</td>
<td>Class A and C</td>
<td><em>P. aeruginosa, K. pneumoniae, Enterobacter spp.</em></td>
<td>[42]</td>
</tr>
<tr>
<td>Boronic acid</td>
<td>Class A and C</td>
<td><em>P. aeruginosa, K. pneumoniae</em></td>
<td>[43]</td>
</tr>
<tr>
<td>RPX7009</td>
<td>Class A</td>
<td><em>Enterobacter spp.</em></td>
<td>[44]</td>
</tr>
<tr>
<td>Cyclobutanone</td>
<td>Class A and B</td>
<td><em>Enterobacter cloacae</em></td>
<td>[45]</td>
</tr>
<tr>
<td>Penicillin sulfone</td>
<td>Class D</td>
<td><em>Acinetobacter spp., P. aeruginosa</em></td>
<td>[46]</td>
</tr>
</tbody>
</table>

Table 3: β-lactamase inhibitors and their activity spectrum.

**Efflux pump inhibitors**

Efflux pumps are the membrane proteins, which expel antimicrobials and other toxic molecules, and thus decrease their concentration inside the cell to sub-toxic levels [47]. These protein pumps recognize and extrude different antibiotics, antiseptics, disinfectants, detergents [48], fatty acids [49, 50], and virulence factors [51]. In gram-negative organisms, MDR mechanisms are more complicated due to their double-membrane cells, which allow the expression of a tripartite efflux pump system such as AcrA/AcrB/TolC in Enterobacteriaceae or MexA/MexB/OprM in *P. aeruginosa* [52]. Multidrug resistance efflux pumps are also important in gram-positive bacteria. Methicillin-Resistant *S. aureus* (MRSA), one of the most notorious multidrug resistant gram-positive bacteria, acquire resistance to various antibiotics including tetracyclines, aminoglycosides and fluoroquinolones, through its efflux machinery. Clinically, drug efflux pumps are attractive targets for inhibition as they have an important role in bacterial pathogenesis, virulence, and biofilm formation [53, 54]. As an efflux pump is involved in export of multiple antibiotics, its inhibition can make the organism simultaneously susceptible to those many antibiotics. Inhibition of the efflux machinery will not only help in mitigating bacterial resistance to antibiotics but will also attenuate its virulence as the efflux inhibitors will not allow efficient secretion of the virulence factors through the efflux pumps. For any agent to qualify as an Efflux Pump Inhibitor (EPI), it should fulfill certain criteria viz., it should not affect the sensitive strains lacking the drug efflux pump; should not reduce MIC of those antibiotics which are not expelled by its target pump [55]. EPIs can serve as the resistance modifying agents by synergizing with currently used antibiotics (Table 4), or by restoring the efficacy of antibiotics to which resistance has arisen.
### Table 4: Some examples of efflux pump inhibitors.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Targeted efflux pump(s)</th>
<th>Organism</th>
<th>Antimicrobial(s) whose effect is enhanced in combination with this EPI</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperine</td>
<td>Nor A</td>
<td><em>S. aureus</em></td>
<td>Ciprofloxacin</td>
<td>[52]</td>
</tr>
<tr>
<td>5-nitro-2-phenyl-1H-indole</td>
<td>Nor A</td>
<td><em>S. aureus</em></td>
<td>Ciprofloxacin</td>
<td></td>
</tr>
<tr>
<td>4-(3-morpholinopropylamino)-quinazoline</td>
<td>AcrAB-ToIC MexAB-OprM</td>
<td><em>E. coli</em> <em>P. aeruginosa</em></td>
<td>Norfloxacin, Nalidixic acid, Sparfloxacin;</td>
<td>[56]</td>
</tr>
<tr>
<td>MBX2319</td>
<td>AcrB</td>
<td><em>E. coli</em></td>
<td>Piperacillin, Levofloxacin</td>
<td>[57]</td>
</tr>
<tr>
<td>Plumbagin</td>
<td>AcrB</td>
<td><em>E. coli</em></td>
<td>Triphenylphosphonium, Erythromycin, Tetracycline</td>
<td>[58]</td>
</tr>
<tr>
<td>(−)-Epigallocatechin Gallate</td>
<td>-</td>
<td><em>Campylobacter spp</em></td>
<td>Erythromycin, Ciprofloxacin</td>
<td>[59]</td>
</tr>
<tr>
<td>Lanatoside C and Diadzein</td>
<td>AcrB, MexB</td>
<td><em>E. coli</em> <em>P. aeruginosa</em></td>
<td>Carbanecillin, Levofloxacin</td>
<td>[60]</td>
</tr>
<tr>
<td>Trimethoprim and Epinephrine</td>
<td>AcrB, MexB</td>
<td><em>S. typhimurium</em> <em>E. cloacaee</em> <em>S. marcescens</em> <em>P. aeruginosa</em> <em>K. pneumoniae</em> <em>E. coli</em></td>
<td>Ciprofloxacin</td>
<td>[61]</td>
</tr>
<tr>
<td>Sertraline</td>
<td>AcrB, AcrEF, MdtEF, and MexAB</td>
<td><em>P. aeruginosa</em> <em>E. coli</em></td>
<td>Inhibition of Nile Red efflux</td>
<td>[62]</td>
</tr>
<tr>
<td>Artesunate</td>
<td>AcrAB-ToIC</td>
<td><em>E. coli</em></td>
<td>Reduces MIC of β-lactam antibiotics</td>
<td>[63]</td>
</tr>
<tr>
<td>Pimozide</td>
<td>AcrAB-ToIC</td>
<td><em>E. coli</em></td>
<td>Oxacillin, Ethidium bromide</td>
<td>[64]</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>NorA and MepA</td>
<td><em>S. aureus</em></td>
<td>-</td>
<td>[52]</td>
</tr>
<tr>
<td>Polymyxins</td>
<td>MexAB-OprM</td>
<td><em>P. aeruginosa</em></td>
<td>-</td>
<td>[65]</td>
</tr>
<tr>
<td>Colistin</td>
<td>MexAB</td>
<td><em>P. aeruginosa</em></td>
<td>-</td>
<td>[66]</td>
</tr>
<tr>
<td>Phe-Arg-β-naphthylamide</td>
<td>MexAB</td>
<td><em>P. aeruginosa</em></td>
<td>Levofloxacin</td>
<td>[65]</td>
</tr>
</tbody>
</table>

### Quorum Sensing Inhibitors

Quorum Sensing (QS) is a phenomenon of intercellular communication among microorganisms, which allows them to modulate their behaviour as a population in accordance with their cell density. QS inhibition, a quorum based method for virulence disruption, is an attractive anti-virulence approach as it can interfere with the bacterial communication leading to decreased or no expression of the virulence coding genes, whose expression is QS-regulated. As QS is a signal-based mechanism, interfering with signaling event can interfere with inherent bacterial protective mechanisms. QS inhibition can also disrupt biofilm formation [67]. Bearing in mind the fact that a wide range of notorious pathogens including *P. aeruginosa*, *V. cholerae* and *S. aureus* use QS for regulation/ expression of their virulence traits, targeting the communication ways or QS circuits seems to be a bright strategy to fight pathogens. Different life forms including algae, lichens, plants, and animals have been reported to possess different QS inhibitors. Particularly many plant products have been reported for their QS inhibitory potential [68]. Phytocompounds such as caffeine, catechin, gingerol, etc. have been reported for their ability to arrest bacterial QS. In most of such studies, QS inhibitory potential of the test compounds has been demonstrated against strains of *E. coli*, *P. aeruginosa*, *P. mirabilis*, *S. marcescens*, and *Chromobacterium violaceum* [69,70].

However, most QS antagonists are reported to have a narrow spectrum of activity that may target only a few pathogens and may have limited clinical value. Therefore, it is required to find broad spectrum QS-inhibitors, which may have activity on multiple gram-positive and gram-negative pathogens. QS modulators can be used in combination with conventional antibiotics for better therapeutic results. One polyherbal ayurvedic formulation namely
panchvalkal possessing QS modulatory property was reported to act as a robust therapeutic agent when administered along with conventional antibiotics [71].

**ROS Generators as Modulators of Virulence**

Reactive oxygen species (ROS) are the partially reduced or activated derivatives of oxygen. These molecules such as superoxide radical (O$_2^•$), Hydrogen Peroxide (H$_2$O$_2$), and hydroxyl radical (•OH), are highly reactive and toxic at high concentrations. Superoxide radical and hydroxyl radical are continuously formed in the process of reduction of oxygen to water. Hydroxyl radicals are generated in the presence of hydrogen peroxide and iron ions, described as Haber-Weiss reaction [72] (Figure 1). The first step of reduction of ferric into a ferrous ion is followed by the second step known as the Fenton reaction [73]. ROS have a regulatory role in basic cellular processes like cell signaling, transcription factor activation, gene expression, differentiation and cellular proliferation. ROS are the by-products of mitochondrial respiration, and key enzymes involved in ROS formation are Nicotine Adenine Diphosphate (NADPH) oxidases, Xanthine Oxidases (XO), arachidonic acid oxygenases, Nitric Oxide Synthases (NOSs), and cytochrome P450 reductase. A cell is said to be in a state of ‘oxidative stress’ when the level of ROS exceeds the capacity of the defense mechanisms. The enhanced ROS production can lead to lipid peroxidation, protein oxidation, nucleic acids damage, enzyme inhibition, apoptosis and eventually cell death. Few reports have shown that antibiotics such as chloramphenicol, trimethoprim, rifampicin [74], aminoglycosides, and ampicillin [75] can promote the bacterial cell death through the formation of ROS. Bactericidal antibiotics generate ROS by destabilizing iron-sulfur clusters and disturbing tricarboxylic acid metabolism. It also involves depletion of NADH [76]. Aminoglycoside antibiotics produce hydroxyl radicals by inducing mistranslation and misfolding of membrane-associated proteins. Kuczyńska-Wiśnik et al., [77] reported that five antibiotics trimethoprim, nalidixic acid, rifampicin, kanamycin, and streptomycin could stimulate ROS production, and thereby inhibit the growth of *E. coli* biofilm. Potentiation of ROS in disease conditions viz. cancer and malaria, using small molecules has been considered as a drug design strategy and attracted attention for new drug development. ROS generators can inhibit the growth of mycobacteria as well as MRSA [78].

![Figure 1: Schematic of Intracellular events involving ROS](image-url)

**Figure 1:** Schematic of Intracellular events involving ROS. O$_2^•$ : Superoxide, •OH: hydroxyl radical; OH$^-$: Hydroxide Ion; SOD: Superoxide dismutase; H$_2$O$_2$: Hyderagen peroxide.
Some flavonoids from the plant kingdom have been reported to generate ROS in presence of transition metals. Pyrogallol has been reported to have a role in the generation of $O_2^{-}$, and thus useful in studying the role of ROS in the biological system [79]. Another bactericidal flavonoid, epigallocatechin gallate has a role in apoptosis and has the ability to produce $H_2O_2$ by reducing $O_2$. Moreover, compounds such as quercetin, morin, and hesperetin have the ability to induce ROS depending on the concentration [80]. Compounds having the ability to generate ROS in pathogens may be useful to treat bacterial infections. However, this ROS-induced stress generates a high degree of selective pressure and bacteria do react to a variety of stresses. Bacteria can generate responses to this selective pressure such as SOS DNA stress response, the heat-shock protein stress response, and the oxidative stress response. As ROS are harmful in nature, cells produce enzymes e.g. catalase, superoxidase dismutase, and peroxidases for ROS removal.

Although ROS have been conventionally viewed as damaging molecules, some reports have also suggested that ROS is beneficial to the organism at a certain level for longevity through the adaptive mechanism [72, 81]. Additionally, ROS also have a role in cell-cell communication, biofilm formation, host-pathogen, or host-symbiont interactions [82]. Besides these, ROS can also directly promote a bacterial infection. First such demonstration was made in *Mycobacterium abscessus*-infected macrophages. In this study, infected macrophages were incubated with $H_2O_2$, a rapid burst inducer, subsequently they observed an enhancement in the growth of *M. abscessus*, whereas growth of organism was inhibited in presence of antioxidants during 4-7 days infection. Similar results have been found with other organisms e.g. *Porphyromonas gingivalis*, *Helicobacter pylori*, *Bacillus anthracis*, etc. However, there are no evidences to the mechanisms by which ROS promotes the growth of the organisms; it seems that ROS may have a role in the establishment of the infection [81].

ROS scavengers have an important role in overcoming the oxidative stress. For example, cells produce enzymes viz. dismutase to detoxify superoxide, but hydroxyl radicals cannot be detoxified by enzymatic reactions. As bacteria are not known to possess any enzymes for detoxifying free hydroxyl radicals, compounds which can trigger generation of these radicals can have appreciable therapeutic potential, with little possibility of the appearance of the resistant strains. Nonenzymatic natural antioxidants are required for the detoxification of hydroxyl radicals. Polyphenols such as vanillic acid, rutin, tannic acid, protocatechuic acid; carotenoids like β-carotene, linoleic acid, zeaxanthin, lutein; and flavonoids viz. quercetin, catechin, kaempferol, tocopherol do possess free radical scavenging antioxidant properties [83-85].

It is clear that exogenous as well as endogenous ROS are important in the microorganisms. Endogenous ROS production can be induced by antibiotics in susceptible bacteria, whereas, exogenous ROS production can be induced at the time of host-pathogen interaction. Many bacterial species release ROS as an oxidative defense, wherein specialised enzymes produce ROS. Inversely, ROS can serve as regulatory factors in signaling pathways, and also act as quorum sensing factors. As ROS-mediated mechanisms are widespread in bacterial domain, improved understanding of ROS-driven mechanisms can provide deeper insight into the complex biological processes, which can be implemented in modern medicine. Both the strategies i.e. implementing ROS-generators or ROS-scavengers, are attractive.

**Riboswitches as Targets of Wide Utility For New Antimicrobials**

The new classes of antibacterial medications are direly required, ideally by recognising new bacterial targets. One potential focus for advancement of such differently acting antibiotics is riboswitches, noncoding RNA structures present in the 5'- untranslated region (5' - UTR) of certain bacterial mRNAs. Riboswitches regulate the bacterial gene expression in accordance with the intracellular concentration of particular metabolites, recognised by them. Proper functioning of the riboswitches is crucial for survival, virulence, and regular metabolism.
of bacteria. Riboswitches have a ligand binding ‘aptamer domain’ and an ‘expression domain’. The ‘aptamer domain’ ties to characteristic metabolites and this coupling event triggers ‘expression stage’, a conformational change switching the bacterial transcription or translation process on/off. Then again, the metabolite, at lower concentration, separates from its related riboswitches and reverses its impact on the bacterial transcription or translation process. The ubiquitous presence of the riboswitches throughout the domain bacteria (Table 5), and their essential role in regulating bacterial gene expression makes them very attractive target for antibiotic development. The binding interaction amongst riboswitches and their related metabolites are accepted to be practically identical to those of protein-ligand interaction in complexity and specificity, which proposes that structure-based drug design strategy might be valuable in planning the use of metabolite mirrors for the target riboswitches [86].

Some riboswitch classes i.e. Flavin Mononucleotide (FMN) and Thiamine Pyrophosphate (TPP) are generally spread across bacterial species, for example, *S. aureus*, *P. aeruginosa* and *K. pneumoniae* [87], and in some bacterial species they are believed to control more than 2% of the genome [88]. Riboswitches hold the possibility of being pathogen-specific therapeutic targets since they have not been found in humans [89]. Suitability of quite a few riboswitch classes to serve as therapeutic target has been explored, including those belonging to TPP, lysine, FMN [90-93], and guanine families [94, 95]. Strangely, these reports have revealed that many of the previously known antibacterial compounds for which the mechanism of action was obscure, target riboswitch structures [96].

<table>
<thead>
<tr>
<th>Riboswitch class</th>
<th>Ligands</th>
<th>Organism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>Glutamine, Glycine, Lysine</td>
<td><em>Bacillus anthracis</em>, <em>Brucella melitensis</em>, <em>Clostridium difficile</em>, <em>A. baumannii</em></td>
<td>[97-99]</td>
</tr>
<tr>
<td>Nucleotides and nucleobases</td>
<td>Adenine, Cyclic di-adenosine monophosphate (c-di-AMP), Cyclic di-guanosine monophosphate (c-di-GMP), Guanine, Prequeuosine-1 (preQ1)</td>
<td><em>Bacillus anthracis</em>, <em>Listeria monocytogenes</em>, <em>C. difficile</em></td>
<td>[98, 100]</td>
</tr>
<tr>
<td>Sugars</td>
<td>Glucosamine-6-phosphate (GlcN6P)</td>
<td><em>S. aureus</em>, <em>Enterococcus faecalis</em></td>
<td>[98, 101]</td>
</tr>
<tr>
<td>Coenzymes</td>
<td>Adenosylcobalamin (AdoCbl), Flavin mononucleotide (FMN), SAM, Tetrahydrofolate (THF), Thiamine pyrophosphate (TPP)</td>
<td><em>K. pneumoniae</em>, <em>Brucella melitensis</em>, <em>Mycobacterium tuberculosis</em>, <em>B. anthracis</em>, <em>E. faecalis</em>, <em>L. monocytogenes</em></td>
<td>[102-104]</td>
</tr>
<tr>
<td>Ions</td>
<td>Fluoride, Magnesium (Mg2+)</td>
<td><em>Pseudomonas syringae</em>, <em>Salmonella enterica</em></td>
<td>[105-106]</td>
</tr>
</tbody>
</table>

*Table 5: Different classes of riboswitches present in pathogenic bacteria.*

Microorganisms may develop resistance to riboswitch-targeting medications through a mutation that upsets binding of the drug to the riboswitch receptor. Resistance to L-aminoethylcysteine (AEC), pyrithiamine and roseoflavin can arise in a laboratory setting through mutation of a lysine, TPP or FMN riboswitch individually. However, frequencies of mutations have not yet been resolved. It is not clear how broad this mechanism would be for developing resistance to riboswitch-targeting compounds. In situations where a few
riboswitches of the same class are targeted by a single compound, one may expect that a single point mutation would be inadequate to present resistance; or a change would need to happen in each riboswitch, that regulates an important gene. This mutation would also disturb the binding of a natural metabolite of that riboswitch, bringing about dysregulation of the related biosynthesis pathway(s). This could impede survival of the bacteria, particularly in an infectious setting [87].

**Siderophores as Targets**

Iron is an essential micronutrient for most life forms including the pathogenic microbes. This metal ion is included in different biological phenomena essential to life such as oxygen transport and respiration, electron transfer, DNA synthesis and repair. Iron also serves as a cofactor for many important enzymes. Iron is commonly found in the Fe$^{2+}$ and Fe$^{3+}$ oxidation states in natural frameworks, and higher oxidation states are accomplished transiently during enzymatic catalysis. Organisms procure this essential supplement by utilising dedicated mechanisms for locating the iron source, its transport and storage. Prokaryotes require micromolar (~$10^{-6}$ M) concentrations of iron to replicate and colonise, and are subsequently confronted with a metabolic difficulty in light of the fact that the concentration of Fe (III) at neutral pH is low (ca. $10^{-18}$ M). In addition, availability of free iron in vertebrate hosts is almost always limited (ca. $10^{-24}$ M in human serum) in view of the inherent toxicity of this metal ion stemming from the scope of formation of harmful free radicals through Fenton reaction. To obtain the concentrations of iron necessary to thrive in nutrient-limited environments, prokaryotes utilise a variety of strategies for iron acquisition. Both gram-negative and gram-positive bacteria produce and export iron scavenging proteins called siderophores [107]. Siderophores, the small iron-chelating molecules, are important virulence factors. Siderophores play an essential role in making the survival of the pathogen possible inside the iron-limited host environment, and thus are crucial for successful expression of its virulence and pathogenesis [108]. The affinity of siderophores for iron surpasses by several orders of magnitude that of transferrin, the main protein in blood for iron transport [109]. When released under the circumstances of iron-starvation, the siderophores scavenge ferric particles and this siderophore-iron complex is moved inside the cell. The mechanism involved in uptake of this complex differs for gram-negative and gram-positive strains, and is better known for the former [110].

A new strategy to tackle AMR, the ‘Trojan Horse Approach’ (THA) proposes to exploit the iron-siderophore uptake system to convey an antibiotic payload. This mechanism is already being practised in nature by a few bacteria, through the production of sideromycins like albomycin, ferritin, and salmycin. These sideromycins comprise of naturally occurring hydroxamate kind of siderophores, covalently connected to an antibiotic moiety [111]. To achieve an enhanced antibiotic uptake by pathogenic bacteria, endeavours have been made for the configuration of siderophore-antibiotic conjugates (Table 6). Generally, such conjugates contain a catechol/hydroxamate siderophore analogue and a β-lactam drug. While implementing this strategy, it has to be observed that the basic mechanism of siderophore recognition and uptake is not hampered. A suitable linker is utilised to make conjugate steady in the extracellular environment, yet the drug is released intracellularly by enzyme activity, in either the cytoplasm or the periplasm, the latter frequently required to augment the action of the conjugate. Fascinating advancements have happened in the
design of Siderophore-Drug (SD) conjugates [112], up to the point where a siderophore monosulfactam, BAL30072, gave results promising enough for clinical trials to be performed [113]. These conjugates combine a suitable antibiotic with a siderophore-mimicking small molecule.

<table>
<thead>
<tr>
<th>Conjugate</th>
<th>Target Site</th>
<th>Target Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxamate-Fluoroquinolone</td>
<td>cytoplasmic DNA gyrase</td>
<td>S. aureus</td>
<td>[114]</td>
</tr>
<tr>
<td>Trisicateolate–Ampicillin</td>
<td>outer membrane permeability</td>
<td>S. aureus</td>
<td></td>
</tr>
<tr>
<td>Trisicateolate–Amoxicillin</td>
<td>outer membrane permeability</td>
<td>P. aeruginosa</td>
<td>[115]</td>
</tr>
<tr>
<td>Biscatecholate-</td>
<td>Penicillin binding proteins</td>
<td>A. baumannii</td>
<td>[116]</td>
</tr>
<tr>
<td>hydroxamate–Carbacephalosporin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxypyridone-β-lactams</td>
<td>Penicillin binding proteins</td>
<td>P. aeruginosa</td>
<td>[117]</td>
</tr>
</tbody>
</table>

Table 6: Few examples of the siderophore-antibiotic conjugates.

Biofilm Inhibition and/or Disruption

Biofilm is a microbial community living attached to a surface [118]. They are like ‘city of microorganisms’, where the microorganisms are embedded in a self-produced extracellular matrix. Bacteria form a biofilm on submerged surfaces e.g. natural aquatic systems, water pipes, living tissues, etc. Biofilm formation on medical devices e.g. catheters, heart valves, pacemakers, prosthetic joints, and contact lenses represent a serious clinical issue. Common examples of biofilm forming gram-negative bacteria are E. coli, K. pneumoniae, P. mirabilis, and P. aeruginosa. The gram-positive counterparts include Enterococcus faecalis, S. aureus, S. epidermidis, and Streptococcus viridans [119]. Microbial biofilms demand special attention as drugs effective against the planktonic cells may not be effective against biofilms of the same species, or may be required to be used at too high concentrations, which may not be therapeutically relevant. Behaviour of microorganisms in biofilm is noticeably different from their planktonic counterparts. A bacterial biofilm can be considered as a population of cells, which shows higher tolerance to antimicrobial agents at a phenotypic level. Different types of microorganisms interact with each other and their environment and coordinate their activities within the biofilm in a mutually beneficial way. Moreover, bacteria within a biofilm show enhanced resistance to environmental stressors, including antimicrobial compounds due to the de novo emergence of antibiotic resistance through elevated mutations. Higher cell densities inside a biofilm prepare the ground for HGT to occur at the higher magnitude, which has been well recognised as a phenomenon contributing heavily towards the spread of resistance and virulence among the members of the microbial world [120]. Not only exchange of genetic material, but also QS is likely to be performed more effectively inside these ‘microbial sanctuaries’, safeguarded against the entry of antimicrobials on account of their intrinsic diffusion-limited structure. The most influential evidence for a specific role for biofilms in pathogenesis and virulence in human originates from direct observational studies wherein microscopy or imaging methods have been used to image biofilm-like structures at infection sites in situ or on infected tissues or devices freshly recovered from patients [121]. Many strategies have been proposed to inhibit biofilm formation and/or affecting its dispersal (Table 7).
<table>
<thead>
<tr>
<th>Strategy</th>
<th>Experimental surface/ effector agent used</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-microbial coating</td>
<td>Silver</td>
<td>[122]</td>
</tr>
<tr>
<td>Polymer modification</td>
<td>N-bromide alkylpyridinium</td>
<td>[123]</td>
</tr>
<tr>
<td>Hydrophobicity modification</td>
<td>Nano silica</td>
<td>[124]</td>
</tr>
<tr>
<td>Surface roughness alteration</td>
<td>Monomeric trimethylsilane Microtopography Sharklet AF™</td>
<td>[125-126]</td>
</tr>
<tr>
<td>Surface charge modification</td>
<td>Poly (ethylene oxide) Pluronic F127</td>
<td>[127]</td>
</tr>
<tr>
<td>Surface modification by plasma treatment</td>
<td>Nonthermal atmospheric plasma treated glass coverslips (S. mutans cultures were grown on plasma treated and non-treated surfaces of glass coverslips)</td>
<td>[128]</td>
</tr>
<tr>
<td>Anti-biofilm polysaccharide</td>
<td>A101 Ec111p Psl</td>
<td>[129-131]</td>
</tr>
<tr>
<td>Anti-biofilm enzymes</td>
<td>B-Dispersin Alginate lyase Thermonuclease</td>
<td>[132]</td>
</tr>
<tr>
<td>Chelating agents</td>
<td>Tetra sodium-EDTA, Sodium citrate, Disodium- EDTA</td>
<td>[133-134]</td>
</tr>
<tr>
<td>Antimicrobial peptides</td>
<td>Lytic peptide</td>
<td>[135]</td>
</tr>
<tr>
<td>Anti-adhesion agents</td>
<td>Plicicides Curlicides</td>
<td>[136-137]</td>
</tr>
</tbody>
</table>

Table 7: Strategies to inhibit biofilm formation and/or effecting its dispersal.

**Simple Animal Models for Preliminary ‘In Vivo’ Efficacy Testing**

Though a large number of natural and synthetic compounds/extracts have been described to possess in vitro antimicrobial property, and new ones are being reported continuously, they cannot be put to actual use, until and unless their in vivo efficacy and safety is demonstrated. This can be achieved through experiments with animal models, followed by human trials. However, animal experiments involve serious ethical considerations. Obtaining regulatory approvals for sacrificing enough number of animals while evaluating in vivo efficacy and safety has not remained easy. This situation can be helped by using simpler life forms for preliminary screening rather than directly doing experiments with large animals. This way many of the compounds which show some toxicity or no appreciable in vivo efficacy, can be ruled out right in the initial phase of the process of drug discovery. This approach not only reduces the number of animal sacrifices but also saves considerable time and money. Only the formulations which are found effective with the simpler models need to be processed further for clinical trials. Simpler animal models such as the nematode *Caenorhabditis elegans*, fruit fly *Drosophila melanogaster*, and zebrafish *Danio rerio* are being used since last few years for identifying the role of virulence determinants in the pathogenesis of microbial infections, as well as for preliminary in vivo efficacy assays [138]. Among all these simpler life forms, the nematode *C. elegans* has been of great significance in various fields of biological research for almost four decades. Though its use in biology labs has remained a widely used practice, its use for efficacy testing of novel antimicrobials has been realized only in the recent past [139]. *C. elegans*, a self-fertilizing hermaphrodite, is attractive as a model organism for a variety of reasons. It has a short life cycle, making it possible for the experiments to be completed in reasonable time. It is an easy-to-handle organism, and its maintenance in the lab does not involve heavy costs. Additionally, comparative studies of the human and *C. elegans* genomes confirmed that many of the human disease genes and disease pathways are present in *C. elegans*, and its genome shares 40% similarity with human genome [140, 141]. Further, considerable knowhow has been generated about lab handling of this organism and relevant protocols, much of which is freely available to the interested researchers (Table 8).
<table>
<thead>
<tr>
<th>Resource</th>
<th>Website Address</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>WormBase</td>
<td><a href="http://www.wormbase.org/">http://www.wormbase.org/</a></td>
<td>Genes, expression, resources, phenotypes, metadata, and publications</td>
</tr>
<tr>
<td>WormBook</td>
<td><a href="http://www.wormbook.org/">http://www.wormbook.org/</a></td>
<td>Basic information about the biology of <em>C. elegans</em> and other nematodes, including methods</td>
</tr>
<tr>
<td>WormAtlas</td>
<td><a href="http://www.wormatlas.org/">http://www.wormatlas.org/</a></td>
<td>Worm anatomy, including neurons and wiring, Electron microscopy sections, and cell lineage</td>
</tr>
<tr>
<td>Caenorhabditis Genetics Center</td>
<td><a href="https://www.cbs.umn.edu/research/resources/cgc">https://www.cbs.umn.edu/research/resources/cgc</a></td>
<td>Stocks of wild-type and mutant nematode strains</td>
</tr>
<tr>
<td>OpenWorm Science</td>
<td><a href="http://www.openworm.org/">http://www.openworm.org/</a></td>
<td>Various online resources for <em>C. elegans</em> research</td>
</tr>
</tbody>
</table>

Table 8: Resources for *C. elegans* researchers.

*C. elegans* can successfully be infected in the lab with many gram-positive and gram-negative bacteria, and fungi of clinical relevance e.g., *Enterococcus, Staphylococcus, Streptococcus, Burkholderia, Pseudomonas, Salmonella, Serratia, Yersinia, Cryptococcus neoformans* [142]. *C. elegans* was used as a model host to find the relationship between QS and virulence of *P. aeruginosa*. Mechanisms of *P. aeruginosa* mediated killing of *C. elegans* involves the production of phenazine pigment as well as ROS generation [138]. Many studies have shown that the microbial virulence factors involved in the killing of *C. elegans* are also required for pathogenesis in mammals [143]. This opens novel opportunities for advancement in the therapies that target specific virulence mechanisms. Traditional *in vitro* assays measuring growth inhibition or killing of pathogens, as well as whole-cell drug screens, are not likely to fully meet the future needs of drug development. *C. elegans* can prove a useful tool for identification of novel antimicrobials/anti-infectives with a higher probability of clearing subsequent clinical trials. Curcumin was reported for its protective effect in *Burkholderia pseudomallei* infected *C. elegans* [144]. Potent QS antagonists were shown to protect *C. elegans* from killing by *P. aeruginosa* [145, 146] and *C. violaceum* [147, 148].

**Role of ‘in silico’ Tools**

The traditional process of drug discovery has relied on *in vitro* efficacy assays followed by *in vivo* animal experiments, and then human trials. The cost of this approach is determined directly by the number of compounds being assayed. More is the number of compounds to be tested, more is the time, money and manpower required to be invested. If among all the available test compounds, some can be ruled out earlier owing to their possible toxicity or low efficacy, this will directly result in lesser compounds undergoing efficacy and toxicity assays, and thus lesser resources will be consumed. This objective in part can be achieved by effective use of *in silico* tools. Molecular docking of all the available test structures with the potential target inside the pathogen using compatible softwares can make it possible to reduce the number of compounds actually undergoing wet-lab assays, and only the compounds with a higher probability of binding to the identified target can be focused on. Though *in silico* results may not always be successfully validated in wet-lab, as the wet-lab results may vary from the *in silico* predictions, overall utility and relevance of the bioinformatics tools cannot be questioned. These are the days when combinatorial chemistry can affect the virtual synthesis of an immensely large number of molecules [149], many more than the ones that can actually be tested in the lab in a reasonable time. Despite the fact that not many success stories are there in the name of combinatorial chemistry based drug-discovery approach, to take full advantage of the potential of combinatorial chemistry, docking tools appear to be a very apt tool for primary screening. *In silico* tools can also be used to validate some hypothesis formed on the basis of wet-lab experiments. For example, the probable binding of the candidate drug molecules to a particular cellular target can be checked through molecular docking, even after that lead molecule has been demonstrated to be effective *in vivo*, with the mode of action being still obscure.
**In silico** tools offer a different type of platform for investigating the lead/receptor interactions. A large number of compounds/extracts have been described in literature having different biological properties. It is essential to use a systematic and standardized approach to validate the real-world potential of these claims [150]. **In silico** tools can be applied for virtual screening and/or validation, deploying dynamic search protocols, priority indexing, systemic categorization, and cross-verification [151]. **In silico** tools include databases of protein as well as small compounds, Quantitative Structure-Activity Relationships (QSAR) tools, pharmacophore modeling tools, homology modeling and ligand-protein docking tools, data mining, machine learning, network and data analysis tools. An informed integration of *in vitro, in vivo,* and *in silico* approaches would be beneficial for effective target-based drug discovery, starting with a rapid screening of novel preparations being explored as alternatives to current antimicrobials [152]. Molecular docking allows the on-screen study of the interactions of lead compounds with target receptors. In this approach, small molecules are evaluated with computer-aided molecular simulation to explore their efficacy for binding the target. Structures of test compounds are designed with tools like ChemSketch or can be retrieved from PubChem type of databases. A list of various structure drawing tools and molecule structure databases is presented in Table 9. Relevant structures can be retrieved from appropriate databases, followed by construction of protein-ligand complexes, and their assessment via molecular dynamics and molecular docking simulations. In addition, QSAR, useful as *in silico* filters, apply force field calculations to 3D structures and correlate the chemical structures with their biological activity.

<table>
<thead>
<tr>
<th>Informatics Tool</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structure drawing tools</strong></td>
<td></td>
</tr>
<tr>
<td>ChemSketch</td>
<td><a href="http://www.acdlabs.com/resources/freeware/chemsketch/">http://www.acdlabs.com/resources/freeware/chemsketch/</a></td>
</tr>
<tr>
<td>MarvinSketch</td>
<td><a href="https://www.chemaxon.com/products/marvin/marvinSketch">https://www.chemaxon.com/products/marvin/marvinSketch</a></td>
</tr>
<tr>
<td>ChemDoodle</td>
<td><a href="https://www.chemdoodle.com">https://www.chemdoodle.com</a></td>
</tr>
<tr>
<td>ChemWriter</td>
<td><a href="http://chemwriter.com/">http://chemwriter.com/</a></td>
</tr>
<tr>
<td>WinDrawChem</td>
<td><a href="http://xdrawchem.sourceforge.net/windrawchem/">http://xdrawchem.sourceforge.net/windrawchem/</a></td>
</tr>
<tr>
<td>BKChem</td>
<td><a href="http://bkchem.zirael.org/">http://bkchem.zirael.org/</a></td>
</tr>
<tr>
<td>Symyx Draw</td>
<td><a href="http://bbruner.org/obc/symyx.htm">http://bbruner.org/obc/symyx.htm</a></td>
</tr>
<tr>
<td>BIOVIA Draw</td>
<td><a href="http://accelrys.com/products/collaborative-science/biovia-draw/">http://accelrys.com/products/collaborative-science/biovia-draw/</a></td>
</tr>
<tr>
<td><strong>Chemical structures (small molecules and proteins) databases</strong></td>
<td></td>
</tr>
<tr>
<td>ZINC</td>
<td><a href="http://zinc.docking.org/">http://zinc.docking.org/</a></td>
</tr>
<tr>
<td>eMolecules</td>
<td><a href="https://www.emolecules.com/">https://www.emolecules.com/</a></td>
</tr>
<tr>
<td>ChEMBL</td>
<td><a href="https://www.ebi.ac.uk/chembldb/">https://www.ebi.ac.uk/chembldb/</a></td>
</tr>
<tr>
<td>ChemSpider</td>
<td><a href="http://www.chemspider.com/">http://www.chemspider.com/</a></td>
</tr>
<tr>
<td>BiAdb</td>
<td><a href="http://crdd.osdd.net/raghava/biadb/">http://crdd.osdd.net/raghava/biadb/</a></td>
</tr>
<tr>
<td>DrugBank</td>
<td><a href="http://www.drugbank.ca/">http://www.drugbank.ca/</a></td>
</tr>
<tr>
<td>Super Natural II</td>
<td><a href="http://bioinf-applied.charite.de/supernatural_new/index.php">http://bioinf-applied.charite.de/supernatural_new/index.php</a></td>
</tr>
<tr>
<td>NPACT</td>
<td><a href="http://crdd.osdd.net/raghava/npact/">http://crdd.osdd.net/raghava/npact/</a></td>
</tr>
<tr>
<td>Super Drug Database</td>
<td><a href="http://bioinf.charite.de/superdrug/">http://bioinf.charite.de/superdrug/</a></td>
</tr>
<tr>
<td>ChemBank</td>
<td><a href="http://chembank.broadinstitute.org/">http://chembank.broadinstitute.org/</a></td>
</tr>
<tr>
<td>MolProt</td>
<td><a href="https://www.molport.com/shop/index">https://www.molport.com/shop/index</a></td>
</tr>
<tr>
<td>PDB</td>
<td><a href="http://www.rcsb.org/">www.rcsb.org/</a></td>
</tr>
<tr>
<td>BindingDB</td>
<td><a href="http://www.bindingdb.org/">http://www.bindingdb.org/</a></td>
</tr>
<tr>
<td>SCOP</td>
<td>scop.mrc-lmb.cam.ac.uk/scop/</td>
</tr>
<tr>
<td>CATH</td>
<td><a href="http://www.cathdb.info/">www.cathdb.info/</a></td>
</tr>
<tr>
<td>DSDBASE</td>
<td><a href="http://caps.ncbs.res.in/dsdbase/dsdbase.html">http://caps.ncbs.res.in/dsdbase/dsdbase.html</a></td>
</tr>
<tr>
<td><strong>Structure viewer</strong></td>
<td></td>
</tr>
<tr>
<td>RasMol</td>
<td><a href="http://www.umass.edu/microbio/rasmol">http://www.umass.edu/microbio/rasmol</a> <a href="http://www.umass.edu/microbio/rasmol">www.umass.edu/microbio/rasmol</a> /</td>
</tr>
</tbody>
</table>
Table 9: Diversity of in silico tools relevant to the process of drug discovery.

<table>
<thead>
<tr>
<th>Tool Name</th>
<th>URL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swiss-PDB Viewer</td>
<td><a href="http://www.expasy.ch/spdbv/">http://www.expasy.ch/spdbv/</a></td>
</tr>
<tr>
<td>Ribbons</td>
<td><a href="http://www.cmc.uab.edu/">http://www.cmc.uab.edu/</a></td>
</tr>
<tr>
<td>MolPOV</td>
<td><a href="http://www.chem.ufl.edu/~der/der_pov2.htm">http://www.chem.ufl.edu/~der/der_pov2.htm</a></td>
</tr>
<tr>
<td>MolScript</td>
<td><a href="http://www.avatar.se/molscript/">http://www.avatar.se/molscript/</a></td>
</tr>
<tr>
<td>Pymol</td>
<td><a href="https://www.pymol.org/">https://www.pymol.org/</a></td>
</tr>
<tr>
<td>AutoDock</td>
<td><a href="http://autodock.scripps.edu/">http://autodock.scripps.edu/</a></td>
</tr>
<tr>
<td>FlexX</td>
<td><a href="http://www.biosolveit.de/FlexX/">http://www.biosolveit.de/FlexX/</a></td>
</tr>
<tr>
<td>Glide</td>
<td><a href="http://www.schrodinger.com">www.schrodinger.com</a></td>
</tr>
<tr>
<td>Dock6</td>
<td><a href="http://dock.compbio.ucsf.edu/">http://dock.compbio.ucsf.edu/</a></td>
</tr>
<tr>
<td>Arguslab</td>
<td><a href="http://www.arguslab.com/">www.arguslab.com/</a></td>
</tr>
<tr>
<td>GOLD</td>
<td><a href="https://www.ccdc.cam.ac.uk/solutions/csd-discovery/components/gold/">https://www.ccdc.cam.ac.uk/solutions/csd-discovery/components/gold/</a></td>
</tr>
<tr>
<td>QSAR tools</td>
<td></td>
</tr>
<tr>
<td>vls3d</td>
<td><a href="http://www.vls3d.com/links/chemoinformatics/qsar">http://www.vls3d.com/links/chemoinformatics/qsar</a></td>
</tr>
<tr>
<td>3D-QSAR</td>
<td><a href="http://www.3d-qsar.com/">http://www.3d-qsar.com/</a></td>
</tr>
<tr>
<td>Hyperchem</td>
<td><a href="http://www.hyper.com/">http://www.hyper.com/</a></td>
</tr>
</tbody>
</table>

Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) are the five parameters that influence the drug kinetics and overall performance of the compound as a drug. As whole compound libraries cannot be tested in vivo, ADMET profiling of the compounds will help to identify the most suitable candidates for further experimentation [153, 154]. In silico studies can help in positively screening the molecules with a high probability of efficacy, and low toxicity. Knowledge of the receptor topography and receptor-ligand interactions can yield promising compounds for preclinical studies and clinical trials.

**Future Perspectives**

The rapid emergence of multidrug resistant strains and their heavy societal impact cannot be denied. The limited arsenal of available antimicrobial agents is not proving enough to deal with the colossal challenge of antimicrobial resistance, making the discovery and development of new antimicrobials/anti-infectives a must for the safeguard of human society against infectious microorganisms. This problem needs to be tackled from various angles at different levels. As the development of new antibiotics is not likely to keep pace with the rapid emergence of resistant strains, the already available antibiotics are to be used in the wisest possible way. The most potent antibiotics have to be reserved for the most serious infections. Appropriate awareness programmes for general public, medical professionals, paramedical staff, and drug-sellers also have an important role to play. Global concerted efforts have already become an absolute necessity if we desire to save our ground against the infectious agents armed with the dangerous capacity to evolve at a rapid pace and enrich their armamentarium with newly acquired virulence and resistance factors. Besides intensifying the research for new antimicrobial formulations with different structures and different targets than the current ones, the focus needs to be put on antibiotic alternatives. Anti-virulence agents, susceptibility modulating agents and adjuvants enhancing the efficacy of antimicrobials etc. need to be developed. In order to achieve this, screening exercises need to expand their reach to less explored terrestrial as well as marine biodiversity resources for finding new classes of antimicrobials. Ensuring that we do not run out of targets (receptors) is equally important, which can be made possible by genome-wide analysis of pathogenic/resistant strains, involving effective use of bioinformatics.
References

6. Antibiotic resistance threats in the United States (2013) Centers for Disease Control and Prevention, Office of Infectious Disease, USA.


Abstract

Bacteria existed on this earth even before antibiotics were invented and so was the Antimicrobial Resistance (AMR) due to constitutional resistance makeup carried by the bacteria themselves. Antibiotics were introduced into the clinical care in mid-20th century. These wonder molecules revolutionized modern medicine drastically by reducing the morbidity and mortality due to infectious diseases. But the emergence of resistance in bacteria threatens the benefits of antibiotics. The crisis is more prominent in the case of Gram negative bacteria with limited number of new antibiotics in the pipeline. Unfortunately, international, medical and political communities were slow to respond and react to this global crisis with far-reaching ramifications and serious implications. The threat of AMR has already crossed national and continental boundaries, involving not only humans but also animal and agricultural sectors. Drug resistant bacteria will result in 10 million additional deaths every year and the global economy may lose 100 trillion US dollars by 2050 due to the AMR crisis. It is our social and political responsibility to optimize the use of antibiotics and conserve the existing antibiotics so that our next generation could harvest the benefits and obtain best care for the patients. So it is a geo-political issue. To unravel it, everyone and people from every sector across the globe should be brought into action - academicians, doctors, pharmacists, veterinarians, agricultural producers, politicians and policymakers should join hands to launch initiatives to control this ever-increasing peril. This chapter describes the policies made at global level and national level to alleviate the crisis due to AMR.

Introduction

Bacteria existed on this earth even before antibiotics were invented and so was the Antimicrobial Resistance (AMR) due to constitutional resistance makeup carried by the bacteria themselves. Antibiotics were introduced into the clinical care in mid 20th century. These wonder molecules revolutionized modern medicine drastically by reducing the morbidity and mortality due to infectious diseases [1,2]. But the emergence of resistance in bacteria threatens the benefits of antibiotics [3]. The crisis is more prominent in the case of Gram negative bacteria with limited number of new antibiotics in the pipeline. Unfortunately,
international, medical and political communities were slow to respond and react to this global crisis with far reaching ramifications and serious implications[4].

The threat of AMR has already crossed national and continental boundaries, involving not only humans but also animal and agricultural sectors. Drug resistant bacteria will result in 10 million additional deaths every year and the global economy may lose 100 trillion US dollars by 2050 due to the AMR crisis[5]. It is our social and political responsibility to optimize the use of antibiotics and conserve the existing antibiotics so that our next generation could harvest the benefits and obtain best care for the patients.

**Why the Antibiotic Stewardship Is Still Going At A Slower Pace?**

Antibiotic-resistant bacteria can spread among human beings and animals through food, water and the environment. Resistant bacteria are found even in animal food and food products destined for consumption by humans. Antimicrobial resistance (and particularly antibiotic resistance) is spreading, at a faster rate as compared to the development of new classes of antibiotics in the short term[6].

In spite of so many proposals and initiatives by medical professionals over many years to mitigate antimicrobial resistance, progress has been slow, in part because of inadequate recognition by all stakeholders of the need for action in their respective areas and also scarce surveillance and proper reporting at national, regional and global levels[4,6].

So it is a geo-political issue. To unravel it, everyone and people from every sector across the globe should be brought into action - academicians, doctors, pharmacists, veterinarians, agricultural producers, politicians and policymakers should join hands to launch initiatives to control this ever-increasing peril. This collaboration will prevent gaps in knowledge and would help in reducing antimicrobial resistance if there is a political will to adopt new policies and strategies[4].

Policy makers of every country, especially developing countries should financially and technically support research for new drugs, encourage microbiology laboratories in development of diagnostic tools, vaccines, infection control practises, strengthen surveillance and reporting systems to assess the burden of AMR and finally regulate appropriate use and access to antimicrobial agents in improving their health care system[4,5,6].

**Steps Already Taken At the Global Level**

**WHO**

In 2013, WHO established the Strategic and Technical Advisory Group (STAG) on AMR, chaired by the UK Chief Medical Officer Dame Sally Davies.WHO also developed tripartite approach with the FAO (Food and Agricultural Organization of the United Nations) and OIE (World Organization for Animal Health) and initiated “WHO Global action plan on AMR”, released in the World Health Assembly meeting in 2015[6].WHO also initiated Global Antimicrobial Resistance Surveillance System (GLASS) to strengthen standardized AMR surveillance globally[7].

**United Nations**

Global leaders met at the United Nations General Assembly in New York on 21st September 2016 to commit to fighting AMR together. This was only the fourth time in the history of the UN that a health topic was discussed at the General Assembly (HIV, non-communicable diseases, and Ebola were the others). Member States have agreed upon a strong political declaration that provides a good basis for the international community to move forward[8-10].
The European Commission action plan against AMR

In 2014, the European Commission (EC) compiled a road map based on detailed overview of the 12 National Actions plan with concrete activities and the deadlines[11].

The Transatlantic Task Force on Antimicrobial Resistance (TATFAR)

This is a joint initiative by the US and the EU established in 2009 and published a progress report in 2014[12].

The European Centre for Disease Control (ECDC)

ECDC was established in 2005 as EU initiative. AMR control is one of the main components of the ECDC programme[13].EAAD, European Antibiotic Awareness Day (18th November) is an ECDC initiative, launched in 2008. EAAD has become more popular now and transformed into WAAW (World Antibiotic Awareness Week) in many countries.

Global Health Security Agenda (GHSA)

GHSA has identified AMR as one of the global health security risks and developed AMR Action Package[14].

Jaipur Declaration on Antimicrobial Resistance

Health ministers of all the member states of the WHO South-East Asia Region signed Jaipur Declaration in 2011. A holistic and multidisciplinary approach towards prevention and containment of antimicrobial resistance to improve public health was adopted by all the health ministers of member states of the WHO South-East Asia region [15].

National Level Action Plan

The US Interagency Task Force on Antimicrobial Resistance, a joint initiative of various organizations such as CDC and FDA[16], France National antibiotic plan[17],the UK five year AMR strategy[18],German Antimicrobial Resistance Strategy (DART), Swedish Strategic Programme against Antibiotic Resistance (STRAMA)[19], are various reputed national initiatives.

Non-Governmental Organizations (NGOs)

Various NGOs such as ReAct[20](Action on Antibiotic Resistance), Alliance for the Prudent Use of Antibiotics (APUA)[21],World Alliance Against Antibiotic Resistance[22],Global Antibiotic Resistance Partnership (GARP)[23],UK Antibiotic Action[24]and Chennai Declaration[25] have made significant contributions and momentum to tackling the AMR cause.

Initiatives in India

In 2011, Indian Ministry of Health released national antibiotic policy to rationalize antibiotic usage and step up infection control practices across the country[26].

In 2013, Ministry released modified H1 rule to regulate over the counter sale of antibiotics without prescription [27].

In 2015, India along with 193 other countries signed the WHO AMR global action plan and Indian Ministry of Health is in the process of preparing a national action plan in accordance with the WHO strategy .

In 2016, Ministry of Health released national treatment guidelines for antimicrobial use in Infectious diseases [28].

Recently, Ministry of Health reorganized the national antibiotic policy core committee into three interlinked committees, for better performance and coordination. These committees have been described below.
Core Working Group on AMR (CWG-AMR) Chaired by the National Centre for Disease Control (NCDC) Director

This committee includes subject experts responsible for coordinating preparation of various documents, surveillance etc.

Technical Advisory Group on AMR (TAG-AMR) Chaired by the Director General of Health Services

The committee includes senior representatives from various Ministries and subject experts. The committee is responsible for reviewing activities of the Core working group committee (by NCDC) and provides advice to the Committee of senior Govt. officials (ICC-AMR).

Inter-sectorial Coordination Committee on AMR (ICC-AMR) Chaired by the Health secretary

The committee comprises senior most officials in Health ministry and other relevant ministries. The committee is responsible for coordination of national efforts and international collaborations.

The Chennai Declaration

In 2012, medical societies in India with the participation of various governmental and non-governmental bodies developed a document and initiative- “The Chennai Declaration” that has since received national and international acclaims [25]. The document has clearly laid out roles and responsibilities of all stakeholders, including the Ministry of Health, medical societies, media, journals, and the insurance industry.

Chennai declaration created a serious attitude change among the medical community and policy makers in India, convincing them not only on the significance of the resistance issue but on a positive way of approaching the problem as well [29-37].

Chennai declaration is based on a realistic understanding of the Indian scenario of the heterogeneity of health care system, with significant sanitation issues in the community and infrastructural issues hindering proper practice of infection control measures. Chennai declaration advocates a step-by-step approach towards implementation of all the major components of tackling the resistance strategy.

“Chennai declaration document and initiative” has helped Indian Ministry of Health in accelerating the tackling of AMR activities and helped India to achieve a highly respectable position in the field of AMR[29]. “Chennai declaration-Five year plan” provided a template for the national policy implementation in India and other developing countries [38].

Concluding Remarks

AMR is a geo-political issue which should be dealt from all aspects and directions like conservation of available antibiotics, rapid and affordable diagnostic approaches from microbiology laboratories, research into newer antibiotics and proper infection control measures to prevent the spread of resistant organisms. We also need measures to strengthen and collaborate national, regional and international surveillance systems. Educational approaches, though an easy strategy will not be fruitful in the absence of regulatory interventions. Multisectorial approach by incorporating human, animal health, food production and supply chains with the sincere participation of public and private organizations and of course law makers is the key for a successful outcome against AMR.
References

17. http://en.strama.se/
18. 20 http://www.reactgroup.org


36. Team C. "Chennai Declaration": 5-year plan to tackle the challenge of anti-microbial resistance. Indian J Med Microbiol [Epub ahead of print]

Abstract

Penicillin, the first antibiotic which saved countless lives and heralded a new era in healthcare, was discovered by an accident which earned Dr. Alexander Fleming the Nobel Prize in 1945. However, indiscriminate use of antibiotics has brought us close to a new age where most minor infections will not be easily treatable with the drugs we have today. We find ourselves facing bacteria which have become resistant to most, if not all antibiotics. MDR - Multiple Drug Resistance - has become a global problem. Bacteria, like any living organism, have evolved and adapted to new environments in their quest for survival. This chapter addresses the problem of MDR and explains how simple remedies and healthy lifestyles could alleviate this problem. Healthy and fresh food and practices like yoga, massage and laughter could lead to holistic health and obviate the need for profligate use of antibiotics, a primary reason for the spread of MDR.

Introduction

Penicillin, the first antibiotic which saved countless lives and heralded a new era in healthcare, was discovered by an accident which earned Dr. Alexander Fleming the Nobel Prize in 1945. But in an interview shortly thereafter, he presciently warned that, “The thoughtless person playing with penicillin treatment is morally responsible for the death of the man who succumbs to infection with the penicillin-resistant organism”[1].

Indiscriminate use of antibiotics has brought us close to a new age where most minor infections will not be easily treatable with the drugs we have today. We find ourselves facing bacteria which have become resistant to most, if not all antibiotics. MDR - Multiple Drug Resistance - has become a global problem. Bacteria, like any living organism, have evolved and adapted to new environments in their quest for survival.

If the medical fraternity had heeded Dr. Fleming’s warning, things would not have so rapidly come to such a pass. The discovery of penicillin as a wonder drug created a paradigm shift in healthcare. But instead of its judicious use, people were encouraged to neglect hitherto viable natural remedies and the power of their own self immunity in favour of the miraculous quick-fix of antibiotics for every possible ailment.

The calculator is undoubtedly a useful device for mathematical calculations, but through
indiscriminate use, people are now losing the capacity for simple mental arithmetic. These skills are still preserved by street vendors, who though uneducated, can swiftly arrive at the change to be returned even as their customers are fumbling through the calculator on their mobile phones. This erosion by the calculator of our mental faculties is similar though less pernicious than the erosion of our self-immunity by antibiotics. While human progress in any field is part of the evolutionary process, we have to follow the Buddha's middle path to ensure that such progress yields meaningful benefits without succumbing under the weight of its own excesses.

**Bacteria, Pathogenicity and Antibiotic Resistance**

Bacteria, also referred to as germs, are the simplest creatures that are considered alive[2]. They are small, but powerful and complex and can survive extreme conditions. The ecosystem relies on bacteria to function properly.

Bacteria serve as tiny recyclers. They decompose organic waste and convert atmospheric nitrogen into forms that can be absorbed by plant life to create amino acids and nucleic acids, the building blocks of DNA. We eat the plants and reap the benefits. On the inside, they help us break down food and improve our nutritional intake. And in or out, exposure to bacteria is what helps us develop the immunity to respond to pathogenic invaders later in life. Children sheltered from bacteria are the ones most prone to asthma and allergies.

The number of bacterial cells in the body is commonly estimated at 10 times the number of human cells. While there are both good and bad bacteria, for a long time science and medicine have treated all bacteria as enemies. Bacteria in conjunction with an acute immune response are the first line of defence against pathogens attacking our body. When the external pathogens attack, the body's immune system begins to respond. These helpful intruder-fighting bacteria that live on in our body are called commensal bacteria, as they benefit from the relationship with the host, while the host neither benefits nor suffers. These symbiotic bacteria are everywhere on the skin, nose, gut, bowels and mouth. The skin bacteria live on a steady diet of dead skin cells and sweat.

As we wipe out one set of bacteria and pick up another batch of bacteria, they could contain a pathogenic gene. Pathogenic bacteria can cause health problems if the good bacterium levels are low. *Clostridium tetani* (causes tetanus) is not very invasive, but it produces a potent toxin that causes damage at a very small concentration. Pathogens are rated on two characteristics – invasiveness and toxigenicity. Invasiveness is a measure of the bacterium's ability to grow inside the host, and toxigenicity measures the capacity of the bacterium to produce toxins (chemical substances that cause damage to the host). The combination of these two characteristics gives the final rating of the bacteria's virulence (ability to cause disease). A species does not necessarily need to have both high invasiveness and high toxigenicity to be rated highly virulent. One or the other can be high enough to cause the bacterium to be very virulent. For example, the bacterium *Streptococcus pneumoniae* (which causes pneumonia) does not produce a toxin, but is highly invasive such that it causes the lungs to fill up with fluid from the immune response required[3]. Pathogenic bacteria will propagate antibiotic resistance traits when repeatedly exposed to antibiotics. Terminal concerns about the spectre of invincible bacteria have already reached hospitals in the USA. American military researchers have recently identified the first patient in the US to be infected with bacteria that are resistant to an antibiotic that was the last resort against drug resistant germs[3].

**Some of the important functions of the good bacteria are:**

- Supplement the digestive process to break down food
- Produce vitamins, short-chain fatty acids and proteins utilized by the body
- Protect against the overgrowth of pathogenic bacteria and yeasts
- Strengthen immune function
• Create beneficial nutrients that prevent weight gain

*Most of these bacteria live on or in our bodies without harming us, until provoked by wrong life style and more recently compounded by the abuse of antibiotics.*

• *Neisseria meningitides* meningitis
• *Streptococcus species* Dental disease
• *Enterococcus faecalis* stomach disease
• *Escherichia coli* diarrhoea
• *Streptococcus pneumoniae* pneumonia
• *Staphylococcus aureus* hospital infection

The harmful effects of bacteria are:

• Produce toxic substances including carcinogens
• Harbour a reservoir of bacterial invaders to create future serious infections
• Produce digestive disturbances
• Promote immune system dysfunction and autoimmune inflammatory diseases
• Promote weight gain

In summary, we need to modify our understanding of germs and re-evaluate our relationship with bacteria. Human-bacterial symbiosis is basically the concept of trillions of bacteria that live on our body and in our gut, and participate in the living ecosystem within us. A proper balance of this symbiotic relationship is essential for good health, which is a process that takes time and energy. Despite the notion that popping a pill gives instant relief, it has serious consequences on our health. In the same light, disease also does not take place over night and we cannot expect to restore our health back over night either.

**Infection, Disease, Antibiotics**

Infections are considered responsible for a large number of diseases affecting health. They can be caused by bacteria and can be treated by antibiotics. Antibiotics can be defined as a variety of substances derived from organic and non-organic sources that kill or control the growth of bacteria.

Antibacterials work in one of two ways:

• A bactericidal antibiotic like penicillin kills the bacteria.
• A bacteriostatic antibiotic stops bacteria from multiplying.

Antibiotics fight or prevent infection caused by bacteria but cannot fight viral infections such as cold and flu.

**Antibiotic Misuse**

Antibiotics can be compared to chemotherapy drugs, producing containment with toxic side effects instead of a cure. There are many factors in modern medicine that contribute to antibiotic resistant bacteria, including:

• Over-prescribing of antibiotics by physicians. For example, Mohan has a cold and the doctor prescribes a one week course. He becomes better in four days and stops the medicine. This creates three big problems which are so common today. First, symptoms of cold are caused by the body's reaction to the viral infection and antibiotics have no effect on viruses. Mohan would likely have become better even without any medicine in four days. Secondly, in the process Mohan would have wiped out a good portion of the symbiotic flora, disturbing his natural symbiotic balance which could lead to some diseases in the future. Thirdly, there might be a pathogenic strain of bacteria left over after dosing of the antibiotic which would grow in numbers over time, if unchecked. Another complication of antibiotics is secondary infection. Research has identified the way that the normal gut flora keep the
immune system ‘primed’ to recognise the cell walls of bacteria so that the slightest change from a normal to a pathogenic bacterium will stimulate an immediate attack. Antibiotics shut this recognition ability down, leaving the body without one of its defence systems[4]. Probiotics can help in reversing this process.

- Other reasons include the rampant use of antibiotics in animals to enhance growth which gets transmitted to people and poor hospital hygiene.

Preservation of good health is better than the abuse of antibiotics in search of an elusive cure. There are nine constituents of good health which are the result of balance and harmony in the following areas:

- Genes
- Symbiotic bacteria
- Immune system
- Food habits
- Life style
- Emotional health
- Spiritual health
- Yoga and meditation

Positive changes in one area will have ripple effects in the other areas as well to get the required balance and harmony.

**Indian Customs and Traditions**

The best protection against infection is the habit of washing hands with ordinary soap and water that washes away germs and viruses. Washing your hands before eating, after using the toilet and before food preparation, reduces the risk of getting sick in the first place. In villages across India, cow dung is used as a sanitizer. Cow dung is a rich source of commensal bacteria, especially *E. coli*, which are harmless and fight off pathogens. In fact *E. coli* is part of the human symbiote, and also produces vitamin K in our bodies. People who live in cow-dung sanitized homes have no allergies or bacterial infections despite their poverty.

Traditional Indian households cook food in a clean kitchen with fresh vegetables and grains as it is offered to the divine before consumption. This served to exclude old and processed food and consequently reduced the onset of food borne illnesses caused by food contaminated by microbial pathogens. Another traditional practice of drinking liquids from above without touching the lips and saliva also prevented infection through saliva. Such healthy traditional habits had food safety practices ingrained in them.

**Massage in Ayurveda**, an ancient Indian healthcare system, is one of the most natural means of relieving pain and discomfort. Some of the positive effects of massage therapy on the human physiology are:

- Dilates the blood vessels, improves the circulation, relieves congestion and increases flow of nutrition to the tissues.
- Helps return venous blood to the heart and so eases the strain on this vital organ.
- Acts as a ‘mechanical cleanser’, stimulating lymph circulation and hastening the elimination of wastes and toxic debris.
- Mildly reduces high blood pressure temporarily.
- Makes you feel good and relieves stress and tensions, mental and physical fatigue.
- Induces deep relaxation and relieves insomnia.
- Tones the skin and helps prevent blemished skin during facial massage.
- Stretches connective tissue, improves its circulation and nutrition and so breaks down or prevents the formation of adhesions and reduces the danger of fibrosis.

A strong immune system can be achieved with a healthy diet. Despite the leaps and
bounds we have made over the years when it comes to medical discovery, the human body and nature continue to adapt and keep one step ahead of us. Finally, according to CDC Director Dr. Thomas Friedmen, reducing the frequency with which antibiotics are used is ‘the single most important action needed to greatly slow the development and spread of antibiotic-resistant infections’.

Wisdom of the Human Body

*Man is the product of Nature. Life manifested in matter. And mind appeared in life in the course of evolution*

The physical body that we experience is the extension of the world of Nature and part of the ‘universal self’. All traditional religions consider the human body as sacred. Hinduism says that all that exists in the outer world, also exists in the body, including the divine consciousness. In the bible it is stated, ‘our bodies are the temple of the Holy spirit which is in us’. Quran says, ‘I breathe into him Ḏadṣm; my spirit’.

The sacredness of the human body with its correspondences to the macrocosm and its significance need not be debated. The holistic view of human beings embraces body, mind and the spirit. So the body is a living psycho-biological entity. The *panchamahabhuta* are the five elements of nature. According to Hindu mythology, every human body essentially is made from five elements which are Earth (Bhumi), Water (Jala), Fire (Agni), Air (Vayu) and Space (Aakash). Hindus believe that upon death, all these 5 elements of the human body are dissolved into the respective elements of nature, so that it can balance the cycle. Each of these five elements has its own character and celestial elements. Each element is responsible for different structures in the body. Earth forms solid structures such as bones, flesh, skin, tissues and hair. Water forms saliva, urine, semen, blood and sweat. Fire forms hunger, thirst and sleep. Air is responsible for all movement, including expansion, contraction and suppression. Space forms physical attraction and repulsion, as well as fear.

If any element is impure or out of balance with another, disease and suffering may occur. Yoga helps us purify these elements and restore balance and health, and unfolds the inner powers and abilities contained in each element. In fact, yoga is one of the most powerful ways to restore health because it gives us the means to bring even those elements that are natural enemies into harmonious relationships with each other.

Focus on our body’s inherent self-healing mechanism can help us avoid popping pills and looking for external solutions. The wisdom of the body anticipates microbial invasion and knows how to handle it; the organism is therefore regarded by Guyenot, as the “best among physicians”. The body is designed to detox naturally through the kidneys, liver, sweat glands, lungs and digestive system. Immunity is established only through exposure to microbes; external hazard promotes the wisdom of the body. We have special white blood cells whose job it is to attack harmful bacteria and help fight off infections. Homeostasis is the body’s natural state. Disease is nature’s attempt at self-healing - it’s an effort of nature to free the system from conditions that result from a violation of the laws of health. We break the homeostasis through wrong life style habits, but the body constantly seeks the simplicity of this balance.

Before the development of antibiotics, there were food and herbs which afforded some protection against infection. These natural antibiotics strengthen your immune system. These are known as ‘astringent’ food, which naturally cleanse your blood without harmful side effects, keeping the good bacteria in the body. If our immune system is working as intended, it is our inner army protecting us through life. Food is our major contact with the external environment so food choices can negatively or positively modulate the immune system.

Fungal infections are quite common and mostly happen on the skin. The digestive tract, genitalia, urinary tract and the respiratory tract too can become infected. Anybody can get
this infection, but the old or critically ill persons, or people with an endangered immune system and individuals who take immunosuppressive medicines are more prone to it. Several species of fungi are seen in our body, but they are harmless. The organism called *Candida albicans* is usually responsible for all sorts of fungal infections. In small amounts it is harmless but when it begins to increase rampantly, multiplying fast then it results in a disease. A weak immune system is unable to handle this unexpected growth and the fungus begins hurting the body. As long as the infection is contained in the top folds of the skin, it is quickly remediable and does not cause much harm. But if it enters the bloodstream, then it can have terrible results.

**Super Immunity and the Natural Antibiotics**

Super immunity can be defined as the body’s immune system working to its full potential. Foods give us energy, the building blocks of life, in the form of nutrients to grow and develop. We have to appreciate the non-caloric micronutrients in food, including those that are neither vitamins nor minerals but phytochemicals - elements that strengthen and support normal immune function. Natural plants are complex packages of biologically active compounds. The term ‘phytochemicals’ means ‘plant chemicals’ which have thousands of plant source compounds that have functional effects on human health and immunity. Herbs were considered as an important medicine throughout the world and are once again becoming popular. Plants contain natural biochemicals to maintain their structure and functions. When we take the herb, any one of its parts heals diseases and leaves back no adverse effects. Modern drugs have achieved a lot in disease treatment but have also contributed to ADR [adverse drug reaction]. Every modern drug comes with a warning of their side effects.

Phytochemicals play the following roles:

- Inducing detoxification enzymes
- Controlling the production of free radicals
- Deactivating and detoxifying cancer causing agents
- Protecting cell structures from damage from toxins
- Fuelling mechanisms to repair damaged DNA sequences
- Impeding the replication of cells with DNA damage
- Inducing beneficial antifungal, antibacterial and antiviral effects
- Protecting cell structures from damage from toxins
- Improving immune cells’ cytotoxic power that is the power to kill microbes and cancer cells.

Scientists have identified different phytochemicals found in vegetables, fruits, nuts and herbs. The following herbs and vegetables are some of the natural immune boosters. They not only stimulate the immune system but exhibit anti-inflammatory and anti-septic properties which inhibit the growth of bacteria and scavenge them.

**Garlic:** Raw garlic when crushed or chewed contains a compound called, ‘allicin’ which has similar properties like penicillin. It is antibiotic, anti-inflammatory, anti-viral, anti-parasitic, anti-fungal and anti-oxidant.

**Honey:** An enzyme found in honey releases hydrogen peroxide. This helps your body fight infection and prevents the growth of bacteria. Honey removes toxins from the blood and helps in the smooth functioning of the liver.

**Cabbage:** Cabbage has sulfur compounds in it and is naturally antibacterial thereby improving digestion and preventing disease.

**Grape seed extract:** It is high in antioxidants, boosts immunity, alkalizes the body naturally and aids in digestion by improving your beneficial gut flora.

**Watermelon:** It’s not only refreshing, but when ripe, also has plenty of antioxidants in
the form of glutathione. It strengthens the immune system so it can fight infection. To get the most glutathione in your watermelon, eat the red pulpy flesh near the rind.

**Spinach:** You'll find lots of nutrients in this ‘super food’. One of them is folate, which helps your body make new cells and repair DNA. It also boosts fiber, antioxidants such as vitamin C, and more. Eat spinach raw or lightly cooked to derive the maximum benefit.

**Sweet Potato:** Like carrots, sweet potatoes have beta-carotene. In your body that turns into vitamin A and mops up damaging free radicals. This helps bolster the immune system and may even improve the aging process.

**Coconut oil:** It has naturally occurring anti-fungal and anti-microbial properties and is packed with antioxidants. It boosts your immune system, balances thyroid, cholesterol and blood sugar levels and even improves brain function.

**Fermented foods:** Probiotics work to boost and preserve the natural gut flora [good bacteria] found in your digestive system.

**Cinnamon:** It also contains antibiotics properties.

**Apple-cider Vinegar:** It contains malic acid which has antibiotic properties.

**Ginger:** Studies have shown that ginger has antibiotic effects against food borne pathogens such as salmonella, listeria, and campylobacter. Fresh ginger also aids stomach acid production and improves digestion.

**Turmeric:** It is recognized to be the most auspicious and useful spice because of its multitudinous therapeutic values which cure a host of diseases and ailments. People, who regularly eat this spice, avoid suffering from fungal and bacterial infections. Turmeric and **Tulsi** (Holy Basil) which are extensively used across the world in Hindu Temples are proven to be the Best Anti Biotic and Anti Oxidants.

**Yogurt:** This has powerful cultures that aid to eradicate the infection causing bacteria and sustain the healthy balance of bacteria in your body. To fight bacterial infections one can take yogurt orally and even apply it right to the infected area. To treat vaginal infection in women, one should include a teaspoon of yogurt every couple of hours for the duration of three to five days. Yogurt or curd is one of the excellent methods of treating vaginal bacterial infection.

**Fenugreek:** Immerse a teaspoonful of fenugreek seeds in some water and allow it to soak overnight. Sip this water the next morning on an empty stomach. Fenugreek aids in controlling the hormonal balance and maintaining the menstrual cycle.

**Eucalyptus:** Eucalyptus has herbal qualities that make it very beneficial in the treatment of the bacterial infection in the vagina. It is an extraordinary remedy for the common cold. Combining few drops of undiluted eucalyptus oil to the bath every day will promote good health. Extracts of the eucalyptus are found in VapoRub and other medications that are used for the treatment of cold and congestion of the chest. Massaging eucalyptus oil on the chest, nose, and back will assist you to breathe easily when suffering from nasal congestion. The oil can be reliably used by pregnant women as well. But, under no conditions should one drink the oil.

From a health perspective, the cost of antibiotic resistance is an increase in the seriousness of disease. For example, treating a person with tuberculosis caused by a strain that is killed by antibiotics is highly effective. In contrast, between 40 and 60% of people who get antibiotic-resistant tuberculosis die.

The cost of misuse of antibiotics can be a weakened immune system. Researchers have found that certain patients taking antibiotics had reduced levels of cytokines, the hormone messengers of the immune system. When your immune system is suppressed, you’re more
likely to develop resistant bacteria or to become sick in the future.

Here are steps to take to use antibiotics properly:
- Take antibiotics only for bacterial infections and not for viral infections.
- Take antibiotics the right way. If you are prescribed an antibiotic, it’s crucial that you take the entire course.
- Don’t use antibiotics to try to prevent infection.
- Don’t save or share antibiotics.
- Sanitation and better nutrition are preventive measures.

**Laughter - The Best Medicine**

Researchers have found that the positive emotions associated with laughter decrease stress hormones and increase certain immune cells while activating others. In one study conducted at Loma Linda University School of Medicine in California, 10 healthy men who watched a funny video for an hour had significant increase in one particular hormone of the immune system that activates other components of the immune system [5]. Find reasons to laugh or join the laughing club which has become so popular today. Read a book of jokes or watch a comedy movie or make friends with people with a good sense of humour.

**Bacteria Quiz**

Knowledge about spreading germs:
1. What is the most germ intense thing in the household:
   - Toilet seat
   - Kitchen sponge
   - Dog’s bowl
2. The only way to catch a cold is from germs that float in the air
   - True
   - False
3. How fast germs travel when you sneeze
   - 10 mph
   - 50 mph
   - 100 mph
4. The cell phone has more germs than toilet seat
   - True
   - False
5. Chlorine keeps pool germ free
   - True
   - False
6. Germs are living things
   - True
   - False
1. False. Kitchen sponge. Cloths are better
2. False. Besides the germs in the air, you can get sick if you touch the door knob or elevator button where germs are present and then you touch your nose, eyes and mouth
3. 100 mph. The best defence against flying germs is staying six feet away from the sick person
4. True. Cell phone has ten times more bacteria than toilet seat because we do not clean the phone
5. False. Most germs die in an hour. But some can live for days. Do not swallow the
water

6. True. Germs are living things which can get into plants, animal and plants. There are four kinds of germs—bacteria, viruses, fungus, and protozoa.

**Conclusions**

What goes around comes back to you. A poor Scottish farmer Mr. Fleming saved a child drowning in a pond. It happened to be Winston Churchill, son of Randolph Churchill. Churchill was so overwhelmed by the farmer’s goodness and wanted to reward him monetarily. The Good Samaritan farmer said there is no price for humanity. Then he saw the farmer’s son Alexander coming out of the house. He requested the father to give him a chance to educate his son which would give some peace and satisfaction. As it turns out, in time, Alexander discovered Penicillin which saved Churchill from pneumonia.

Let us all use with discretion any antibiotics and not fall in the trap of monetary gains. Then we have to catch the bad ball which we have thrown at the universe. Awareness alone can help us in our decision. So the choices you make today can protect you or punish you later. The end of illness resides within all of us. As Plato said, “The part can never be well, unless the whole is well.”

There is no one right answer on health issues. Each and every individual is unique and maintaining health and preventing disease must be tailored to suit each individual. There is no replacement for a good life style as the primary focus for holistic health.

Dealing with MDR is a multi-disciplinary task involving health professionals, policy makers, livestock farmers, veterinarians, scientists, civil society and environment groups. In future the study of the human microbiome may revolutionize health and medical treatment.

**References**

2. Bacteria, Dr. Sayeed Ahmad D. I. Hom. (London)
3. The superbug, Sabrina Tavernise & Denise Grady, Times of India May 28 2016
Scientists working with clinical and environmental samples in their laboratories are not directly exposed to the real scenario of MDR in the hospitals and communities. How a pathogenic bacterium resistant to multiple drugs wreaks havoc in ICUs, clinics and refugee/soldier camps is something witnessed in real time by clinicians. We are fast heading towards a post-antibiotic era. Is this scare real or is it a ghost with no real existence? Are our fears baseless? This section presents views/experiences from two seasoned clinicians (Dr. Ghafur and Dr. Taneja) and two seasoned researchers (Dr. Ghosh and Dr. Ramamurthy) to address the above mentioned questions.

**Philosophy and Resistance**

**Dr Abdul Ghafur**

**Corresponding Author:** Dr Abdul Ghafur MD(Med) MRCP(UK) FRCPath (UK), Senior Consultant in Infectious Diseases, Apollo Hospitals, Chennai, Phone: +91 9710506285; E-mail: drghafur@hotmail.com

Working in an oncology centre with severely immunocompromised patients, there is nothing more realistic than superbugs and the devastating effect of these tiny but mightier creatures!

Have you ever thought all your medical qualifications will be unhelpful when you face a child with neutropenic sepsis with a pan drug resistant bug in his blood?

All the wonders of modern medicine will appear so futile when the blood pressure of the child drops further and further with not even a single antibiotic marked sensitive in the microbiology culture report.

Who is to blame for this catastrophic scenario?

Is it the doctors - for prescribing antibiotics as if these drugs are candies?

Are patients innocent with all those over the counter purchases without prescription, whilst shopping for vegetables?

Is it the fault of the Government, for not improving sanitation scenario in the community?

Are hospitals to blame for not implementing antibiotic and infection control policies?

Should we crucify pharmaceutical companies for not investing in new antibiotics?

Should we blame everybody? Should we accuse the society?

Rationalising antibiotic usage and educating doctors, considered to be major components of tackling the AMR
strategy, may be less significant than sanitation issues in the community and the lack of cleanliness in hospitals. It is time we should focus our attention to the root cause of the AMR problem.

Am I being philosophical? Well...the bug is Pan drug resistant…I pleaded and the God looked away!!!

The Havoc of Multi Drug Resistant *Klebsiella pneumoniae*: A Young Life Snuffed In Bed

**Dr. Neelam Taneja**

**Corresponding Author:** Dr. Neelam Taneja, Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh, 160012, Phone: +91-7087008160 Extn. 5160; E-mail: drneelampgi@yahoo.com

This is a really tragic story of a young seven year old girl who was admitted to pediatric emergency 5 years back. The girl presented with acute watery diarrhea, suspected to be cholera. At admission, she had moderate dehydration, was conscious and talking with her parents. She was immediately started on rehydration therapy using intravenous fluids with electrolytes. By next day, when we visited her to tell that we had confirmed cholera bugs in her stools, she had completely sprang back to life. She was chatting happily with our resident and telling about her favourite cartoon show. She was to be discharged same evening. Three days later when we contacted her family to fill up our cholera form, we were shocked to know that she was in septic shock and was receiving imipenem. Her blood culture grew *Klebsiella pneumoniae*. We thought imipenem will save her as this was Rambaan (panacea) and all our isolates were susceptible. From where this *Klebsiella* came, I was wondering. Could gut microbiota have translocated to blood as cholera could have led to a leaky gut. Or was it a hospital transmission? By the time we got antimicrobial susceptibility data showing that it was carbapenem resistant, we had lost the precious life. Whole of the unit felt very sad and lamented at our inadequacy to conquer this formidable bug.

This case has become an example for me to quote when I teach the residents and nurses about hospital acquired infections. You come to the hospital with a highly treatable or innocuous problem and die because of the multi drug resistant bug the hospital gives you.

Is The Scare Of Microbial Drug Resistance Real?

**Dr. Amit Ghosh**

**Corresponding Author:** Dr. Amit Ghosh, National Institute of Cholera and Enteric Diseases (ICMR), P-33 CIT Road, Scheme XM, Beliaghata, Kolkata 700010, Phone: +91-33-23537470; E-mail: amitghosh24@yahoo.com

A short answer to this question is yes. Antimicrobial drug resistance which is the resistance of a variety of microorganisms (bacteria, viruses, parasite) to drugs used in the treatment of infections caused by them, has become a major problem worldwide and because of this, infections which could be treated easily until very recently are now sometimes becoming very difficult to tackle. According to the World Health Organization, it no longer is a problem of the future and today there is no part of the world that is totally free from this menace. A report published a few years ago by the Centre of Diseases Control, Atlanta, showed that in the USA, infections caused by *Enterobacteriaceae* resistant to carbapenems, considered to be among the last-resort antibiotics, registered a 3.5 fold rise between 2001 and 2011, a matter of deep concern as the fatality due to infections caused by the resistant bugs range between 40-50%. Recently *Plasmodium falciparum* the causative agent of falciparum malaria resistant to Artemisinin has emerged in Greater Mekong Sub-region, casting a shadow on the global malaria control programme. It is estimated that bacterial infections caused by resistant bacteria which are responsible for about 50000 deaths a year in Europe and USA alone, could in 2050, claim upto 10 million lives and cause a 2-3.5 percent reduction in global GDP.

Evolution of drug resistance in bacteria is a natural phenomenon. But under the selection pressure exerted by the antibiotics this process is accelerated and the proportion of resistant bacteria in a population increases manifold. Scientists have always been aware of this and indeed Fleming in his Nobel lecture warned about the possibility
of Penicilin becoming ineffective one day because of this. Yet initially, the development of antibiotic-resistance was never considered a serious problem because it was thought that resistance against an antibiotic occurred only due to mutation in its ‘target’ gene and hence the possibility of bacteria developing resistance to more than one antibiotic as the result of simultaneous occurrence of other independent mutations in the target genes of other antibiotics was considered to be rather remote. Besides, there was a steady flow of newer and newer antibiotics in the market. Over the years however, this flow has come down almost to a trickle because the development of antibiotics is no longer a priority for the pharma industry due to insufficient returns on investment. Also it has been discovered that bacteria have many “means” of developing resistance. Excessive use, that too often of broad-spectrum antibiotics, have led to very accelerated emergence and spread of multi-drug resistant bacteria; Multi drug resistance in bacteria is most often caused by their acquiring many genes responsible for resistance to different antibiotics through the mediation of mobile genetic elements and horizontal gene transfer. An element called “integron” allows bacteria to “capture” and accumulate exogenous genes. Thus excessive, inappropriate and indiscriminate use of antibiotics are pushing mankind slowly towards a “post-antibiotic era”. Unless some “drastic” innovative approaches are taken to halt this process, we may once again find ourselves in a situation where every common, safe medical treatment could present a life-threatening scenario.

**Practical considerations in use of antibiotics**

**Dr. Thandavarayan Ramamurthy**

**Corresponding Author:** Dr. Thandavarayan Ramamurthy, Translational Health Science and Technology Institute, Faridabad, Haryana. Phone: +91-129-2876485; E-mail: tramu@thsti.res.in

The rising risk to global health caused by pathogens resistant to several antibiotics remains a serious concern. Therapeutic failures in numerous life threatening antibiotic resistant microbial infections are increasing. The resultant effects have been regarded in prolonged hospital stay of the patients, higher cost of alternative treatment, the spread of pathogens in outbreak situations, etc. In the case of acute diarrhoeal disease and during outbreak situations, it is becoming mandatory to use antibiotics to reduce the volume of the stool and the duration of the hospital stay. As a microbiologist, I have seen the incorrect use of antibiotics in several clinical settings. During my outbreak investigation visits to several rural hospitals, I have seen stockpiling of inappropriate antibiotics to be distributed to the wards or the use of less effective drugs to the patients. The whole effort in controlling the disease will be futile if such practise continues. Healthcare providers should have adequate knowledge on the etiology and the severe signs of dehydration associated with diarrhoea. More importantly, the health care professionals must also know the contemporary resistance trend of the suspected pathogen in the clinical management. Such practises will save the cost and deliver effective outcomes.
To get to know, to discover, to Publish - this is the destiny.

Americas: OMICS International
5716 Corsa Ave, Suite 110, Westlake, Los Angeles, CA 91362-7354, USA,

Reach us at:
omics.ebooks@omicsonline.org
ebooks@omicsonline.org

please visit: http://www.esciencecentral.org/ebooks/