A Generalized Overview of Antibiotic Resistance on the Context of South Asian Countries

by

Ahsab Rahman
18136002
Fatima Fidatur Rafey
18136004

A dissertation submitted to the Department of Mathematics and Natural Sciences
in partial fulfillment of the requirements for the degree of
Bachelor in Science in Biotechnology

Department of Mathematics and Natural Sciences
BRAC University
July 2022

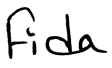
Declaration

It is hereby declared that

1. That the research work reported in this thesis title **'A Generalized Overview of Antibiotic Resistance on the Context of South Asian Countries'** has been carried out under the supervision of Dr. Iftekhar Bin Naser, Assistant Professor, Program Coordinator, Biotechnology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka.
2. This research work presented here is our original work while completing our degree at BRAC University.
3. The thesis has not been submitted to any other institution for any degree or diploma.
4. All the main sources of help have been acknowledged.

Student's Full Name & Signature:

Ahsab Rahman
ID: 18136002



Fatima Fidatur Rafey
ID: 18136004

Approval

The thesis/project titled **"A Generalized Overview of Antibiotic Resistance on the Context of South Asian Countries"** submitted by

1. Ahsab Rahman (18136002)
2. Fatima Fidatur Rafey (18136004)

Of Spring, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor in Science in Biotechnology on July 30, 2021.

Examining Committee:

Supervisor:
(Member)

Dr. Iftekhar Bin Naser

Assistant Professor, Program Coordinator, Biotechnology Program
Department of Mathematics and Natural Sciences
BRAC University

Program Coordinator:
(Chair)

A F M Yusuf Haider, PhD

Professor and Chairperson
Department of Mathematics and Natural Sciences
Brac University

Abstract

Antibiotic resistance has become a global health concern, marking its prevalence in the South Asian countries as well due to a number of underlying factors. With its manifold effects in the public health sector, it's high time that a sense of awareness and general knowledge created among the community to combat this phenomenon. This article aims to provide some comprehensive insights into the mechanism, management, and overall scenario of antibiotic resistance in the South Asian nations.

Keywords: Antibiotic, Resistance, Factors, Mechanism, Effects, Examples, Management

Dedication

Dedicated to our family and friends

Acknowledgement

First and foremost, we are very thankful to Almighty Allah for blessing and helping us in every aspect of our lives. We are also thankful to Almighty Allah that we were blessed with cooperating mentors, who had helped us throughout the whole process. We are grateful to **Professor Dr. A F M Yusuf Haider**, (Chairperson Department of Mathematics and Natural Sciences, BRAC University) for looking after all the students and teachers under his department and always providing his helping hands whenever needed. Our most sincere acknowledgment goes to all the faculties of Mathematics and Natural Sciences Department who had helped us in our undergraduate journey. This study would not have been possible without our supervisor **Dr. Iftekhar Bin Naser**. It was he who provided us with the adequate knowledge and guidelines required to complete this project. He constantly motivated and guided us in the required manner with his critical feedback and pleasant regards. It was a privilege and honor for us to be able to work under his guidance. In addition to that, we would also like to thank our friends and peers, Rubaiya Doula, Mustafa Galib and others who were constantly here for us in our times of need and motivated us throughout this journey.

Ahsab Rahman
Fatima Fidatur Rafey

Table of Contents

List of Figures

List of Tables

Nomenclature

The next list describes several abbreviations that will be later used within the body of the document

CAT Chloramphenicol acetyl transferase

CDC Centers for Disease Control and Prevention

CRE Carbapenem-resistant Enterobacteriaceae

$ESBL$ Extended spectrum beta-lactamase

FDA Food and Drug Administration

$HCAI$ Healthcare Associated Infections

$icddr, b$ International Centre for Diarrhoeal Disease Research, Bangladesh

$IEDCR$ Institute of Epidemiology Disease Control And Research

MDR Multi-drug resistant

$MRSA$ Methicillin-resistant *Staphylococcus aureus*

$NHSL$ National Hospital of Sri Lanka

OTC Over the counter

TB Tuberculosis

VAP Ventilator Associated Pneumonia

WHO World Health Organization

XDR Extremely drug-resistant

Chapter 1

Introduction

Antibiotic, which has been monumental in transforming modern medicine, is endangered due to the increase of resistant bacteria [1]. There has been an impact on the efficacy of the antibiotics due to the emergence of resistant genes, rendering some drugs useless against certain bacteria. While the discovery of these wonder drugs has been pivotal and revolutionary in the human history, the current antibiotic resistance scenario has the scientists and clinicians concerned due to the lack of efficiency of these antibacterial substances [2]. The necessity of antibiotics is immense since these are the medicines involved in the prevention and cure of many bacterial infections [3].

The commencement of modern antibiotic era began with the finding of penicillin by Sir Alexander Fleming in 1928. Penicillin was proven to be super effective during the time of World War II, helping the soldiers to combat various bacterial infections [4]. However, by the 1950s, penicillins soon started to become ineffective as penicillin resistance became a serious clinical issue— limiting its use to treat the bacterial infections. Consequently, scientists had to look for an alternative to penicillin, which resulted in the discovery and development of beta-lactam antibiotics [4][5].

However, resistance cases were increasing simultaneously as the first case of methicillin-resistant *Staphylococcus aureus* (MRSA) was found in the United Kingdom and the United States during the '60s [4][6]. Antibiotic resistance to multiple antimicrobial agents was detected first during the late 1950s and early 1960s among enteric bacteria namely *Salmonella*, *Shigella*, and *Escherichia coli*. These multidrug resistant strains caused a huge loss of life and clinical and financial losses. In the developing countries, these drugs were available over the counter which resulted in increasing use and ultimately an accelerated resistance. The lack of proper hygiene also helped in the transmission of resistance and the insufficient funds for healthcare restricted the access to novel antibiotics that would be effective. Eventually, resistance was found to be evident in nearly all the antibiotics that have been developed during that time frame. It was then when vancomycin was presented in the clinical setting to treat methicillin-resistance in both *Staphylococcus aureus* and coagulase-negative *Staphylococci* [6]. Inducing vancomycin resistance was proven to be very difficult by the scientists; hence, it was thought to be highly unlikely for the antibiotic to show resistance in clinical practices. However, the unexpected occurred when vancomycin resistance cases started to pile up during 1979 and 1983 [7]. During the late 1960s to

early 1980s, medical pundits have tried their heart and soul to keep on introducing new antibiotics as a way of finding solution to the resistance issue.

However, very few new classes of drugs have developed since then and the advancement has stalled in recent years. As a result, bacterial infections have again become a global threat in modern time [5][8]. A plethora of bacterial infections— from the likes of pneumonia and tuberculosis to gonorrhoea and salmonellosis— are becoming more troublesome to treat as antibiotics that used to treat these infections are becoming less effective day by day. As for some Gram-negative bacteria like *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and some *Enterobacteriaceae* like *Escherichia coli*, therapeutic options have become worryingly limited [3][9]. Clinicians and scientists are now indicating a return to the pre-antibiotic era as a recent database confirms the existence of approximately 20,000 resistance genes of nearly 400 types [10].

Antibiotic resistance is a phenomenon where antibiotics become ineffective against bacteria they were designated to kill. Therefore, the bacteria can easily survive inside the host (human and animal) and the infections occurring as a result of it are harder to treat than those that are caused by non-resistant bacteria [11]. We know for a fact that antibiotics act on microorganism rather than the individual; hence, microorganisms transferring readily from one individual to another can spread resistance from one person or environment to another. Basically, resistance will occur if a bacterium is able to survive and thrive in the presence of an antibiotic concentration that is adequate to destroy the bacteria of the same species. Global resistance is caused generally by the increase in global migration and overpopulation of humans, overuse of antibiotics in hospitals and agriculture, inadequate sewage disposal system and sanitation practices [12]. Resistance can be intrinsic or acquired (after being exposed to antibiotic) and it can develop because of mutation and direct transfer of resistance gene [13].

The phenomenon of antibiotic resistance has been regarded as one of the biggest global threats of the current century given the lack of and decline in new antibiotics as well as the overprescription of existing antimicrobial agents [14]. More than 2.8 million antibiotic resistance cases are found in the United States alone every year. And according to the Centers for Disease Control and Prevention (CDC), more than 35,000 people die due to bacterial infections that are resistant to antibiotics [15]. Overprescribing existing antibiotics has accelerated this threat and it is high time physicians and patients changed the way they prescribe and consume antibiotics respectively. Despite the development of new antibiotics, antimicrobial resistance will continue to be a problem as long as there is no behavioural change [16]. Behavioural changes indicate the need to minimise the transmission of infection by means of vaccination, handwashing, practicing safer sex, decent food hygiene, not taking antibiotic in viral infections, and taking it only when it is necessary [3][17]. If necessary initiatives aren't taken to reduce the antibiotic abuse, there is a possibility we might be heading towards the post-antibiotic era where many treatable bacterial infections might become untreatable again [18].

In the past 10 years, microbiologists were mostly anxious regarding Gram-positive bacteria like methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus spp.* Currently, it is concluded that multi-drug resistant Gram-negative bacteria pose a bigger threat to public health. Gram-negative bacteria can increase resistance faster than their counterparts with fewer developmental antibiotics that work against it [19-24]. The drug development does not sufficiently provide a therapeutic cover for the next 10–20 years [25-27]. The mobile genes on the plasmids of Gram-negative bacteria allow them to become resistive and aid to spread through populations of bacteria. Our understanding of the host ranges for these elements and their alarming distribution have enhanced significantly thanks to standardized plasmid typing methods [28][29]. Besides, bacterial plasmids and clones can transport much quicker all over the world by means of air travel. Since the resistant clones can be carried via human flora, most of the time, the spread stays undetected. They become noticeable only when causing endogenous infections [30-32].

Anywhere, at any time, novel antibiotic resistance factors can emerge. Due to the staggering number of bacterial cells on earth, there is an immense genetic variability, and greater opportunities for mutations, horizontal gene transfer and rearrangements. Mutations that lead to antibiotic resistance occur in types of genes that encode the targets of the antibiotic, encode their transporters, and those that encode regulators which can repress the antibiotic-decontaminating elements expression. Similarly, a mutation within regulatory genes, such as MarA, can increase expression of genes of efflux pump. Again, there have been reports of environmental bacteria being their origin. As a number of environmental microorganisms are synthesized to antibiotics, it was reported that the origin could be within antibiotic producers. The origin of resistance genes that have been discovered till now are human pathogens i.e., the quinolone resistance gene QnrA originated from Shewanella algae, and the CTX-M beta-lactamase family originated from Kluyvera. It is to note that these microorganisms are not antibiotic producers. The increase of antibiotic resistance has impacted human health in mainly two ways. It is directly affecting the infection treatment by delaying antimicrobial therapy and forcing the use of primitive and toxic antibiotics, ultimately decreasing the quality-of-life while increasing the mortality rate. Besides, it also compromises the treatment procedure needed for immunosuppression, such as, transplantation, catheterization, intubation, or chemotherapy. Most pharmaceutical companies have gradually turned away from antimicrobial research since the 1980s, and inclined more towards the treatment of chronic non-communicable diseases. Hence, steps should be taken to reconsider the antimicrobial research and control and monitor antibiotic resistance not only in humans but also in agriculture, aquaculture, and animal husbandry [33].

Chapter 2

Underlying Factors of Antibiotic Resistance

Currently, the multifarious etiology of antibiotic resistance has a plethora of underlying factors that come into play. However, they are broadly separated into two categories— (i) microbial behaviour (ii) human behaviour.

(i) Microbial Behaviour

Selective Pressure: Selective pressure, by definition, is the effects of external agents in an organism which gives the organism an edge to survive in a given environment. Basically, antibiotics will induce a selective pressure by destroying susceptible bac-

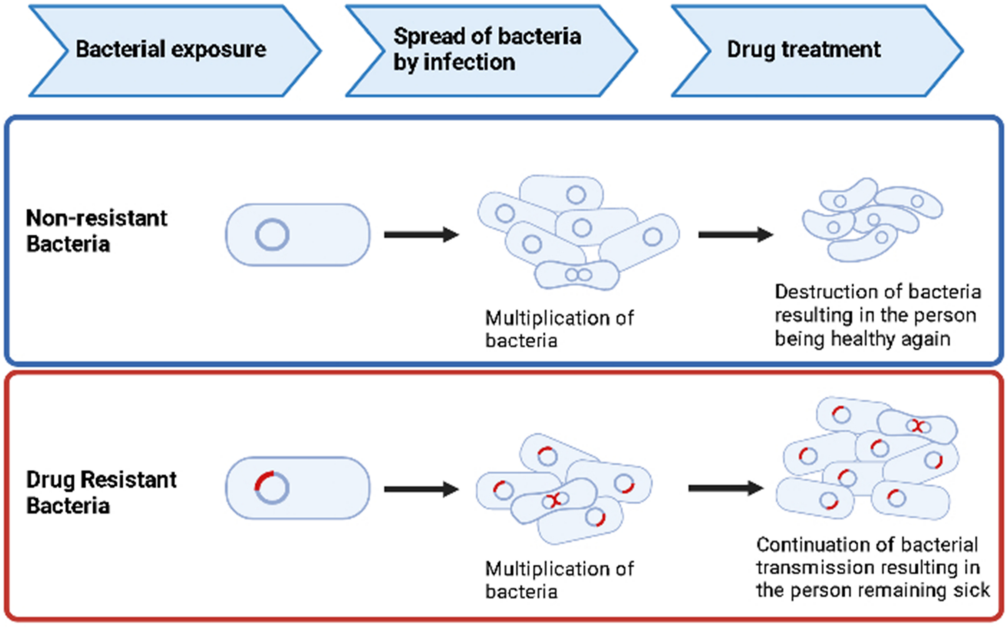


Figure 2.1: Difference between non-resistant bacteria and drug-resistant bacteria. Non-resistant bacteria undergo replication and upon antibiotic treatment, the bacteria die whereas drug-resistant bacteria multiply and survive upon antibiotic treatment. This figure was generated with BioRender premium subscription.

teria, allowing the survival and multiplication of antibiotic resistant bacteria. It is

considered to be a force which causes a specific organism to evolve in a particular direction. Due to the selective pressure, antibiotic resistant bacteria which underwent change in their DNA for their survival will outgrow non-resistant bacteria. To put it simply, non-antibiotic resistant bacteria will multiply, and upon antibiotic treatment, the bacteria will die. On the contrary, antibiotic resistant bacteria will multiply as well, and upon antibiotic treatment, the bacteria will survive and continue to spread. Selected resistant genes and their hosts will spread and multiply under continued antibiotic selection to accelerate and expand the problem to other hosts and other geographic locations. There are likely to be two outcomes when an antibiotic is exposed to a microbe— a) microbes that do not carry any resistance gene will be killed b) microbes that carry resistance genes will survive. Antibiotics are very much likely to be influencing plasmid transmission by inducing the transmission of resistance genes, additionally exerting a "selective pressure". These surviving bacteria will replicate and soon the newly generated resistant bacteria will become the dominant type throughout the microbial population [34].

Mutation: The propagation of bacteria means that one cell will divide into two cells. Before the bacterium can divide, it has to create two identical copies of the DNA in its chromosome; one for each cell. There is a chance that everytime the bacterium undergoes this process, an error might occur. That error is "mutation". Mutations can be random and its location can be anywhere within the DNA. External stimuli like radiation or detrimental chemicals can also result in mutations. Bacterial hypermutator strains have mutations in genes affecting DNA repair and replication fidelity, thus allowing increased mutation rates. Usually, mutations will

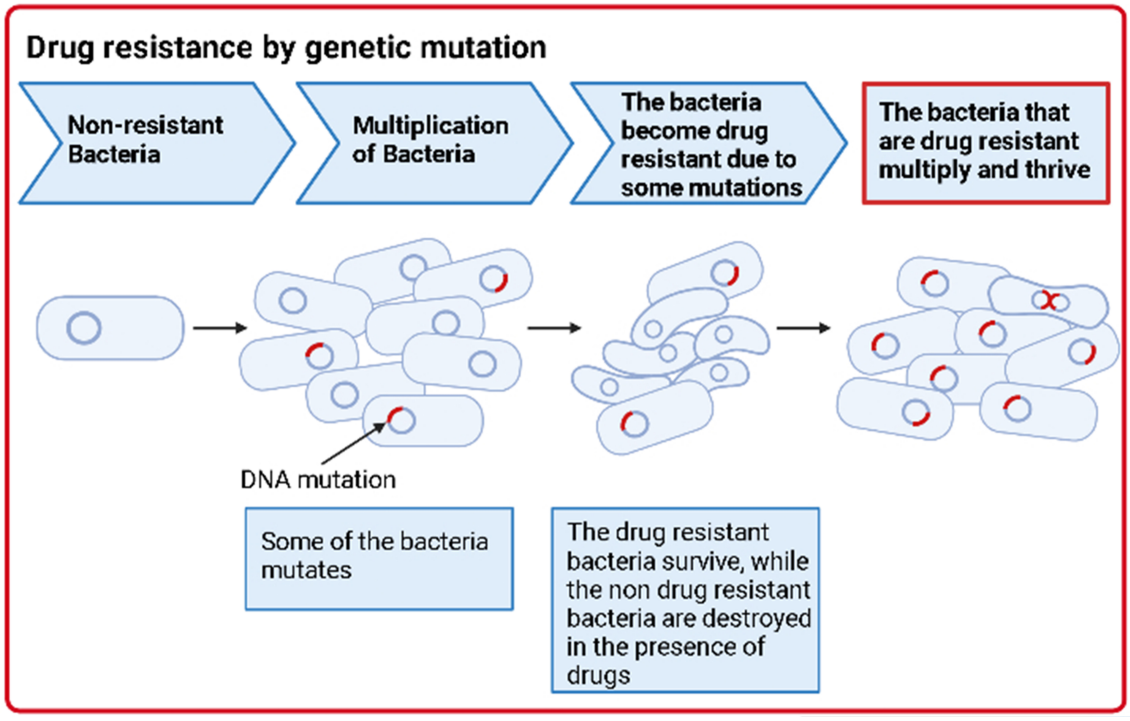


Figure 2.2: Upon multiplication, some of the bacteria mutate and become resistant due to mutation. The drug-resistant bacteria multiply and thrive. This figure was generated with BioRender premium subscription.

occur de novo in such hypermutable strains, and they will generate a good host background for the acquired resistance gene evolution in vitro. In particular species like Mycobacterium tuberculosis, mutation is the core reason behind clinical resistance problems. For example, resistance to all the antibiotics in Mycobacterium tuberculosis is induced by mutations, i.e., rifampicin resistance in rpoB, isoniazid resistance in katC, inhA, oxyR, ahpC and furA; resistance to streptomycin in rrs and rpsL; resistant to pyrazinamide in pncA; resistance to ethambutol in embB; and resistance to fluoroquinolone in gyrA and gyrB [35]. Non-resistant bacteria will replicate and multiply by millions, among which some of them will mutate. Some of these mutations will cause them to adapt to new environmental conditions and survive in the face of antibiotics. These antibiotic resistant bacteria will later replicate and multiply themselves initiating a serious concern. For millions of years now mutations and horizontal gene sharing have been a cause for evolution for bacteria. But depending on the bacterium and antibiotic, the frequency of mutations causing antibiotic resistance can vary. It is seen that mutations frequently occur in bacteria that lack the DNA repair system (hyper mutators) [36].

Gene Transfer: A bacterium can pick up genetic material from another bacterium, including genetic material containing antibiotic resistance. Gene transfer can be of

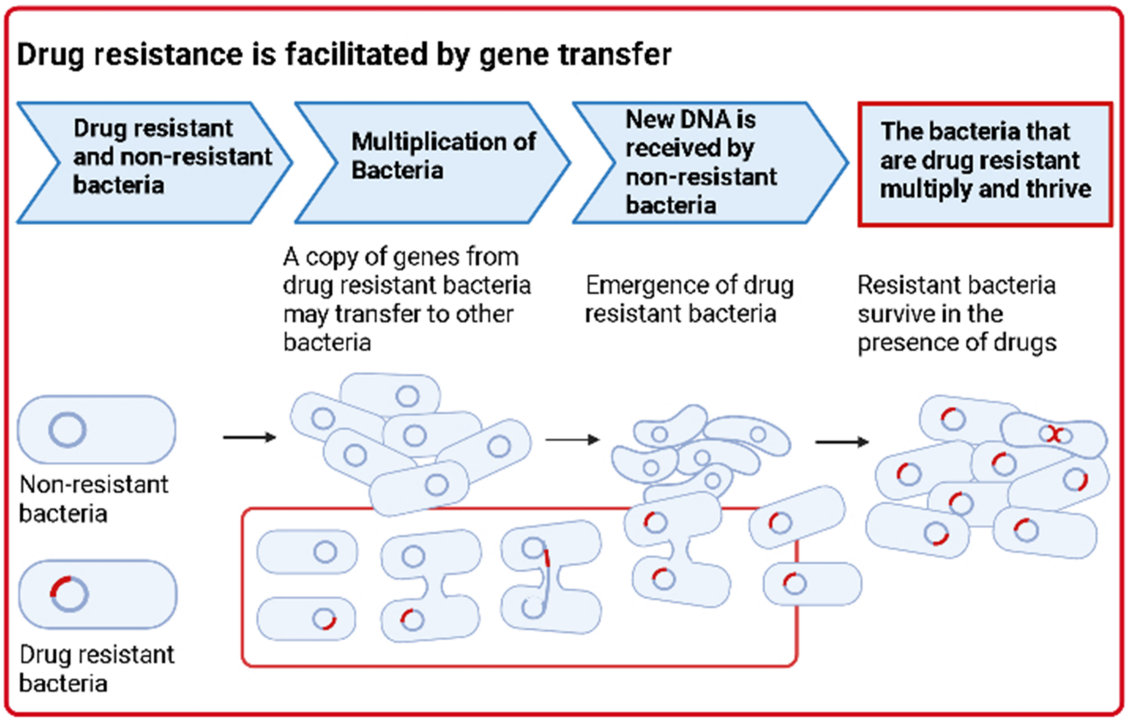


Figure 2.3: Graphical representation of antibiotic resistance transmission by means of gene transfer. This figure was generated with BioRender premium subscription.

two types: a) horizontal gene transfer, b) vertical gene transfer. Horizontal transfer is responsible for the acquired resistance. And usually, this phenomenon can be seen in community settings where horizontal transfer of genes occur between bacteria. On the contrary, vertical transfer can take place between mother and her offspring during birth and at times of lactation, or spontaneously due to the exposure of antibiotics. Vertical gene transfer is a crucial event as it might result in

early colonisation of bacteria among the infants which would later have a significant impact on their immune system. In case of horizontal gene transfer, transmission of genes can occur between same bacterial species and different bacterial species. Primarily, there are three ways for a bacterium to transfer genes and they are: a) conjugation: Pairing can happen between two bacteria and they can connect with each other with the help of cell membrane structures. Later, DNA can be transferred from one bacterial cell to another b) transduction: bacteriophages are viruses that can infect bacteria. They sometimes carry genes obtained during infection of another bacterium. These genes can later be introduced into the new bacterial host, and thus, if any of the genes are antibiotic resistant, it will surely result in the antibiotic resistance phenomenon c) transformation: Some bacteria are just capable of uptaking DNA pieces from the environment around the cell [37]. By means of plasmid transmission, resistance genes can be exchanged between bacteria, ultimately playing a role in the antibiotic resistance distribution. The exchange of antibiotic resistance genes between environmental bacteria and human pathogens has recently been proven. Dissemination also occurs by the genetic sequences, where the mobilized genes are present amongst the environmental bacteria [38].

(ii) Human Behaviour

Fault in Diagnosis: When a patient is suffering from a serious bacterial infection, physicians are oftentimes forced to prescribe an antibiotic without testing how resistant or susceptible the best-guessed condition is to the antibiotics they have prescribed. That happens due to a lack of rapid, reliable, and affordable diagnostic tests. In most cases, infections are diagnosed by sending the sample of the patient to a laboratory for it to be cultivated and tested against a variety of antimicrobial agents to check which one kills the bacteria. Over the last ten years, a number of molecular techniques have been discovered to speed up the diagnosis process. These molecular diagnostic tests can identify the presence or absence of genes or gene mutations for resistance to antibiotics. However, the drawback of such techniques is that they are only available in well-resourced healthcare facilities, are costly, and require trained professionals to interpret the results. Researchers at the University of Cambridge are developing a far more cheaper and simpler portable antibiotic resistance diagnostic kit packaged into a portable hand-held sized briefcase with a solar-panelled battery that can be used any place a person wants. The diagnostic kit is modelled on a cholera test trialled in Malawi and India. The biggest advantage of that kit is that its result can be easily read off a molecular dipstick. As a result, it can directly guide a person to clinical decision-making. Unlike the traditional testing, Cambridge test can detect antimicrobial resistance profile of a particular sample without needing to identify the bacteria. The Cambridge test will also be extremely dynamic and customisable due to the dipstick probes which can be rapidly changed to identify a specific range of antibiotic-resistant genes found locally. The tools and kits to diagnose bacterial infections have hardly been improved since the 1940s and most of the existing testing methods are the methods developed by Louis Pasteur and Robert Koch in the 19th century. It can take 36 to 48 hours to know if someone has a particular bacterial infection. But this is too long of a time as some patients need to be treated immediately with antibiotics. Hence, wrong antibiotics

are prescribed and oftentimes broad-spectrum antibiotics are prescribed in case of infections that can be treated with narrow-spectrum antibiotics. If there was a rapid detection tool, appropriate antibiotics could be prescribed in correct dosages upon identifying a bacterial infection. That's why, more kits like the one in University of Cambridge need to be discovered. Oftentimes, medical care professionals will utilise incorrect and/or inadequate information to diagnose an infection and in turn provide antibiotics "just in case" or prescribe broad-spectrum antibiotic where a particular antibiotic can work better in that condition. As a result, this might cause selective pressure and fasten up antibiotic resistance [39].

Hospital Use: Most of the resistant strains are common in healthcare facilities rather than the community, and as a result, one of the greatest risks of developing an antibiotic resistant infection is staying in a hospital. Every day, thousands of patients bring with them their own range of bacteria - on their clothes, and on/inside their bodies. Some of the bacteria are definitely resistant to antibiotics, hence, the hospital environment soon becomes a pool of antibiotic resistant bacteria. The bacteria can spread from patient to patient, patient to non-patients via various means like lack of hand hygiene, transfer of bacteria to sterile surgical equipments, and clothes as well. Healthcare professionals should test samples prior to an antibiotic prescription. Trial and error method until one antibiotic works is very risky and enhances the chances of antibiotic resistance. The use of broad-spectrum antibiotics to treat an infection which can be treated with a narrow-spectrum one can facilitate antibiotic resistance as well. Physicians prescribe antibiotics before even getting the result of a test, and then there is the pressure from unknowledgeable patients to receive antibiotics in any given case forcing the doctors to prescribe antibiotics. Inappropriate empirical therapy, use of broad-spectrum antibiotics, and lack of rapid diagnostic technique, and lack of doctor and patient education regarding antibiotic resistance result in the antibiotic resistance scenario in hospitals [40]. Along with its misuse in viral infections, overuse of antibiotics can be also seen in the postnatal women and newborns in hospitals which definitely facilitate antibiotic resistance to some extent. There are critically ill patients who oftentimes need antibiotics immediately due to their extreme susceptibility to infections. However, receiving higher doses of antibiotics can contribute to the distribution of antibiotic resistance, worsening the issue by selecting for antibiotic resistant bacteria. This creates an environment for antibiotic resistant bacterial genes to spread and generate serious concern in the community. At the same time, poor hygiene policy and practices contribute when it comes to spreading antibiotic resistant bacteria. Sometimes, doctors even end up prescribing antibiotics even if it's not a critically ill patient. There is evidence where doctors prescribed antibiotic for a sore throat. However, we do know for a fact that only 15% of sore throats are direct results of bacterial infections. In most other cases, antibiotic doesn't even work against a sore throat. Prescribing antibiotics unnecessarily holds no benefit, rather it can induce antimicrobial resistance in some cases [36][41].

Agricultural and Poultry Use: The overall health of stock and crops can be significantly improved if they are treated with antibiotics; hence, ultimately, increasing the agricultural yield. There is a rising demand for animal proteins in developing countries, as a result, intensive farming is instigated. This will later result in

antibiotic residues getting incorporated into animal-derived products, causing antibiotic resistance. Antibiotic resistant bacterial genes associated with animals can be easily transferred between humans by means of food chains. Additionally, animal wastes in the environment will play a vital role in the transmission process as well. This will cause untreatable, prolonged bacterial infections in humans which will ultimately result in hospital stay and maybe even death. Basically, antibiotic is used in agriculture and poultry for a plethora of reasons including livestock farming, animal growth promotion, improved feed conversion efficiency, and disease prevention. There is a reason why antibiotic use in agriculture and poultry varies geographically depending on several factors including regional production patterns, forms of production system, extensive or intensive farming, food animal species, farming purpose, lack of proper policies or legislative framework, and the socioeconomic status of the population of the given region. In case of underdeveloped countries, the incorporation of antibiotics in animal feeds for growth promotion remains unmonitored and unregulated. Lack of government policies, over-the-counter antibiotic sale, reduced use of infection control measues, and the unenthusiastic behaviour among the farmers to modify their farming practices are some of the core reasons behind the antibiotic resistance phenomenon happening due to antibiotic use in agriculture and poultry. Underdeveloped countries utilise antibiotics like tylosin, chloramphenicol, and TCN (a mixture of powder consisting of oxytetracycline, chloramphenicol, and neomycin) in animal feed whereas these antimicrobial agents are banned in developed countries. Antibiotic can be released in the environment by means of farming wastes and veterinary use remains. Thus, they contaminate soil and water. As a result, if any of the wastes contain antibiotic resistant genes, they can be transmitted to humans easily and cause significant harm [42]. Researchers and scientists have warned against the practice of using antibiotics in farm animals as it might promote antibiotic resistance. It has been found that more than half of the antimicrobial agents generated in the United States are utilised for agricultural purposes and livestock treatment. However, the possibility of antibiotic resistant bacteria in animals causing noteworthy threat in public health is up to debate. But it should also be mentioned that antibiotic resistant bacteria have been found in meat and food crops. So, exposing them to fertilisers and contaminated water might contribute to the distribution of antibiotic resistance from animals to humans [43].

Inappropriate Use: Inappropriate use of antimicrobial agents have accelerated the process of antibiotic resistance. Self-medication, along with inappropriate medication of family members without the prescription of physicians, and use of leftover drugs are some of the key factors of causing antibiotic resistance in the long run. There are many reasons behind the improper use of antibiotics including lack of education regarding the phenomenon, low socioeconomic status, younger age, lack of satisfaction with the healthcare services, busy and hectic life with job engagement, etc. Over reliance on empirical therapy when treating infections without laboratory confirmation, inappropriate use of broad-spectrum antibiotics for treating very susceptible bacteria, wrong dosages of antibiotics, prolonged use of antibiotics when it's not needed after dosage completion, use of wrong antibiotic to treat an infection, using somebody else's antibiotic without visiting the doctors are some other inappropriate uses of antibiotic which enhance antibiotic resistance [44]. There are other factors as well like promotional activities by pharmaceutical companies and source

of income for pharmacies in small hospitals. An example of unnecessary antibiotic use can be a bronchitis patient being prescribed an antibiotic even though national guidelines prohibit the use of antibiotics for treating bronchitis. Unqualified doctors, bad influence of pharmacists in the drug centers, and immense dependency on antibiotics result in this antibiotic resistance phenomenon spreading further. Sometimes physicians and medical care givers prescribe antibiotics to patients suffering from viral infection. Sometimes a patient doesn't even complete the course of antibiotics which may cause some bacteria to survive and develop resistance to the drug. Additionally, antibiotics are inappropriately prescribed when there is an undiagnosed condition or when the patient is too insistent on getting antibiotic. Overprescription of antibiotics is one of the key factors of resistance evolution. Despite not having any impact on viral diseases, antibiotics are commonly prescribed for this purpose [36][45]. Lack of regulations and awareness policy have also made antibiotic resistance more prevalent. Besides, low-grade antibiotics are easily accessible now thanks to online marketing. So, there is a lack of restriction there as well [46][47].

Additionally, some other factors contributing to antibiotic resistance include disease control standards, sanitation settings, water hygiene settings, migration and traveling, drug quality and diagnostics [46][48].

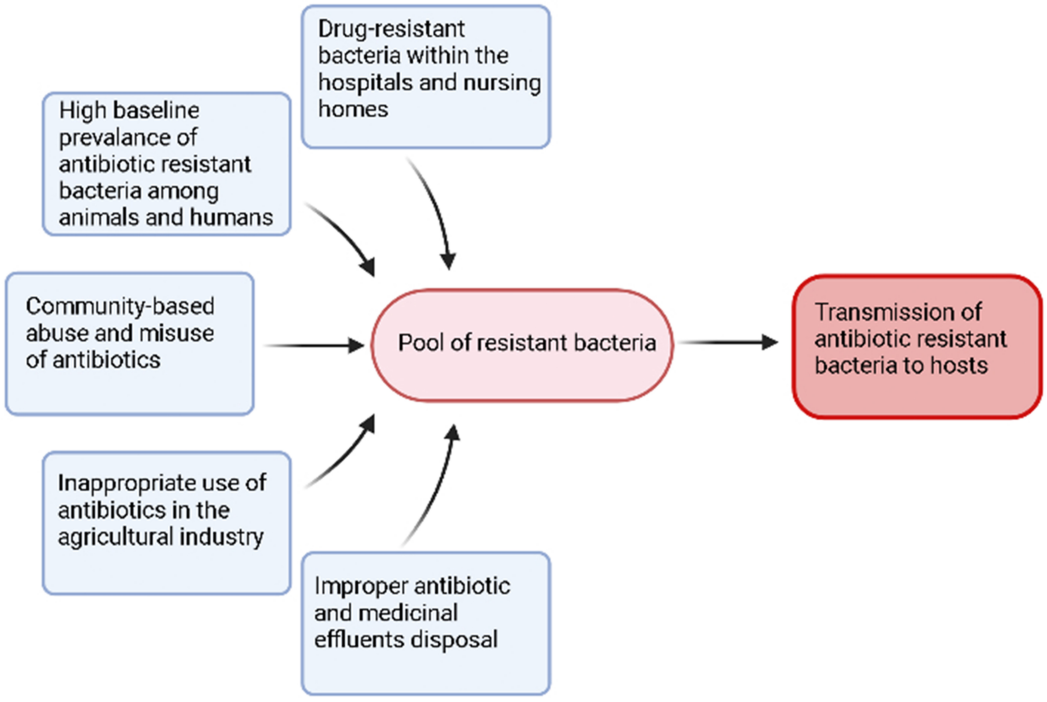


Figure 2.4: Major sources of drug-resistant bacteria in the context of South Asian countries.

Causes of Antibiotic Resistance	Implications
Selective Pressure	Antibiotics are very much likely to be influencing plasmid transmission by inducing the transmission of resistance genes, additionally exerting a "selective pressure". These surviving bacteria will replicate and soon the newly generated resistant bacteria will become the dominant type throughout the microbial population
Mutation	Non-resistant bacteria will replicate and multiply by millions, among which some of them will mutate. Some of these mutations will cause them to adapt to new environmental conditions and survive in the face of antibiotics
Gene Transfer	A bacterium can pick up genetic material from another bacterium, including genetic material containing antibiotic resistance. By means of plasmid transmission, resistance genes can be exchanged between bacteria, ultimately playing a role in the antibiotic resistance distribution
Poor Diagnosis	Oftentimes, medical care professionals will utilise incorrect and/or inadequate information to diagnose an infection and in turn provide antibiotics "just in case" or prescribe broad-spectrum antibiotic where a particular antibiotic can work better in that condition
Hospital Use	Receiving higher doses of antibiotics can contribute to the distribution of antibiotic resistance. This creates an environment for antibiotic resistant bacterial genes to spread and generate serious concern in the community. At the same time, poor hygiene policy and practices contribute when it comes to spreading antibiotic resistant bacteria
Agricultural and Poultry Use	Researchers and scientists have warned against the practice of using antibiotics in farm animals as it might promote antibiotic resistance
Inappropriate Use	Sometimes physicians and medical care givers prescribe antibiotics to patients suffering from viral infection. Sometimes a patient doesn't even complete the course of antibiotics which may cause some bacteria to survive and develop resistance to the drug

Table 2.1: Causes of Antibiotic Resistance [34-47]

Chapter 3

The Manifold Effects of Antibiotic Resistance

The effect of antibiotic resistance is manifold— ranging from clinical outcomes to economic consequences. The magnitude of such outcomes can be further visualised in the future based on the increase of severity of diseases, virulence of strains, and vulnerability of hosts. Here, we will briefly categorise the effects and learn how they can have a significant impact on human health and economy—

(i) Morbidity and Mortality

An increased level of morbidity and mortality will be witnessed due to antibiotic resistance. Failure of antibiotic treatment to cure infections will lead to more chronic health issues. Infections occurring from resistant bacterial strains are more likely to cause two-fold higher rates of detrimental outcomes compared to infections occurring from non-resistant bacterial strains. The outcomes could be manifold, ranging from death or treatment failure to increased length of stay and hospital cost. The magnitude of such outcomes is based on the severity of the disease, virulence of strain, or host vulnerability increase. An example of resistant bacterial strain causing more harm can be observed in bacteremia and other crucial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Infections caused by MRSA will have far more fatality rate compared to methicillin-susceptible *Staphylococcus aureus* infections. Same can be seen in *Enterobacteriaceae* that are capable of producing extended-spectrum beta lactamase (ESBL). ESBL producing *Enterobacteriaceae* will have more fatality rate and treatment failure compared to bacteremia caused by non-ESBL producers. Even in case of ESBL producing *Klebsiella pneumoniae*, we can see that the treatment failure is higher compared to non-ESBL producing *Klebsiella pneumoniae*. Moreover, in recent times, it has been found that carbapenem-resistant *Klebsiella pneumoniae* have approximately two to five fold higher risk of death than infections caused by carbapenem-susceptible *Klebsiella pneumoniae*. Carbapenem-resistant *Enterobacteriaceae* are now linked with crude in-hospital death rate of 48%-71%. On the other hand, the mortality rate for crude in-hospital carbapenem-resistant *Acinetobacter baumannii* infections causing bacteremia is 45%. Other than death, there are other complications as well when it comes to antibiotic resistance, such as penicillin-resistant pneumococci being associated with greater than four times the risk of suppurative complications. Even in case

of antibiotic resistant *Neisseria gonorrhoeae*, infections caused by this bacterium can cause treatment failure, frequent reproductive tract disease, infertility, and facilitation of other sexually transmitted diseases [49].Then there are outcomes that happen due to alternative treatments, which end up being more toxic and less effective. The chronic problems arising from treatment failure can either result in the death of the patient or leave him with extremely weakened immune system which would take a long time to recover from. This can also result in decreased societal productivity. Antibiotic resistance might have secondary effects as well such as cancer patients suffering from antibiotic resistant bacterial infection cannot undergo chemotherapy. Chemotherapy can impair the immune system and make cancer patients vulnerable to various infections. Same can be said for patients needing organ transplantation. It will be challenging to do if the patient is going through antibiotic resistant bacterial infection. Overall, the phenomenon will have a significant impact on morbidity and mortality. There are a number of reasons why such adverse outcomes occur from the likes of bacterial fitness and greater severity of illness to lack of effective therapy and delays in initiation of the therapy. Deaths happening because of antibiotic resistant bacterial infections reduce the workforce, which again impacts the population size and overall quality of the nation's human capital. This will also have an effect on the livestock as more antibiotics will be ineffective against many infections in animals. As a result, there will be a decrease in the production and trade of the livestock and increase in protein price due to reduced protein sources such as meat, egg, and milk. Due to antibiotics not working for specific bacterial infections, people are more likely to be infected by them— which will have a severe impact on their health. This will result in an increase in the length of hospital stay, more usage of mechanical ventilation, and an extended need for intensive care and invasive devices. Overall, there will be a functional decline due to the post-treatment relapse of the infection, use of antimicrobial agents with minimised efficacy, loss of narrow-spectrum antibiotic classes, and use of antibiotics with increased toxicity [50].

(ii) Excess Health Care Costs

Cost of antibiotic resistance every year is 55 billion dollars in the United States, among which 20 billion dollars is for health care and 35 billion dollars for loss of productivity. 6 (Vancomycin-resistant *Enterococcus*, Carbapenem-resistant *Acinetobacter* species, Methicillin-resistant *Staphylococcus aureus*, Carbapenem-resistant *Enterobacterales*, Extended-spectrum cephalosporin resistance in *Enterobacterales* suggestive of extended-spectrum -lactamase (ESBL) production, and Multidrug-resistant *Pseudomonas aeruginosa* of the 18 most alarming antimicrobial resistance threats cost the United States more than 4.6 billion dollars annually.
It has been found that in the United States alone, antibiotic resistance could result in the addition of 1400 dollars to the hospital bill for the treatment of any bacterial infections. In addition to that, the current projection is that antibiotic resistance could cost from 300 billion dollars to more than 1 trillion dollar on annual basis by 2050 globally. The direct monetary effects of such cost include expensive treatments and hospital resources along with admission and length of stay. The length of stay is far greater in case of patients suffering from antibiotic resistant bacterial infections because combination of regimens being utilised might be ineffective, which will result in lengthier hospital stay, greater use of intensive care units and isola-

tion beds. Moreover, resistant bacteria might cause nosocomial outbreaks, which might result in the closure of a hospital wing and cancellation of elective surgeries, costing healthcare money. It has been found that antibiotic resistance can amplify the rate of poverty and have a greater impact on low-income countries compared to the high-income countries of the world. Worldwide gross domestic product could decrease by approximately 1% and the possibility is that there is likely to be 5%-7% lose in developing countries by 2050. This could mean that antibiotic resistance might cost around 100-210 trillion US dollars. Multidrug-resistant *tuberculosis* itself could end up costing 16.7 trillion dollars globally by 2050 [51]. The financial gap between low-income and high-income countries will significantly increase, resulting in substantial inequity. Basically, underdeveloped and developing countries will take the most blow and that's very alarming because most of the South Asian countries fall into this category. As mentioned earlier, antibiotic resistance will result in people being infected by common bacterial infections and not getting cured through antibiotic treatment. Consequently, alternative ways of treatment need to be used which will prolong hospital stay— additionally increasing the cost for the patients for nursing care, mechanical ventilation, other supporting devices, diagnostic tests, imaging, intensive care units, post-acute care beds, and so on [52].

(iii) Lack of Availability of Clinically Effective Antibiotics and Increased Use of Broad-Spectrum Antibiotics

Loss of narrow-spectrum antibiotic classes due to the current resistance scenario has caused a scarcity of antibiotics that are clinically effective. At the same time, it has increased the use of broad-spectrum antibiotic classes for empirical therapy since many of the common bacterial infections are now resistant to narrow-spectrum antibiotics. Narrow-spectrum antibiotics are very specific and work on a selected group of bacterial type. They will either act against gram-positive bacteria or gram-negative bacteria, but not both. For instance, penicillin G is super effective at destroying gram-positive bacteria, but not very effective against gram-negative bacteria. On the other hand, broad-spectrum antibiotics can act against both gram-positive and gram-negative bacteria. Usually, narrow-spectrum antibiotics are applied when there is a specific infection and the microorganism causing that infection is known. They don't destroy as many of the normal bacteria in the body as broad-spectrum antibiotics. Since narrow-spectrum antibiotics target specific bacteria, they don't have an impact in the increase of antibiotic resistance. The problem with broad-spectrum antibiotics is that they are contributing to the development of antibiotic resistance. At the same time, along with killing harmful bacteria, they end up killing some useful native bacteria residing inside our body as well, thus, causing a problem in our immune system. Broad-spectrum antibiotics disrupt the normal intestinal flora and this disruption facilitates overgrowth of bacteria with emergence of resistant microorganisms. And these microorganisms themselves can cause serious infections, and at the same time, they can carry the resistance factors to other bacteria. The more broad-spectrum an antibiotic is, the more effect it will have on the intestinal flora. Carbapenems are linked with an increase in hospital-acquired infections due to *Stenotrophomonas maltophilia*. This antibiotic resistant gram-negative bacteria will also overgrow in response to treatment with third generation cephalosporins, as well as with a host of other microbes

namely *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus* (MRSA), yeasts and *Enterococci*. This resistance can be transferred to other patients and cause outbreaks, ultimately put a harmful effect on the environment [53]. The problem with broad-spectrum antibiotic is manifold: a) they are expensive b) they have more detrimental effect on protective microflora c) they are occasionally more toxic, and d) they are less effective compared to the narrow-spectrum antibiotics. An example of the usage of broad-spectrum antibiotic instead of narrow-spectrum antibiotic can be seen in case of community-acquired pneumonia where third generation cephalosporins or fluoroquinolones are used instead of penicillin [54].

Chapter 4

Examples of Drug-resistant Bacteria in Current Scenario

According to the Centers for Disease Control and Prevention (CDC), antibiotic resistance threats can be divided into two categories— a) urgent threats b) serious threats. Below, we will briefly discuss about these two categories and learn the potential hazards associated.

(i) Urgent Threats

Carbapenem-resistant Acinetobacter: Acinetobacter are microorganisms that have the capability of surviving long-term on surfaces. Immunocompromised individuals along with hospitalised patients are at greater risk of obtaining *Acinetobacter* infection. In case of antibiotic-susceptible *Acinetobacter* infections, there are a number of therapeutic options including a combination beta-lactam/beta-lactamase inhibitor (i.e, one that includes sulbactam), carbapenems (i.e, meropenem and imipenem), or broad-spectrum cephalosporin (ceftazidime or cefepime). The problem arises when *Acinetobacter* becomes resistant to carbapenems. If resistance occurs, alternative therapeutic options need to be considered. Polymyxins (polymyxin B and polymyxin E aka colistin) are taken as the main therapeutic drug for combating carbapenem-resistant *Acinetobacter*. Colistin was very effective against *Acinetobacter* pneumonia, bacteremia, and meningitis. However, the drug has its drawbacks as nephrotoxicity seems to be associated with systemic colistin. Tetracyclines like monocycline and tigecycline can play crucial roles in fighting antibiotic-resistant *Acinetobacter* as well. Multiple resistant strains of *Acinetobacter baumannii* is susceptible in vitro to monocycline, which can be given intravenously. Although clinical data is limited, so far favourable outcomes have been found with monocycline and tigecycline in treating drug-resistant *Acinetobacter* infections. This bacterium can be resistant to a plethora of antibiotics that are commonly prescribed by physicians. Carbapenem-resistant *Acinetobacter* can cause pneumonia and wound, as well as urinary tract and bloodstream infections, especially in patients that are in the intensive care unit (ICU) [15][55][56]. Carbapenem-resistant *Acinetobacter* carry mobile genetic elements that are transferred between bacteria. Some of the *Acinetobacter* can generate Carbapenemase enzyme which invalidates the action of Carbapenem antibiotics, ultimately making these drugs ineffective against *Acinetobacter*. The bacterium can transmit by means of direct contact and are usually found in food,

water, skin, or soil [57].

Drug-resistant Candida auris: Candida auris is a type of fungi which is resistant to several drugs. This yeast can cause critical infections and spread between health care facilities and hospitalised patients with ease. *Candida auris* can cause candidiasis which is a type of fungal infection. Oftentimes, candidiasis is acquired in healthcare facilities and especially in patients with weakened immune system. *Candida auris* is resistant to a plethora of antibiotics including fluconazole and voriconazole. First identified in 2009, *Candida auris* can cause a havoc in hospitals as healthcare disinfectants are not effective against it. Generally, it can be carried on the skin of patients and spread to others from there. To make matters more, misdiagnosis of *Candida auris* is very common because diagnostic platforms that many public health laboratories and clinics have depend on reference databases, which have not fully incorporated *Candida auris*. However, new diagnosis techniques are being tested but inadequate treatment options and limited disinfection strategies have made this a threat to think about. At this point, understanding its epidemiology and minimising the transmission in patients are two biggest challenges to overcome as soon as possible. It cannot be eliminated through common disinfectants, and it is resistant to almost all antifungal drugs from the likes of azoles to polyenes. 90% of the *Candida auris* show resistance towards at least one antifungal medicine and 30% of them show resistance towards at least two antifungal agents [58].

Clostridioides difficile: This bacterium is known to cause life-threatening bloody or watery diarrhoea and a type of colon inflammation (more commonly known as colitis). Usually, the infection is more prevalent in patients who have consumed antibiotics for other conditions [59]. A higher chance of being affected by this bacterium is when you have been taking antibiotics like clindamycin, cephalosporins, and fluoroquinolones. The older individuals are more at risk and patients with cancer or compromised immune system are more likely to affected by it. Some of the symptoms that *Clostridioides difficile* has affected you would be severe diarrhoea, stomach pain, fever, loss of appetite, and nausea. If there is infection, on-going antibiotic treatment for other infections needs to be stopped and specific antibiotics like vancomycin or fidaxomicin should be used for 10 days to combat this. *Clostridioides difficile* is contagious, so extra caution should be taken in hospitals including basic hygiene, having separate bathrooms, etc. *Clostridioides difficile* can be repetitive in some patients, and for them the fecal microbiota transplants have shown promise. With time however, health care associated *Clostridioides difficile* cases are getting reduced whereas community associated cases are still very much causing trouble. Patients infected with this bacterium must be isolated and non-infected individuals taking care of such patients should be instructed to maintain hygiene and caution as it can spread from patient to patient [60][61].

Carbapenem-resistant Enterobacteriaceae: Enterobacteriaceae are typically those bacteria that are part of our digestive system, not causing any harm. However, they have the ability to become resistant to an antibiotic class named Carbapenem. Carbapenem-resistant *Enterobacteriaceae* can cause pneumonia, urinary tract infections, blood stream infections, abdominal infections, neutropenia, meningitis, etc. Which is why it being resistant to antibiotics can be very detrimental [62].

Enterobacteriaceae are bacteria causing a a plethora of infections, including bloodstream infections, community-acquired pneumonia, hospital-acquired pneumonia, ventilator-associated pneumonia, complicated urinary tract infections, and complicated intra-abdominal infections. As a result, antibiotic resistance in these bacteria can bring about severe socioeconomic and clinical impacts. Carbapenems are used as the first-line empirical drug therapy for *Enterobacteriaceae*. However, its increased use has led to the emergence of carbapenamese generating *Enterobacteriaceae*, which can be very hazardous. Therapeutic options to carbapenem-resistant *Enterobacteriaceae* are very limited with polymyxins and tigecycline being the most viable choice. But resistance to these antibiotics have been found as well. Alternative treatment options need to be considered and that's where the novel beta lactamase inhibitor combinations come into play. However, they are not effective against all classes of carbapenameses. Older antibiotics like minocycline, doxycycline, trimethoprim/sulfamethoxazole, and chloramphenicol can also come handy in fighting carbapenem-resistant *Enterobacteriaceae*. Carbapenem-resistant *Enterobacteriaceae* infections are most likely to occur in healthcare facilities, especially in hospitalised patients requiring devices like catheters and those taking long courses of antibiotics. Carbapenem-resistant *Enterobacteriaceae* are known to possess mobile genetic elements that can be easily shared between bacteria, hence, making it a matter of concern [63].

Drug-resistant Neisseria gonorrhoea: Neisseria gonorrhoea causes gonorrhoea, which is a sexually transmitted disease. Gonorrhoea can cause severe health problems like ectopic pregnancy and infertility if left untreated. In case of drug-resistant *Neisseria gonorrhoeae*, single therapy will not work and hence, based on the local resistance data dual therapy should be recommended. It can be ceftriaxone 250 mg intramuscular as a single dose along with azithromycin 1 g orally as a single dose or it can be cefixime 400 mg orally as a single dose along with azithromycin 1 g orally as a single dose. *Neisseria gonorrhoeae* can be passed from one person to another by means of having vaginal, oral, or anal sex with anyone has the disease. It can also be transmitted from an infected mother to the baby during childbirth. Symptoms in males may include buring sensation while urinating, a green, white, or yellow discharge from the penis, and swollen testicles. Symptoms in females include burning sensation when urinating, increased vaginal discharge, and vaginal bleeding between periods [64][65]. Sadly, but concerningly, gonorrhoea has become resistant to almost all the antibiotics used for its treatment, such as penicillins, tetracyclines, fluoroquinolones, macrolides, etc. It is now treated with ceftriaxone injection without combining it with azithromycin as a result of resistance [66].

(ii) Serious Threats

Methicillin-resistant Staphylococcus aureus: Staphylococcus aureus can cause infections in multiple areas such as skin and soft tissue (cellulitis, abscess), lung (pneumonia), brain (meningitis), bone (osteomyelitis), urinary tract, heart (endocarditis), etc. Methicillin-resistant *Staphylococcus aureus* is the strain of *Staphylococcus aureus* that has developed (by means of natural selection) or acquired (by means of horizontal gene transfer) a multiple drug resistance to beta lactam antibiotics. Strains unable to resist these antibiotics are classified as methicillin-susceptible *Staphy-*

lococcus aureus. Individuals with indwelling implants, prostheses, catheters, and drains along with patients with weakened immune response are more likely to be affected severely by it. Then patients with diabetes, intravenous drug users, abuser of quinolone antibiotics, elderly people, people having thoracic surgery are also at risk. Prison inmates and military personnel can acquire it too as military barracks and prisons are crowded and less hygienic. Ceftaroline, a fifth-generation cephalosporin, is the first beta lactam antibiotic approved in the United States to treat methicillin-resistant Staphylococcus aureus infections in skin and soft tissue or community-acquired pneumonia. Some antibiotics like linezolid belonging to oxazolidinone have seen some success as well [67]. This bacterium has become resistant to a plethora of commonly used beta-lactam antibiotics such as methicillin, amoxicillin, oxacillin, nafcillin, penicillin, and cephalosporins. Methicillin-resistant *Staphylococcus aureus* is generally transmitted through contact. It is known to cause blood and lung infections and specifically affect surgical sites. Patients in the healthcare facilities and nursing homes are more in risk of getting affected by this microbe [68][69].

Drug-resistant Streptococcus pneumoniae: Streptococcus pneumoniae is a bacterium that is mainly responsible for pneumococcal diseases, especially community-acquired pneumonia. *Streptococcus pneumoniae* are gram-positive, lancet-shaped bacteria with more than 100 serotypes. Most of these serotypes cause disease but only few of them generate majority of pneumococcal infections. The bacteria can be isolated from the nasopharynx of 5%-90% of the healthy individuals based on the setting and population. 5%-10% of adults without children are carriers, 20%-60% of the school-aged children might be carriers, and 50%-60% of the military personnel might be carriers. The symptoms of pneumonia caused by *Streptococcus pneumoniae* include chills and fever, breathing difficulty, cough, and chest pain. Pneumococcal meningitis can cause stiff neck, headache, fever, and confusion. Due to its severity, several vaccines have been developed and recommended to combat this bacteria [70]. It can also cause meningitis in children and adults. Additionally, the bacterium is responsible for causing otitis media, bacteremia, sinus infections, septic arthritis, endocarditis, etc. Since the bacteria is responsible for so many infections, drug-resistant *Streptococcus pneumoniae* is a terror in the medical field as drugs might not work in patients who are infected by it [71][72].

Vancomycin-resistant Enterococcus: Enterococci refer to those bacteria that are present inside human body in areas like female genitalia and intestines. Vancomycin-resistant *Enterococcus*, as the name suggests, are resistant to Vancomycin [73]. Generally, *Enterococci* are not that harmful or virulent. This is applicable to both antibiotic-resistant as well as nonresistant or sensitive strains. However, when vancomycin-resistant *Enterococcus* infects the urinary tract, surgical wounds or the bloodstream of hospitalised patients, it may be difficult to treat and, sometimes, can be life threatening. Currently, new antibiotics are under development to treat such infections. Ceftriaxone, which is a third generation cephalosporin, is considered to be a risk factor for the colonisation and infection by vancomycin-resistant *Enterococcus*. Decreased use of cephalosporin can reduce the infection rate of vancomycin-resistant *Enterococcus* and its transmission in hospitals. In the United States, linezolid is commonly used to treat these infections. Individuals who are in greater risk of developing vancomycin-resistant *Enterococcus* include: individuals treated with vancomycin or

other antimicrobial agent for prolonged period, individuals who recently had abdomen or chest surgery, individuals who use catheters, elderly people, and people with weak immune system. This bacterium can cause different kinds of infection such as blood stream infections, urinary tract infections, catheter related infections, endocarditis, bacteremia, etc. And they are commonly found in healthcare settings [73-75].

Multidrug-resistant Pseudomonas aeruginosa: Pseudomonas aeruginosa can cause infections in various sites in the body including blood stream (bacteremia), lungs (pneumonia), skin and soft tissue (burns), abdomen, heart (endocarditis), brain (abscess, meningitis), eyes, urinary tract etc. [76]. *Pseudomonas aeruginosa* is a Gram-negative bacterium which can be found commonly in the environment. It is an opportunistic bacterium which mostly causes infections in patients who are immunocompromised. This bacterium continually tries to find new ways to resist and avoid antibiotics, which in turn might lead some of them to become multidrug resistant. The *Pseudomonas aeruginosa* have a range of mechanisms for adaptation, survival and resistance to multiple antibiotics, thus the infections caused by this bacterium can be life-threatening and it is appearing worldwide as a public health concern. There have also been reports of strains which are resistant to almost all classes of commonly used antibiotics. The bacterium can spread through contaminated water, soil or any other contaminated surface. To control the spread of this microbe, surfaces should be kept clean and disinfected, and proper sanitary measures should be followed. Infections occur a lot in hospitalised patients and especially in patients with weakened immune system. This microbe has been reported resistant to piperacillin, gentamicin, ciprofloxacin, aztreonam, cefepime, ceftazidime, etc. [77].

Multidrug-resistant Mycobacterium tuberculosis: Multidrug-resistant *Mycobacterium tuberculosis* is a serious threat as far as the current antibiotic resistance scenario is concerned. *Mycobacterium tuberculosis* causes tuberculosis (TB) which is a lung infection that can be life-threatening sometimes [78]. *Mycobacterium tuberculosis* is resistant to a set of antibiotics including isoniazid and rifampin which are often utilised as first-line agents usually spreads through the air by aerosol droplets. For people with immunocompromised immune systems, the risk of contracting TB is much higher than for people who are healthy. Despite the widespread use of vaccines and several antibiotics, the worldwide epidemic of TB still kills about 2 million people each year. To prevent getting affected, one should take necessary precautions so that one does not have prolonged contact with known TB patients. Besides, people working in healthcare settings should take measures as not to expose themselves to TB patients and should use personal protections such as masks, aside from the other administrative procedures [79].

Chapter 5

Mechanism of Antibiotic Resistance

There are two main ways for a bacterium to combat the effects of an antimicrobial agent— (i) to prevent the antibiotic from accessing target sites at a high concentration and (ii) to modify the target molecule an antimicrobial agent acts on [80].

(i) Preventing antibiotic from acting on target site: In order to disrupt the overall function of a bacterial cell, it is necessary for an antibiotic to have access to the bacterial cell in the first place where they can reach the target site easily. Usually, antibiotics pass bacterial outer membrane through porin channels; hence, some bacteria keep themselves protected by working on such channels— modifying their frequency, size, and selectivity (basically, decreasing permeability). This mechanism has been discovered in *Enterobacter aerogenes* and *Klebsiella spp.* against carbapenems (beta-lactam antibiotics), *Pseudomonas aeruginosa* against carbapenems, many gram-positive bacteria against amino glycosides, many gram-negative bacteria against quinolones, etc. [81].

Additionally, bacteria are able to produce complex machineries that are capable of thrusting antibiotics out of the bacterial cell. These complex machineries are more commonly known as "efflux pumps" which sit on bacterial membrane or cell wall [66]. For an antibiotic to be effective, it must be in high concentration inside a bacterial cell. However, with the help of efflux pumps, bacteria can extrude antibiotics, thus lowering the antibiotic concentration inside the bacterial cell [82][83]. An instance of this phenomenon can be seen in *E. coli* where an efflux system can pump tetracycline out of the bacterium's cytoplasm [84].

Another way of preventing antibiotic from entering the bacterial cell is the destruction of the antibiotic itself. There are bacterial enzymes capable of inactivating antibiotics— notably beta lactamases, which destroy the amide bond of the beta-lactam ring of penicillins. Through this way, bacteria become resistant to beta-lactam antibiotics [85].

In addition to that, chemical alterations in the antibiotic molecule can result in the development of antibiotic resistance. Bacteria can produce enzymes that will add different chemical groups in an antibiotic molecule, thus, hindering its bind-

ing to a target site in the bacterial cell. This acquired antibiotic resistance can be observed in both gram-negative and gram-positive bacteria [86]. An example of antibiotic modification can be the modification of chloramphenicol, an antibiotic which interacts with the peptidyl-transfer center of 50S ribosomal subunit and in turn inhibits protein synthesis. The enzymatic alternation of chloramphenicol depends on the production of chloramphenicol acetyl transferases— more commonly known as CATs [87].

(ii) Modification of the antimicrobial target site: Structural alternation of the target site in the bacterium can prohibit the antimicrobial agent from binding or acting on the target site. This structural alternation can be achieved by means of point mutations in the bacterial DNA [88]. At the same time, bacteria can even add a variety of chemical groups in the target site, rending an antibiotic ineffective against that bacterium [80][89]. Another example of structural variation can be vancomycin resistant bacteria producing a different kind of cell wall which has low affinity towards vancomycin [83][90].

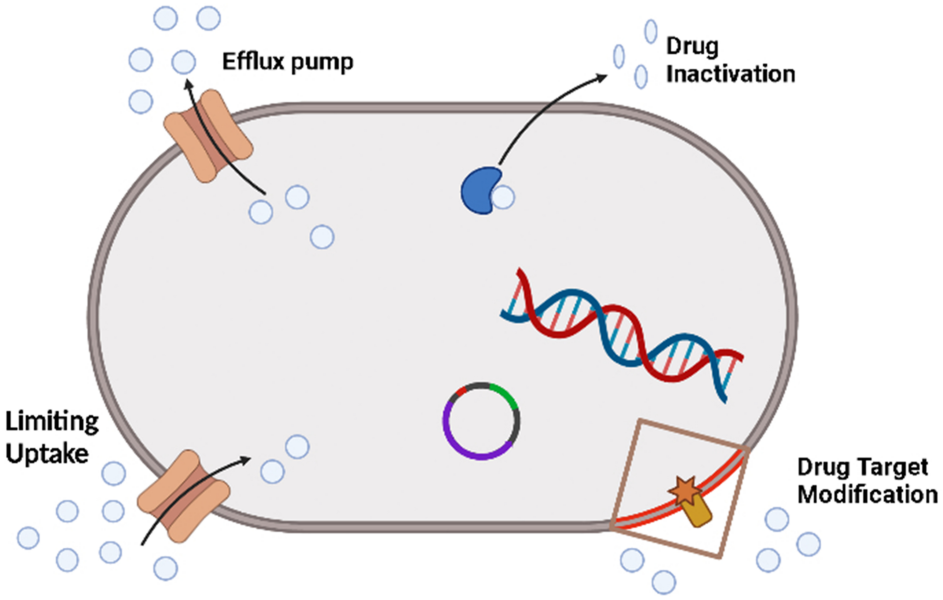


Figure 5.1: Mechanism of antibiotic resistance. This figure was generated with BioRender premium subscription.

Additionally, bacteria are capable of generating alternative proteins that can be used instead of the ones that are acted on by the antibiotics. For instance, *Staphylococcus aureus* obtains the mecA resistance gene and generates a new protein that is penicillin-bound. This protein is required for the synthesis of bacterial cell wall, and this is the one that is used as the target by beta-lactam antibiotics [91]. However, the trick here is that this new penicillin-binding protein has low affinity towards beta-lactam antibiotics; hence, beta-lactam antibiotics do not bind to them. As a result, the bacteria develop resistance against beta-lactam antibiotics [92].

Chapter 6

Management of Antibiotic Resistance

Attempts for the management of antibiotic resistance should be taken to slow down the spread of resistance, which is an important aim of antibiotic stewardship programs. The White House created a National Action Plan for Combatting Antibiotic Resistance. Some of the recommendations that they provided include the development of new antibiotics, production of new effective vaccines, and proper identification of drug-resistant bacteria by means of diagnostic tests. At the same time, public health professionals are advised to monitor the rate of antibiotic resistance. Widespread use of antibiotics unnecessarily in hospitals and agricultural industry needs to be stopped as soon possible. The World Health Organization (WHO) also launched a global action plan to tackle antimicrobial resistance in 2015. It was based on a one health approach prioritizing the importance of the interdependence of human health, animal health and the environment. The five objectives of the global action plan are- to improve awareness on AMR, conduct surveillance and research, reduce infection rates by taking proper infection control measures, optimize the use of antibiotics and develop sustainable investment case for new medicines, vaccines or diagnostic tools [93]. Infections are one of the main reasons of high mortality rates. But, some of the studies have suggested that reducing the use of antibiotics to combat bacterial resistance is achievable in human populations, both in healthcare settings and community level [94]. However, due to its immense need to treat bacterial infections, reducing the antibiotic use to completely zero would be neither feasible nor beneficial. The aim should be to keep a balance between the minimisation of the resistance development and maximisation of the patient outcome [95].

Another viable way of managing antibiotic resistance would be stimulating pharmacological studies which can bring about comprehensive ideas and possibilities of halting resistance emergence and limit its transmission and development [96]. The antibiotics under development should be evaluated for potential chances of not being effective against a bacterium and compared with the current antibiotic classes to predict and generate better therapeutic schemes [95][97]. Food and Drug Administration (FDA) is also trying to take a number of initiatives to fight antibiotic resistance, such as— developing and approving new set of antibiotics, labelling regulations for the sake of appropriate use of antibiotics and promoting public awareness. These steps will encourage people to take antibiotics only when clinically required

[98].

In recent times different initiatives have been taken to increase awareness i.e., celebrating World Antibiotic Awareness Week, holding advocacy meetings of key stakeholders, and arranging awareness-raising materials to sub-districts, districts, and tertiary level health facilities. But most of these activities are arranged in large cities, and the posters are made when only necessary so the awareness among the overall population still remains poor. Moreover, the implementation of different policies and strategies to tackle antibiotic resistance remains a challenge due to inadequacy of resources, insufficient knowledge and lack of national surveillance [99-101].

One crucial way to prevent antibiotic resistance is to let physicians decide when to use antibiotics. Antibiotics combat bacteria, not viruses; hence, utilising antibiotics against viral infection will not help in treating that infection, will not help in minimising its spread, will not make a person feel better, and overall will not have any tangible benefit. On the contrary, it might result in detrimental side effects within the individual consuming that antibiotic [98][102].

In some cases, physicians themselves prescribe antibiotics unnecessarily for viral infections such as common colds, bronchitis, and various ear infections and even sinusitis. Antibiotics should not be taken unless you are absolutely certain that you need them. Through this way, reduction of antibiotic use can play a significant role in preventing antibiotic resistance [103]. Depending on the type and amount of infection and antibiotic involved, Bacteria have developed various strategies to improve their resistance. Thus, antibiotic doses should be taken as prescribed and should not be skipped or taken as per individual wish. The consumption of antibiotic should not be stopped even if one starts feeling better because if the infection is not wiped out completely, chances are high that those bacteria are likely to become antibiotic resistant [104].

Another way of combating antibiotic resistance would be to get vaccinated. Vaccination can give protection against many diseases that are cured with antibiotics. For example, tetanus and whopping cough. A vaccine can help to decrease the rate of infections, decrease the use of antibiotics, and can extend the effectiveness of active drugs [105]. On top of that, hospital hygiene should be maintained strictly because drug-resistant bacteria are commonly found in healthcare settings. It should be monitored whether the health care professionals and nurses are frequently washing their hands. At the same time, surgical equipments need to be sterilised after using every time [106].

Lastly, antibiotics should never be shared with others and leftover medications must not be consumed. It should be made sure that one is getting the right dosage of antibiotic, for the right amount of time, prescribed by a healthcare professional. To minimize the spread of resistance, a combined effort needs to be taken by all levels of the society. The healthcare professionals, health industries, agriculture sectors, policy makers and each individual person must all play their own part to help in limiting the spread of resistance. Individuals must always follow their physician's advice when taking antibiotics, and must never demand antibiotics if not necessary.

Everyone should maintain basic hygiene to prevent unnecessary infections. Besides, people must be conscious and eager to educate themselves on how to use antibiotics and limit the spread of resistance. Healthcare industries must dispose of the wastes cautiously so that it does not spread in the environment. Investments should be done for the research and development of new antibiotics. In the agriculture sector, antibiotics should not be used as growth promoters and hygienic practices should be maintained. Biosecurity should be improved and all animals should be vaccinated to prevent diseases or infections. Policy makers should ensure an effective national action plan to tackle antibiotic resistance. They should strengthen policies and implement various infection prevention and control measures. These are some of the important considerations we need to make in order to minimise antibiotic resistance [107].

Chapter 7

Antibiotic Resistance Scenario in South Asian Countries

7.1 Bangladesh

Bangladesh is a developing country situated on the Bay of Bengal. Due to the poverty and unsatisfactory developmental works, the healthcare system of Bangladesh is also full of flaws. Antibiotic resistance has been found to be prevalent in Bangladesh due to its misuse, overprescription, and lack of awareness. A study from a slum in Dhaka showed that proper awareness campaigns and education have the chance to improve rational use of antimicrobials and reduce the indiscriminate uses [108]. Studies also showed that more than 1 or 2 antimicrobials are being prescribed at a time to patients every now and then in Bangladesh. Due to lack of testing facilities, there have been reports of prescribing antibiotics to patients without legitimate laboratory tests as well [109-112]. More than 50% of the patients used to stop taking antimicrobials as soon as they started to see symptoms getting reduced. Another recent study showed that each child in Dhaka who are under 2 years of age has taken over 10 antibiotic courses per year on average, which is a very high rate compared to global standards [113]. A study showed also that the prevalence of taking medicine without consultation was quite high and common for dysentery, diarrhea and food poisoning (36%), cough and fever (28%); and presumed infection of some sort (13%). Other than that, one other major reason this practice was due to poverty [99][100][114-116]. A surveillance study conducted by Institute of Epidemiology, Disease Control and Research (IEDCR) showed that 6 of the most critical medicines listed by World Health Organisation (WHO) have become ineffective against a lot of bacteria. 10 of the bacteria were tested against 21 antibiotics, among which it was found that 9% of the bacteria were resistant to all available antibiotics [117].

Another study was conducted to generate data on the current antibiotic resistance scenario in Bangladesh where 27,069 clinical samples were collected, out of which 22,119 were gram negative bacteria (namely *E. coli*, *Klebsiella spp.*, *Salmonella spp.*, *Pseudomonas spp.*, and *Acinetobacter spp.*) and 4353 were gram negative bacteria. Gram negative bacteria showed resistance to a plethora of antibiotics, including ciprofloxacin (56-90.1%), third generation cephalosporin (61.6%-94.9%), cotrimoxazole (58-80.3%), tazobactum + piperacillin (20.8-81.4%), imipenem (3-84%), aminoglycosides (10.8-88.6%), nitrofurantoin (14.3-91.7%), and colistin (2.2-16.4%). 92%

Name	2017	2018	2019	2020	2021
Ciprofloxacin	57%	68%	70%	68%	67%
Azithromycin	66%	81%	79%	82%	50%
Cefepime	57%	60%	60%	57%	61%
Ceftazidime	62%	68%	67%	61%	70%
Ceftriaxone	52%	63%	67%	60%	65%
Cefixime	52%	86%	92%	73%	58%

Table 7.1: 5 year resistant trends of critically important drugs [117]

of *Salmonella* was nalidixic acid resistant whereas carbapenem resistant *Enterobacteriaceae* was 9.8%. There was no case of vancomycin resistance in *Staphylococcus aureus* and *Enterococci*, however, *Enterococci* showed significant resistance to gentamicin [118].

According to research, an increased level of antibiotic resistant bacterial infections has been found in children under five years in Bangladesh with pneumonia. Based on this study, approximately 18% of bacterial samples isolated from children with pneumonia showcased resistance to all commonly utilised antibiotics [119]. Unsafe and unhygienic drinking water, widespread availability of over-the-counter antibiotics, and significant issues related with sanitation have been deemed as some of the reasons behind antibiotic resistance in Bangladesh. The spread of antimicrobial resistance can be reduced to some extent by making safe drinking water available for people and taking steps to make various settings clean and sanitary. Besides, proper implementation of laws regarding the availability and usage of antibiotics should be also be carried out. Based on the data generated from 4007 children admitted to the Dhaka Hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) within 2014 and 2017 timeframe, it has been found that there were resistance cases among the 108 (6%) positive cases (1814 children provided sample for bacterial infection check-up). Among the 108 positive cases, 20 of them (18%) showed resistance to all the commonly used antibiotics like ceftriaxone, gentamicin, ciprofloxacin, and ampicillin [120]. A few other reasons for such high resistance scenario in developing countries like Bangladesh would be antibiotic use in agriculture and poultry industry, poor drug quality, insufficient surveillance studies, and poverty [121][122]. A study conducted in Chittagong in 2003 showed how patients with typhoid were not responding to second-line antimicrobial therapy (ciprofloxacin). Due to the existing resistance, first-line antimicrobial therapy was not even performed or applied [123].

Another research found that there was 60% and 70% prevalence of *Salmonella* species in different street foods in Chittagong and multi-drug resistant *Salmonella* in food items tested. 24% of goat farmers were aware of antibiotics but had no prior knowledge regarding antimicrobial resistance. It is high time that the use of antibiotics in agricultural settings should be monitored properly. Antibiotics are used in livestock to increase their growth, but this is extremely harmful and leads to the spread of AMR. The government and policy makers must be vigilant towards this and stop the unnecessary use of antibiotics in the agricultural sector. The farmers can also be made aware of this misuse. Besides, vaccinations or natural immunity boost-

ers can also help a lot to prevent common diseases in the first place [124][125]. The hospitals of Dhaka city discharge untreated medical wastes in nearby water bodies that breed high-levels of resistant *E. coli*. This environmental contamination contributes to increasing the presence of multi-drug resistant *Enterococcus* and Shiga Toxin- producing *E. coli*. There are laws regarding the discharge of medical wastes that need to be put into effect properly. Hospitals and other healthcare industries should abide by those laws and not release any untreated medical waste into the environment [124][126]. There are several factors that lead to the growth and widespread of resistant and nonresistant bacteria. Among them the most notable is the dense population accompanied by the tropical and moist weather [127]. Some other aspects that contributed to the prevalence of antibiotic resistance in Bangladesh is the irrational prescription of antibiotics by doctors and a tendency among patients to take self-medication. These can be limited to some extent by educating the general people regarding the use and misuse of antibiotics. Doctors should also be held liable if they suggest unnecessary antibiotics to any patient and should be given basic training on these [128][129]. An in-depth systematic study was conducted in Bangladesh where 46 literature reviews were included, and based on that, a comprehensive data was generated. Through this study, it was found that *E. coli* showed significant resistance to common antibiotics like ampicillin (MR 94.6%, IQR 85.9–100%), amoxiclav (MR 67.1%, IQR 52–85.5%), ciprofloxacin (MR 65.2%, IQR 52.4–80.5%), and co-trimoxazole (MR 72%, IQR 56.6–82.2%); *Klebsiella spp.* showed high resistance to ampicillin (MR 100%, IQR 100–100%), amoxiclav (MR 58%, IQR 14.3–84.6%), ciprofloxacin (MR 67.4%, IQR 43.6–80.9%), and co-trimoxazole (MR 72.7%, IQR 48–78.9%); and *Staphylococcus aureus* showcased significant resistance to penicillin (MR 89.7%, IQR 58.2–96.5%), ampicillin (MR 83.8%, IQR 74–93.1%), co-trimoxazole (MR 43.2%, IQR 31–65%), and amoxicillin (MR 64.3%, IQR 44.7–84.9%). Resistance of *Klebsiella spp.* to cefotaxime, ceftazidime, and ceftriaxone was 97.8%, 82.5%, and 78% whereas *E. coli* showed 55.4%, 65.3%, and 59% resistance to these antibiotics. *Enterococcus* spp. showed high resistance to co-trimoxazole (MR 87.5%, IQR 74.2–100%) and ciprofloxacin (MR 66%, IQR 64.3–87.7%) whereas the resistance to co-trimoxazole and ciprofloxacin in *Pseudomonas spp.* were (MR 86.6%, IQR 34.1–97.8%) and (MR 59.3% IQR 22–87.3%) respectively. *Acinetobacter spp.* exhibited high resistance (more than 55%) to all of the tested antibiotics except imipenem. The study also found that most of the first-line antibiotics are ineffective against most of the common urinary tract bacterial infections, and alternative drugs like fosfomycin, tigecycline, and nitrofurantoin have been suggested to combat this resistance scenario in Bangladesh. The government of Bangladesh has already taken various steps such as, monitoring the sale of drugs without prescription, setting a standard treatment guideline for antibiotics and conducting antibiotic stewardship programs to combat antimicrobial resistance [130].

7.2 Pakistan

Pakistan is also a developing country and it is the fifth-most populous country in the world. Over the last 16 years, the use of antibiotics in Pakistan has increased by 65%. This overuse of antibiotics nationwide has increased antimicrobial resistance

cases, resulting in severe conditions and even deaths. According to a systematic research based on 93 studies in Pakistan, *E. coli* was found to be showing significant resistance to first-line antibiotics in 28 (30.11%) studies. Amongst the *Staphylococcus aureus* cases, 49% of them were reported to be methicillin. The most widespread resistant genes were found to be blaTEM (78.94% in *Shigella spp.*) and blaNDM-1 (32.75% in *Klebsiella spp.*), followed by VanA (45.53% in *Enterococcus spp.*, mcr-1 (1.61% in Acinetobacter), and blaKPC-2 (31.67% in *E. coli*). Lack of management, unsafe and unhygienic environment, unskilled healthcare professionals, misuse and abuse of antibiotics are some of the key factors that are responsible for antibiotic resistance as previously mentioned, and the scenario is no different in Pakistan [131].

South Asia has been deemed as the core region for antimicrobial resistance. It has been anticipated that approximately 70% of the antimicrobial resistance is ascending in the Asia region. However, in many of these countries, the public health infrastructures to control the problem are very poor [132]. Pakistan is one of the South Asian countries, and hence, chances are high that antibiotic resistance scenario will only worsen in this nation. Already multi-drug resistant (MDR) and extremely drug resistant (XDR) bacteria have been found in Pakistan over the last few years. *Enterobacteriaceae* has become more resistant to antibiotics like quinolones over the last decade [133]. An XDR outbreak has been witnessed in the year of 2016 when *Salmonella* was found to be 100% resistant to fluoroquinolones [134].

As mentioned earlier, misuse and abuse of antibiotics as well as unqualified staff contribute to the development of antibiotic resistance in Pakistan. Along with that, self-medication, overprescription of antibiotics by physicians, irregular and incomplete doses of antibiotics, and non-entity of culture sensitivity tests are also causing resistance in this country [135].

Again, based on the systematic study mentioned earlier, it has been found that out of 93 studies, *E. coli* (found in 28 studies) showed resistance to penicillin, cephradine, ampicillin, and amoxicillin whereas *Klebsiella spp.* (found in 13 studies) showed resistance to cefaclor and cefotaxime. Additionally, *Proteus spp.* (found in 2 studies) were resistant to cefotaxime, ceftriaxone, and tobramycin whereas *Salmonella spp.* (found in 10 studies) showed resistance to ciprofloxacin. *Shigella spp.* (found in 4 studies) showed resistance to co-trimoxazole and ampicillin; *Helicobacter pylori.* (found in 3 studies) showed resistance to metronidazole and *Acinetobacter spp.* (found in 15 studies) showed resistance to all tested antibiotics except tigecycline and colistin. *Pseudomonas spp.* (found in 13 studies) showed resistance to ceftazidime and aztreonam; *Staphylococcus aureus* (found in 20 studies) showed resistance to penicillin, and finally, *Enterococcus spp.* (found in 4 studies) were found to be highly resistant to oxacillin. So, it can be said that antibiotic resistance is very much prevalent in Pakistan. Steps should be taken to lessen the spread of antimicrobial resistance here. Laws should be implemented strictly to control the availability and use of antibiotics. Besides, hygiene and sanitation should be maintained properly. Along with that, the general public must be educated on the use and misuse of antibiotics. Healthcare professionals should also be trained on those and should be accountable on whatever they prescribe a patient [131][136].

7.3 Sri Lanka

Sri Lanka is a developing country having a multinational population. It is an island nation which is ravaged by natural calamities every so often. Antibiotic resistance has become a public health threat over the years in Sri Lanka. The emergence of multi-drug resistant *Acinetobacter spp.* has become a significant problem in the clinical setting. Research performed at the National Hospital of Sri Lanka (NHSL) in 2014 showcased that all *Acinetobacter spp.* as well as 25% of the coliforms which cause Ventilator Associated Pneumonia (VAP) were multi-drug resistant. Moreover, the study also found that all the coliform isolates showed resistance to third-generation cephalosporins [137].

Another study conducted during 2009 at Teaching Hospital, Anuradhapura showed that 70% of the *Acinetobacter spp.* isolated were resistant to two or more antibiotics and the bacteria was regarded as multi-drug resistant by the authors. While none of the *Acinetobacter spp.* isolated showed resistance to colistin, the intermediate resistance rate for cefotaxime, ceftazidime, imipenem, and cefoperazone sulbactum found in this research were 90%, 73.3%, 70%, and 50% respectively [138]. Additionally, extended spectrum beta lactamase (ESBL) generating *Enterobacteriaceae* have been prevalent among the health care facilities in Sri Lanka which are contributing to the development of antibiotic resistance [139].

The prevalence of carbapenem resistant *Enterobacteriaceae* (CRE) can also be observed in the Sri Lankan health care settings as of late which is a matter of serious concern. The emergence of carbapenamese generating coliforms from the blood samples and respiratory specimens obtained at the NHSL and North Colombo Teaching Hospital were 7.9% and 0% respectively [140][141].

In addition to Gram negative bacteria becoming resistant to antibiotics, resistance cases in Gram-positive bacteria have also seen a rise. Based on the data obtained from a private hospital in Sri Lanka within 2013 and 2014 timeframe, the identified rate of multi-drug resistant *Staphylococcus aureus* (MRSA) was 42% (n=156) [142]. As mentioned earlier, ESBL producers have become a problem in the health care sector and now research performed at a private hospital in the Western province showed that the rate of ESBL producing *Enterobacteriaceae* was 26% (n=386) [143]. On top of that, there are cases reported of *Salmonella spp.* showing significant resistance in the community level. Based on the data generated at the National Reference Laboratory of the Medical Research Institute in 2013, *Salmonella spp.* showed 66.6% resistance to ciprofloxacin [144].

Despite the legal provisions, antibiotics are frequently used in animal husbandry in Sri Lanka. The use of antibiotics in animal feed has led to the prevalence of resistant microbes in the farm animals. This, along with other common reasons of antibiotic resistance mentioned earlier in this research have contributed to the development of antibiotic resistance in Sri Lanka. The government and policy makers should implement the laws strictly in the agricultural setting so that everybody is bound to follow them. The laws regarding antibiotic use and availability should also be implemented properly. Moreover, awareness campaigns should be done for the

mass people so that everybody can learn about the general proper use of antibiotics [145][146].

7.4 Nepal

Nepal is a landlocked country situated on the southern part of the Himalayas. It is a poor and developing country having a weak infrastructure. In recent times, Nepal has seen significant amount of antibiotic resistance cases, especially because of misuse and abuse of antimicrobial agents among the community and healthcare. Some reports suggest that pathogenic bacteria are becoming highly resistant to most of the first-line and some of the second-line antibiotics. A study was conducted consisting of 516 individuals, among which 324 of them were patients, 87 of them were clinicians, 33 of them were private drug dispensers, and the rest (77 of them) were livestock and poultry farmers, public health care facilities, and laboratories. The study showed that 139 of the patients thought antibiotics can cure viral fever whereas 256 of the patients bought antibiotics over-the-counter (OTC). Also, the study showed that adult patients were more informed about antibiotic resistance [147].

Nepal suffers from a plethora of bacterial infections including enteric fever, urinary tract infections, respiratory tract infections, etc. And studies have shown that most of these bacteria are becoming drug/multi-drug resistant. The high rates of infectious diseases, poverty, weak governance and health systems, and lack of awareness remain the major challenges to fight against antimicrobial resistance [148][149]. Just like in other South Asian countries, overprescription and over the counter use of antibiotics have contributed to the development of antibiotic resistance in Nepal. These can be lessened by some extent by arranging campaigns for the general people and trying to educate them on the misuse of antibiotics. Besides, healthcare facilities need to be upgraded and made more widely available for the people. Sanitation and hygienic measures should also be ensured in all healthcare settings to prevent the spread of infections in the settings [150].

Referring to the study consisting of 516 individuals, 114 of the patients thought that antibiotics were utilised for curing flu and common cold whereas 56 of the patients thought they were used for sore throat, and 72 of the patients thought that antimicrobial agents can be used for the treatment of headache, and skin or wound infection. 272 of the patients had no idea what antibiotic resistance was and 52 of the patients had various terms for antimicrobial agents, hence, showcasing the lack of awareness. Amongst the 33 private drug dispensers, 6 of them believed that antibiotics could treat all sorts of diseases. Some key factors come into play for this situation such as misuse and abuse of antibiotics, lack of awareness, lack of proper policy which will provide guidance for empirical therapy, pressure within healthcare facilities, lack of appropriate diagnostic facilities, and other socioeconomic factors [147][151].

A study was conducted between 2014 and 2015 at the intensive care unit of Tribhuvan University of Teaching Hospital, Kathmandu, Nepal where 491 clinical sam-

ples were taken. Among the 491 samples, 149 samples obtained from 135 patients potentially infected with healthcare associated infections (HCAI) were found with bacterial growth. Samples were taken from patients who might be potentially infected with hospital acquired pneumonia (79/491), surgical site infections (23/491), bloodstream infections (28/491), and urinary tract infections (19/491). *Acinetobacter spp.*, *Klebsiella spp.*, and *E. coli* were some of the prevalent bacteria. Almost 96% of the Gram-negative bacteria responsible for nosocomial infections were multidrug resistant whereas 43.3% of them were extremely drug resistant (XDR). Beta lactamase generating bacteria were high too (ESBL; 43.7% AmpC; 27.5%, Metallo beta lactamases MBL; 50.2%, and *Klebsiella pneumoniae* carbapenamese KPC; 4.2%). The research also showed *Enterobacteriaceae* being highly resistant to third-generation cephalosporins, *Klebsiella spp.* being resistant to ciprofloxacin (86.4%), gentamicin (83.7%), piperacillin tazobactum (81.0%), and imipenem (48.6%). *E. coli* showed identical rates of resistance apart from carbapenems (19.3% resistance) whereas *Citrobacter spp.* showed resistance to cephalosporins, fluoroquinolones, and aminoglycosides [152].

7.5 India

India is the second-most populous country, and the seventh largest country in the world. It is also one of the fastest developing countries in the world. The geography of India is very diverse and has great physical variations. Just like in other South Asian countries, antibiotic resistance is significantly prevalent in India as well [153]. Over-the-counter antibiotic sales, especially in case of carbapenems, has significantly contributed to the development of carbapenem resistance among the gram-negative bacteria [154]. According to a research, community acquired *E. coli* (n=1,815) showed significant resistance to a plethora of antibiotics, including naladixic acid, co-trimoxazole, and ampicillin (73%, 59%, and 75% respectively) within the 2004 to 2007 timeframe [155]. Between 2008 and 2013, *E. coli* resistance to third-generation cephalosporins have seen a drastic rise from 70% to 83% whereas resistance to fluoroquinolones rose from 78% to 85%. Carbapenem resistance was 10% in 2008 where it slightly increased to 13% on 2013 [156].

In case of *Klebsiella pneumoniae* isolates, there was a significant increase in fluoroquinolone resistance from 57% to 73%, and based on a study in New Delhi, carbapenem resistance became extremely high from 2002 to 2009, generating a whopping 52% resistance in 2009 compared to 2% in 2002 [156][157]. *Salmonella typhi* isolates showed a high resistance to fluoroquinolones as well (28% in 2014 compared to 8% in 2008). Although ampicillin and co-trimoxazole resistance decreased for *Salmonella typhi* due to low consumption of these drugs, resistance to naladixic acid is increasing slowly (20%-30%). It was also found that *Enterococcus faecium* was 11% resistant to vancomycin [158].

Methicillin-resistant *Staphylococcus aureus* is also increasing, showing a resistance rate of 54.8% (ranging between 32% and 80%) [159]. Some of the key factors revolving around the current antibiotic resistance scenario in India include inappropriate prescription of antibiotics by physicians (they receive compensation from

medicine companies and pharmaceutical employees), poor monitoring of infection control within the hospitals, and access to over-the-counter antibiotics. These challenges can be tackled by implementing strict laws on the availability, dispensing and usage of antibiotics. Moreover, there should be laws on infection control and sanitation in hospitals [160-162]. SMART study conducted in Asia-Pacific region showed a very high prevalence of extended spectrum beta lactamases from Indian region. The rate of extended beta spectrum positive beta lactamases for *E. coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* were 79.0%, 69.4%, and 100%, respectively in India [163].

In another study, it was found that more than half of the healthcare acquired infections are carbapenem resistant, with a deadly case fatality rate of approximately 50%. People should be made aware regarding the inappropriate use of antibiotics. Sanitation and cleanliness in hospitals should be maintained strictly so that there is less chance of post-operative infections. The CDC along with the health ministry of India is working together to slow the spread of antibiotic resistance. They are working hard to develop lab facilities to detect pathogenic antibiotic resistance strains, using standardized surveillance to monitor infections, developing strategies to prevent infections, offering various training and maintaining antibiotic stewardship programs [164].

7.6 Afghanistan, Bhutan, and Maldives

Afghanistan is a landlocked country, having very underdeveloped infrastructure. It is one of the least developed countries. The country has been going through antimicrobial resistance problem for years. Based on a study, out of 105 *Staphylococcus aureus* strains obtained from various samples, 59 of them (56.2%) were methicillin-resistant. In addition to that, almost all of the strains (104; 99.05%) were resistant to penicillin G. Another interesting find from this study was that 74.6% of the methicillin-resistant strains were also resistant to azithromycin. Studies also showed a widespread use of ceftriaxone and other similar broad spectrum antibiotics in clinical settings and increased resistance towards cephalosporins of third generation. According to some reports, higher antibiotic doses or new drug combinations were used in some hospitals for different infections as the common antibiotics were not working. But these lead to severe side effects and many people even suffered from repeat or prolonged infections [165].

Another research conducted between 2007 and 2008 showed the prevalence of antibiotic resistant bacteria as well. 411 bacterial isolates were obtained from 266 patients where 211 of them were multi-drug resistant (51%). 70% of the gram-negative bacteria isolated in this study were found to be multi-drug resistant, and the most common ones were *Acinetobacter* (90% multi-drug resistant), *Escherichia coli* (53% multi-drug resistant), and *Klebsiella* (63% multi-drug resistant). Like other countries, Afghanistan also lacks proper health education about the use of antibiotics. Easy availability and misuse also add to the main causes of antibiotic resistance here. The government and policy makers should implement strict protocols on the availability and use of antibiotics. Moreover, various health campaigns and awareness

programs will also go a long way to spread the knowledge among general people [166].

Bhutan is a small and mountainous landlocked country situated on the eastern edge of the Himalayas. It is also one of the least developed countries. Antibiotic resistance is common in Bhutan as well. There is a high prevalence of Helicobacter pylori infection and gastric cancer-related mortality in Bhutan. A study conducting 1178 dyspeptic Bhutanese patients (664 females and 514 males) showed 66.2% of *Helicobacter pylori* infection. It was found that Punakha had the highest prevalence of *Helicobacter pylori* infection with a rate of 85.6%, followed by Wangdue (75.4%), whereas Haa showcased the lowest prevalence. Among the 1178 patients, 357 underwent antibiotic susceptibility testing. 65 out of 357 patients had no antimicrobial resistance, and there was no amoxicillin resistance found in this study. Metronidazole-resistant strains showed the highest resistance rate (81%), followed by levofloxacin (8.1%), clarithromycin (2%), and tetracycline (0.6%). More than one resistant strain was found in 29 of the patients (8.1%). For example, a dual resistance of metronidazole and levofloxacin was found to be the most prevalent (6.2%). The key factors of antibiotic resistance in Bhutan are easy availability of OTC antibiotics in the dispensaries and general misunderstanding about antibiotic use along with lack of awareness and health education. These challenges can be overcome by strict rules and laws on dispensing and prescribing antibiotics. Besides, health education programs and awareness campaigns will also help the general people learn about the basic and proper use of antibiotics [167].

Maldives is an island country, consisting of a chain of about 1200 coral or sand islands and is located in the Indian Ocean. It is also one of the developing countries. The resistance phenomenon can be seen in Maldives as well. In a study consisting of 471 culture-positive samples where 278 (58%) of them were males and 193 (41%) of them were females, significant prevalence of antibiotic resistance was observed. Among the 471 samples, 338 (71.8%) were gram-positive bacteria whereas 133 of them (28.2%) were gram-negative bacteria. It was observed that coagulase-negative *Staphylococcus* showcased a high rate of resistance to antibiotics like ampicillin, cephalexin, cefotaxime, and gentamicin. Evidences of *Klebsiella spp.* showing significant resistance to ampicillin, cephalexin, piperacillin tazobactum, cefuroxime, cefotaxime, ceftriaxone, cefepime, and ciprofloxacin; *E. coli* showing high resistance to ampicillin, cephalexin, cefuroxime, cefotaxime, ceftazidime, ceftriaxone, and ciprofloxacin; and *Acinetobacter spp.* showing a high resistance to cephalosporins cefotaxime, ceftriaxone, and aminoglycoside gentamicin (as well as fluoroquinolone and ciprofloxacin). Again, the main challenges here include lack of awareness, lack of health education and misuse and easy availability of antibiotics. Even though there are healthcare centers on most of the islands, they mostly provide the basic services. There is a lack of healthcare facilities and unsanitary conditions in these settings which makes people more prone to infections. People have to go to the capital to receive better treatment and facilities in hospitals. For serious patients this is very challenging and might lead to further deterioration of health even before reaching the hospital. These can be tackled by mass awareness campaigns and making health education available easily to all classes of people. The government can also try to increase upgraded healthcare facilities having hygienic conditions, and also implement strict laws on the use and availability of antibiotics. However, it should also be brought

into light that the government of Maldives has made significant advances in the area of infection prevention and control. Besides, Maldives has also made significant development in making clean water available and providing sanitation and vaccination campaigns [168].

Chapter 8

Future Scenario of Antibiotic Resistance

There are additional circumstances besides the obvious health hazards associated with increased prevalence of resistance genes among human pathogens. Many resistance genes have small fitness costs for their hosts that are transferred between bacteria and between plasmids [169]. Studies on the selection of antibiotic resistance can help us to predict the evolution and appearance of resistant bacterial populations. Low-cost resistance genes can evolve with response to more efficient variants of similar antibiotics. This can be observed in cephalosporins and the TEM beta-lactamases and tetracycline resistance genes [170][171]. Furthermore, there are reports of resistance genes being against several different antibiotics that can get accumulated. But such co-localization is more unlikely to emerge [172].

Thus, from observations among clinical isolates, it was observed that we might see the rate of bacteria with plasmid-borne multi-resistance phenotypes increasing, which would increase more with time [173]. Globally the use of antibiotics has been rising. Using biocides and metals as antibacterial also adds to the promotion of multi-drug resistance through co-selection. This can occur when the resistance gene to both the antibiotic and metal resides in the same cell, or a single resistance mechanism tends to both the antibiotic and metal leading to co-selection of the bacterial strain [174][175]. Exposure to antibiotics increases chances of mutation in bacteria [176]. Again, environmental bacteria being exposed to different levels of antibiotics is therefore likely to generate variants with genetic change. So, these populations of bacteria with high mutations acquire beneficial mutations [177]. In addition to that, when antibiotics are released to the environment with bacteria, this creates the chance of increasing bacterial evolution and generation of more complex genetic changes. Besides, the over use of antibiotics results in strains that are increasingly drug resistant. This contributes to their ability to evolve and adapt faster than the development of new antibiotics to combat them [178-180].

It is quite not possible to predict certainly what results this may have for bacterial pangenomes. Integrons and other mobile genetic elements allow bacteria to adapt faster to new niches [180]. Therefore, these genes help bacteria to survive extreme abiotic conditions, toxicants, use primary carbon sources, fight other microbes, adjust to different surfaces, re-engineer their ecosystems, and allow formation of highly

durable spores [172][181].

The achievements of modern medicine are at risk due to antibiotic resistance. Medical procedures such as organ transplantations, chemotherapy, caesarean sections and other surgeries are becoming more dangerous due to the risk of post operative infections without effective antibiotics. From health perspectives, selection by antibiotics seem like a more favorable situation where strains with attributes are beneficial for colonization and invasion of the human host. This could include mobilization of genes involved in virulence, transmission and pathogenicity, but also genes that increase competitive ability with human commensals. This shows a bleak future in which human pathogens would become untreatable by most antibiotics. As a result, causing them to become more aggressive and spread more easily between humans. We might already be witnessing a foretaste of what is to come, with hypervirulent *Klebsiella pneumoniae* resistant to all antibiotics tested recently appearing in Chinese hospitals [182]. It is thus important to understand not only the risks for resistance transmission, but also the evolutionary consequences of antibiotic releases into the environment. There is substantial uncertainty about the unexpected emergence and spread of new resistance genes for future economic estimates and for evaluating models for antimicrobial resistance. There is limitation in the models to predict future resistance rates because of the complexities of antimicrobial resistance. Even if more extensive surveillance data were found regarding the usage and resistance of antibiotics, there are still many social and behavioral considerations such as, compliance of the patient to antibiotics and methods of infection control. These are unpredictable and thus bring uncertainty into the models of antimicrobial resistance. However, some broad assumptions can be made regarding the probable factors contributing to future resistance rates. The antimicrobial resistance crisis is an expected and predictable outcome of how we have been using antibiotics since their discovery. If we continue to use them in the same way as we have been using them for the last 80 years, the future of this problem is simple to predict. Resistance will continue to emerge and the treatment options will continue to decline. To have a long-term availability of an effective antimicrobial, we need to be innovative and challenge the long prevailing assumptions. If we do not take immediate steps now, we might be heading into a post-antibiotic era, where even common infections and minor injuries can once again increase the mortality rates. The discovery and implementation of new antibiotics are challenging in itself. But what is equally as challenging, is protecting the effectiveness of those antibiotics for a longer period of time. We should stop repeating the same mistakes and protect these new antibiotics by not misusing them. Until and unless we can bring changes in the way our society deals with the discovery, development, use and protection of these life-saving drugs, the problem of antimicrobial resistance will continue to get worse. Due to the rapid scientific advances for diagnosis, research and new policies aiming at the psychology behind the misuse of antibiotics, the current high prevalence of inappropriate use could be lessened drastically over the coming decade. The eventual success might depend on a complete reevaluation of our relationship with microbes. The final aim would be to stop looking for their eradication, and rather trying to achieve a harmonious coexistence. If we want to succeed in reversing the antimicrobial resistance crisis, we not only need newly developed drugs, but we also have to improve how we use them [172][183].

Chapter 9

Summary & Concluding Remarks

Antibiotic resistance has become a serious threat worldwide, especially in the South Asian countries. With each year passing by, the rate of resistance to all the available antibiotics is significantly increasing. And it goes without saying that if this rate continues, the future generations are in for a major problem as scientists and clinicians have to rethink their strategy to combat the commonly observed bacterial infections. In this article, we tried to demonstrate the causes of antibiotic resistance, especially in the South Asian nations that were our core focus. We have shown that lack of awareness, overprescription of antibiotics, abuse and misuse of antibiotics, poor diagnosis, lack of hygiene, poor drug quality, agricultural and poultry use are some of the notable causes of antibiotic resistance.

Along with that, mutation and gene transfer also play a significant role in contributing to the development of antibiotic resistance. We have also explored the effects antibiotic resistance might have on the current global scenario where we have shown how it can have an impact on morbidity and mortality. At the same time, we have shown how antibiotic resistance might affect the economy and overall spread of bacterial infections. In addition to that, our article also provided insights into the mechanism of antibiotic resistance; how bacteria become resistant to antibiotics. We have also discussed about the bacteria which are currently resistant to various drugs and are very much prevalent in the environment.

Our article also sheds some light on the management of antibiotic resistance where we mentioned how development of new drugs, restricting improper use of antibiotics, completing doses, creating awareness policies, and general action plans can mitigate the impact of antibiotic resistance. Finally, we talked about the current antibiotic resistance scenario in South Asian nations where we found that antibiotic resistance is very much prevalent in every single South Asian country.

In our current times, antibiotic resistance is not an ignored subject anymore and the knowledge that we have is fragmentary. Given that antibiotic resistance genes originated in environmental microorganisms, understanding the processes leading to their emergence, spread and evolution requires the analysis of clinical and non-clinical habitats if the bottlenecks that regulate such processes are to be deciphered. We should continue to increase our knowledge with detailed and strong extensive data collection for antibiotic resistance control, multifaceted strategies like proper

implementation of rules and regulations, development of new screening and diagnostic tools, efficient infection control, and immunization should be done with strict monitoring of antibiotic use. The alternative use of probiotics and lytic bacteriophages instead of antibiotics can help to decrease the effect of antibiotic resistance globally, and especially in developing countries. Our battle with AMR will not end even with the availability of new antibiotics. It is only upto us to make the proper use of the effective antibiotics that are available to us. It is time to break the cycle and learn how not to misuse the new antibiotics.

Chapter 10

Reference

1. Gould I, Bal A. New antibiotic agents in the pipeline and how they can help overcome microbial resistance. Virulence. 2013;4(2):185-191.

2. Davies J. Origins and evolution of antibiotic resistance. Microbiologia. 1996;12(1):9–16

3. Antibiotic resistance [Internet]. Who.int. 2022 [cited 26 May 2022]. Available from: https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance

4. Sengupta S, Chattopadhyay M, Grossart H. The multifaceted roles of antibiotics and antibiotic resistance in nature. Frontiers in Microbiology. 2013;4.

5. Spellberg B, Gilbert D. The Future of Antibiotics and Resistance: A Tribute to a Career of Leadership by John Bartlett. Clinical Infectious Diseases. 2014;59(suppl 2):S71-S75.

6. [Internet]. 2022 [cited 26 May 2022]. Available from: https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf

7. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and therapeutics. 2015 Apr;40(4):277.

8. [Internet]. Bsac.org.uk. 2022 [cited 26 May 2022]. Available from: https://bsac.org.uk/wp-content/uploads/2013/10/Combined-report-for-stakeholder-day.pdf

9. Livermore DM. Has the era of untreatable infections arrived?. Journal of Antimicrobial Chemotherapy. 2009 Sep 1;64(suppl_1):i29-36.

10. Liu B, Pop M. ARDB—antibiotic resistance genes database. Nucleic acids research. 2009 Jan 1;37(suppl_1):D443-7.

11. [Internet]. 2022 [cited 26 May 2022]. Available from: https://www.cdc.gov/drugresistance/index.html

12. Sabtu N, Enoch D, Brown N. Antibiotic resistance: what, why, where, when and how?. British Medical Bulletin. 2015;:ldv041.

13. Livermore D. Can better prescribing turn the tide of resistance?. Nature Reviews Microbiology. 2004 Jan;2(1):73-8.

14. Conly JM, Johnston BL. Where are all the new antibiotics? The new antibiotic paradox. Canadian Journal of Infectious Diseases and Medical Microbiology. 2005 May 1;16(3):159-60.

15. [Internet]. 2022 [cited 26 May 2022]. Available from: https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf

16. Llor C, Bjerrum L. Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. Therapeutic advancesin drug safety. 2014 Dec;5(6):229-41.

17. Ramachandran P, Rachuri NK, Martha S, Shakthivel R, Gundala A, Battu TS. Implications of overprescription of antibiotics: A cross-sectional study. Journal of Pharmacy Bioallied Sciences. 2019 May;11(Suppl 2):S434.

18. Michael CA, Dominey-Howes D, Labbate M. The antimicrobial resistance crisis: causes, consequences, and management. Frontiers in public health. 2014 Sep 16;2:145.

19. Baiden F, Owusu-Agyei S, Webster J, Chandramohan D. The need for new antibiotics. Lancet [Internet]. 2010;375(9715):637–8. Available from: http://dx.doi.org/10.1016/S0140-6736(10)60265-6

20. Heddini A, Cars O, Qiang S, Tomson G. Antibiotic resistance in China-a major future challenge. Lancet. 2009;373(9657):30.

21. Vento S, Cainelli F. The need for new antibiotics. Lancet [Internet]. 2010;375(9715):637. Available from: http://dx.doi.org/10.1016/S0140-6736(10)60264-4

22. Wise R, Piddock L. The need for new antibiotics. Lancet [Internet]. 2010;375(9715):638. Available from: http://dx.doi.org/10.1016/S0140-6736(10)60266-8

23. Cornaglia G. Fighting infections due to multidrug-resistant Gram-positive pathogens. Clin Microbiol Infect. 2009;15(3):209–11.

24. Thuan TT. "Future" threat of gram-negative resistance in Singapore. Ann Acad Med Singapore. 2008;37(10):884–90.

25. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: No ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis. 2009;48(1):1–12.

26. Rice LB. The clinical consequences of antimicrobial resistance. Curr Opin Microbiol. 2009;12(5):476–81.

27. Page MG, Heim J. Prospects for the next anti-Pseudomonas drug. Curr Opin Pharmacol. 2009;9(5):558–65.

28. Carattoli A. Resistance plasmid families in Enterobacteriaceae. Antimicrob Agents Chemother. 2009;53(6):2227–38.

29. Carattoli A, Miriagou V, Bertini A, Loli A, Colinon C, Villa L, et al. Replicon typing of plasmids encoding resistance to newer -lactams. Emerg Infect Dis. 2006;12(7):1145–8.

30. Walsh TR. Combinatorial genetic evolution of multiresistance. Curr Opin Microbiol. 2006;9(5):476–82.

31. Hawkey PM, Jones AM. The changing epidemiology of resistance. J Antimicrob Chemother. 2009;64(SUPPL.1).

32. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. Lancet Infect Dis. 2010;10(9):597–602.

33. [Internet]. Assets.publishing.service.gov.uk. 2022 [cited 26 May 2022].
Available from: https://assets.publishing.service.gov.uk/government/
uploads/system/uploads/attachment_data/file/138331/CMO_Annual_Report_Volume_2_2011.pdf

34. Causes of Antimicrobial (Drug) Resistance [Internet]. Niaid.nih.gov. 2022 [cited 26 May 2022]. Available from: https://www.niaid.nih.gov/research/antimicrobial-resistance-causes

35. Woodford N, Ellington MJ. The emergence of antibiotic resistance by mutation. Clinical Microbiology and Infection. 2007 Jan 1;13(1):5-18.

36. Antibiotic resistance: What causes it? [Internet]. Medicalnewstoday.com. 2022 [cited 26 May 2022].
Available from: https://www.medicalnewstoday.com/articles/
320070: :text=Antibiotic%20resistance%20has%20become
%20a,antibiotic%20resistance%20in%20the%20gut.

37. Transfer of antibiotic resistance – Antibiotic resistance – ReAct [Internet]. ReAct. 2022 [cited 20 July 2022]. Available from: https://www.reactgroup.org/toolbox/understand/ant
resistance/transfer-of-antibiotic-resistance/

38. Holmes AH, Moore LS, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, Guerin PJ, Piddock LJ. Understanding the mechanisms and drivers of antimicrobial resistance. The Lancet. 2016 Jan 9;387(10014):176-87.

39. Antibiotic resistance: cheap diagnostic test could be a saviour [Internet]. The Conversation. 2022 [cited 20 July 2022]. Available from: https://theconversation.com/antibiotic-resistance-cheap-diagnostic-test-could-be-a-saviour-142778.

40. [Internet]. 2022 [cited 26 May 2022].
Available from: https://www.cdc.gov/hai/patientsafety/ar-hospitals.html

41. Battle of the Bugs: Fighting Antibiotic Resistance [Internet]. U.S. Food and Drug Administration. 2022 [cited 26 May 2022].
Available from: https://www.fda.gov/drugs/information-consumers-and-patients-drugs/battle-bugs-fighting-antibiotic-resistance: :text=A%20nationwide%20ad%20campaign%20developed,brochure%20to%20distribute%20to%20patients.

42. Manyi-Loh C, Mamphweli S, Meyer E, Okoh A. Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. Molecules. 2018 Mar 30;23(4):795.

43. The Use of Drugs in Food Animals. 1999;.

44. What danger will result from not completing a course of prescribed antibiotics? — Notes and Queries — guardian.co.uk [Internet]. Theguardian.com. 2022 [cited 26 May 2022]. Available from: https://www.theguardian.com/notesandqueries/query/0,,-1112,00.html: :text=THE%20BODY%20BEAUTIFUL-,What%20danger%20will%20result%20from%20not%20completing%20a%20course%20of,to%20treat%20when%20it%20does.

45. Causes of Antibiotic Resistance — Antibiotic Research UK [Internet]. Antibiotic Research UK. 2022 [cited 26 May 2022].
Available from: https://www.antibioticresearch.org.uk/causes-antibiotic-resistance/

46. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, Nisar MA, Alvi RF, Aslam MA, Qamar MU, Salamat MK. Antibiotic resistance: a rundown of a global crisis. Infection and drug resistance. 2018;11:1645.

47. Bartlett JG, Gilbert DN, Spellberg B. Seven ways to preserve the miracle of antibiotics. Clinical infectious diseases. 2013 May 15;56(10):1445-50.

48. Bürgmann H, Frigon D, H Gaze W, M Manaia C, Pruden A, Singer AC, F Smets B, Zhang T. Water and sanitation: an essential battlefront in the war on antimicrobial resistance. FEMS microbiology ecology. 2018 Sep;94(9):fiy101.

49. Friedman ND, Temkin E, Carmeli Y. The negative impact of antibiotic resistance. Clinical Microbiology and Infection. 2016 May 1;22(5):416-22.

50. Consequences, implications effects of antibiotic resistance — BioMérieux [Internet]. ANTIMICROBIAL RESISTANCE. 2022 [cited 26 May 2022]. Available from: https://amr.biomerieux.com/en/about-amr/

how-do-bacteria-become-resistant-to-antibiotics/

51. Dadgostar P. Antimicrobial resistance: implications and costs. Infection and drug resistance. 2019;12:3903.

52. Mauldin PD, Salgado CD, Hansen IS, Durup DT, Bosso JA. Attributable hospital cost and length of stay associated with health care-associated infections caused by antibiotic-resistant gram-negative bacteria. Antimicrobial agents and chemotherapy. 2010 Jan;54(1):109-15.

53. Melander RJ, Zurawski DV, Melander C. Narrow-spectrum antibacterial agents. Medchemcomm. 2018;9(1):12-21.

54. Eliopoulos GM, Cosgrove SE, Carmeli Y. The impact of antimicrobial resistance on health and economic outcomes. Clinical infectious diseases. 2003 Jun 1;36(11):1433-7.

55. Li S, Duan X, Peng Y, Rui Y. Molecular characteristics of carbapenem-resistant Acinetobacter spp. from clinical infection samples and fecal survey samples in Southern China. BMC Infectious Diseases. 2019;19(1).

56. Cerqueira GM, Peleg AY. Insights into Acinetobacter baumannii pathogenicity. IUBMB life. 2011 Dec;63(12):1055-60.

57. Carbapenem-resistant Acinetobacter baumannii (CRAB) - Minnesota Dept. of Health [Internet]. Health.state.mn.us. 2022 [cited 26 May 2022]. Available from: https://www.health.state.mn.us/diseases/crab/index.html: :text=Carbapenem%2Dresistant%20Acinetobacter%20baumannii%20(CRAB)%20is%20a%20type%20of,infections%20very%20difficult%20to%20treat.

58. [Internet].2022 [cited 26 May 2022]. Available from: https://www.drugs.com/article/antibiotic-resistance.html

59. Dilnessa T, Getaneh A, Hailu W, Moges F, Gelaw B. Prevalence and antimicrobial resistance pattern of Clostridium difficile among hospitalized diarrheal patients: A systematic review and meta-analysis. PloS one. 2022 Jan 13;17(1):e0262597.

60. Sholeh M, Krutova M, Forouzesh M, Mironov S, Sadeghifard N, Molaeipour L, Maleki A, Kouhsari E. Antimicrobial resistance in Clostridioides (Clostridium) difficile derived from humans: a systematic review and meta-analysis. Antimicrobial Resistance Infection Control. 2020 Dec;9(1):1-1.

61. Spigaglia P, Mastrantonio P, Barbanti F. Antibiotic Resistances of Clostridium difficile. Advances in Experimental Medicine and Biology. 2018;:137-159.

62. Superbugs [Internet]. Mayo Clinic. 2022 [cited 26 May 2022]. Available from: https://www.mayoclinic.org/diseases-conditions/infectious-diseases/in-depth/cre-bacteria/art-20166387: :text=Carbapenem%2Dresistant%20Enterobacteriaceae

%20(CRE),cases%20to%20all%20available%20antibiotics.

63. [Internet]. 2022 [cited 26 May 2022].
Available from: https://www.cdc.gov/hai/organisms
/cre/index.html: :text=Carbapenem%2Dresistant%20Enterobacterales%20(CRE),-
Related%20Pagestext=Enterobacterales%20are%20a%20large%20order,coli)
%20and%20Klebsiella%20pneumoniae.

64. [Internet]. 2022 [cited 26 May 2022].
Available from: https://www.cdc.gov/std/gonorrhea/stdfact-gonorrhea-detailed.
htm: :text=Gonorrhea%20is%20a%20sexually%20transmitted,
urethra%20in%20women%20and%20men.

65. Reekie J, Donovan B, Guy R, Hocking JS, Kaldor JM, Mak D, Preen D, Ward J, Liu B, Liu B, Preen D. Risk of ectopic pregnancy and tubal infertility following gonorrhea and Chlamydia infections. Clinical Infectious Diseases. 2019 Oct 15;69(9):1621-3.

66. Multi-drug resistant gonorrhoea [Internet].
Who.int. 2022 [cited 26 May 2022]. Available from: https://www.who.int/news-room/fact-sheets/detail/
multi-drug-resistant-gonorrhoea: :text=WHO%20estimates%20that%20in%202020,
and%20the%20Western%20Pacific%20region.

67. Taylor T, Unakal C. Staphylococcus Aureus [Internet]. Ncbi.nlm.nih.gov. 2022 [cited 26 May 2022]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441868/

68. Foster TJ. Antibiotic resistance in Staphylococcus aureus. Current status and future prospects. FEMS microbiology reviews. 2017 May 1;41(3):430-49.

69. Deyno S, Fekadu S, Astatkie A. Resistance of Staphylococcus aureus to antimicrobial agents in Ethiopia: a meta-analysis. Antimicrobial Resistance Infection Control. 2017 Dec;6(1):1-5.

70. [Internet]. 2022 [cited 26 May 2022].
Available from: https://wwwnc.cdc.gov/travel/diseases/
pneumococcal-disease-streptococcus-pneumoniae: :text=What%20is
%20pneumococcal%20disease%3F,when%20they
%20cough%20or%20sneeze.text=Symptoms%20of%20pneumococcal%20infection
%20depend%20on%20the%20part%20of%20the%20body%20affected.

71. Van de Beek D, de Gans J, Tunkel AR, Wijdicks EF. Community-acquired bacterial meningitis in adults. New England Journal of Medicine. 2006 Jan 5;354(1):44-53.

72. Pneumococcal Infections (Streptococcus pneumoniae): Practice Essentials, Background, Pathophysiology [Internet]. Emedicine.medscape.com. 2022 [cited 26 May 2022]. Available from: https://emedicine.medscape.com/article/225811-

overview

73. [Internet]. 2022 [cited 26 May 2022].
Available from: https://www.cdc.gov/hai/organisms/
vre/vre.html: :text=CDC%20tracks
%20VRE%20infections%20using,central%2Dline%20associated
%20bloodstream%20infections.

74. Enterococcal Infections: Practice Essentials, Background, Pathophysiology [Internet].
Emedicine.medscape.com. 2022 [cited 26 May 2022].
Available from: https://emedicine.medscape.com/article/
216993-overview: :text=Tables-,Practice%20Essentials,
of%20choice%20for%20susceptible%20strains
.
75. Enterococcal Infections - Infectious Diseases - MSD Manual Professional Edition [Internet]. MSD Manual Professional Edition. 2022 [cited 26 May 2022]. Available from: https://www.msdmanuals.com/professional/infectious-diseases/gram-positive-cocci/enterococcal-infections

76. Pseudomonas aeruginosa Infections: Practice Essentials, Background, Pathophysiology [Internet]. Emedicine.medscape.com. 2022 [cited 26 May 2022]. Available from: https://emedicine.medscape.com/article/226748-overview

77. Aloush V, Navon-Venezia S, Seigman-Igra Y, Cabili S, Carmeli Y. Multidrug-resistant Pseudomonas aeruginosa: risk factors and clinical impact. Antimicrobial agents and chemotherapy. 2006 Jan;50(1):43-8.

78. [Internet]. 2022 [cited 26 May 2022].
Available from: https://www.cdc.gov/tb/topic/basics/default.htm

79. it? W. Tuberculosis: Multidrug-resistant tuberculosis (MDR-TB)
ternet
. Who.int. 2022 [cited 26 May 2022].
Available from: https://www.who.int/news-room/questions-and-answers/
item/tuberculosis-multidrug-resistant-tuberculosis-(mdr-tb): :text=
Multidrug%2Dresistant%20TB%20(MDR%2D,person
%2Dto%2Dperson%20transmission.

80. Resistance mechanisms – Antibiotic resistance – ReAct [Internet]. ReAct. 2022 [cited 26 May 2022].
Available from: https://www.reactgroup.org/toolbox/understand/
antibiotic-resistance/resistance-mechanisms-in-bacteria/

81. Microbiology — Antimicrobial Resistance Learning Site [Internet]. Amrls.umn.edu. 2022 [cited 26 May 2022]. Available from: https://amrls.umn.edu/microbiology

82. Webber MA, Piddock LJ. The importance of efflux pumps in bacterial antibiotic resistance. Journal of antimicrobial chemotherapy. 2003 Jan 1;51(1):9-11.

83. Walsh C. Molecular mechanisms that confer antibacterial drug resistance. Nature. 2000 Aug;406(6797):775-81.

84. McMurry L, Petrucci RE, Levy S. Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in Escherichia coli. Proceedings of the national academy of sciences. 1980 Jul 1;77(7):3974-7.

85. Abraham EP, Chain E. An enzyme from bacteria able to destroy penicillin. Nature. 1940 Dec;146(3713):837-.

86. Munita JM, Arias CA. Mechanisms of antibiotic resistance. Microbiology spectrum. 2016 Apr 8;4(2):4-2.

87. Schwarz S, Kehrenberg C, Doublet B, Cloeckaert A. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. FEMS microbiology reviews. 2004 Nov 1;28(5):519-42.

88. Woodford N, Ellington MJ. The emergence of antibiotic resistance by mutation. Clinical Microbiology and Infection. 2007 Jan 1;13(1):5-18.

89. Reygaert WC. An overview of the antimicrobial resistance mechanisms of bacteria. AIMS microbiology. 2018;4(3):482.

90. Brandon Reynolds B, More L. Vancomycin Mechanism of Action — Resistance and More — DoseMeRx [Internet]. DoseMeRx. 2022 [cited 26 May 2022]. Available from: https://doseme-rx.com/vancomycin/articles/vancomycin-mechanisms-of-action: :text=When%20it%20reaches%20the%20cell,necessary%20to%20keep%20it%20strong.

91. Chambers HF. Penicillin-binding protein-mediated resistance in pneumococci and staphylococci. The Journal of infectious diseases. 1999 Mar 1;179(Supplement$_2$) : $S353 - 9$.

92. Navratna V, Nadig S, Sood V, Prasad K, Arakere G, Gopal B. Molecular basis for the role of Staphylococcus aureus penicillin binding protein 4 in antimicrobial resistance. Journal of bacteriology. 2010 Jan 1;192(1):134-44.

93. What you need to know about antibiotic resistance – and how to protect yourself [Internet]. WebMD. 2022 [cited 26 May 2022]. Available from: https://www.webmd.com/cold-and-flu/antibiotic-resistance

94. Mebis J, Goossens H, Bruyneel P, Sion JP, Meeus I, Van Droogenbroeck J, Schroyens W, Berneman ZN. Decreasing antibiotic resistance of Enterobacteriaceae by introducing a new antibiotic combination therapy for neutropenic fever patients. Leukemia. 1998 Oct;12(10):1627-9.

95. Guillemot D, Courvalin P, French Working Party to Promote Research to Con-

trol Bacterial Resistance. Better control of antibiotic resistance. Clinical infectious diseases. 2001 Aug 15;33(4):542-7.

96. Rahal JJ, Urban C, Horn D, Freeman K, Segal-Maurer S, Maurer J, Mariano N, Marks S, Burns JM, Dominick D, Lim M. Class restriction of cephalosporin use to control total cephalosporin resistance in nosocomial Klebsiella. Jama. 1998 Oct 14;280(14):1233-7.

97. Årdal C, Balasegaram M, Laxminarayan R, McAdams D, Outterson K, Rex JH, Sumpradit N. Antibiotic development—economic, regulatory and societal challenges. Nature Reviews Microbiology. 2020 May;18(5):267-74.

98. Combating Antibiotic Resistance [Internet]. U.S. Food and Drug Administration. 2022 [cited 26 May 2022]. Available from: https://www.fda.gov/consumers/consumer-updates/combating-antibiotic-resistance

99. Hoque R, Ahmed SM, Naher N, Islam MA, Rousham EK, Islam BZ, et al. Tackling antimicrobial resistance in Bangladesh: A scoping review of policy and practice in human, animal and environment sectors. PLoS One. 2020;15(1):1–22.

100. Saha T, Saha T. Awareness Level of Patients Regarding usage of Antibiotics in a Slum Area of Dhaka City, Bangladesh. Int J Med Sci. 2018;5(9):10–6.

101. Nahar P, Unicomb L, Lucas PJ, Uddin MR, Islam MA, Nizame FA, et al. What contributes to inappropriate antibiotic dispensing among qualified and unqualified healthcare providers in Bangladesh? A qualitative study. BMC Health Serv Res. 2020;20(1):1–11.

102. [Internet]. Medicinenet.com. 2022 [cited 26 May 2022]. Available from: https://www.medicinenet.com/antibiotic$_r esistance/article.htm$

103. CDC A. Recommendations for preventing the spread of vancomycin resistance: recommendations of the Hospital Infection Control Practices Advisory Committee (HICPAC). Morb. Mort. Wkly. Rep.. 1995 Sep 22;44.

104. Maron D. Should Fighting Antibiotic Resistance Always Include Finishing a Prescribed Medication? [Internet]. Scientific American. 2022 [cited 26 May 2022]. Available from: https://www.scientificamerican.com/article/should-fighting-antibiotic-resistance-always-include-finishing-a-prescribed-medication/

105. Jernigan DB, Cetron MS, Breiman RF. Minimizing the impact of drug-resistant Streptococcus pneumoniae (DRSP): a strategy from the DRSP working group. Jama. 1996 Jan 17;275(3):206-9.
106. Murray BE. Can antibiotic resistance be controlled?. New England journal of medicine. 1994 Apr 28;330(17):1229-30.

107. How to Prevent Antibiotic Resistance [Internet]. National Foundation for Infectious Diseases. 2022 [cited 26 May 2022]. Available from: https://www.nfid.org/antibiotic-

resistance/how-to-prevent-antibiotic-resistance/

108. Chowdhury F, Sturm-Ramirez K, Mamun A Al, Iuliano AD, Chisti MJ, Ahmed M, et al. Effectiveness of an educational intervention to improve antibiotic dispensing practices for acute respiratory illness among drug sellers in pharmacies, a pilot study in Bangladesh. BMC Health Serv Res. 2018;18(1):1–11.

109. Fahad BM, Matin A, Shill MC, Asish KD. Antibiotic usage at a primary health care unit in Bangladesh. Australas Med J. 2010 Jul;3(7):414–21.

110. Sayeed MA, Iqbal N, Ali MS, Rahman MM, Islam MR, Jakaria M. Survey on Antibiotic Practices in Chittagong City of Bangladesh. Bangladesh Pharm J. 2015;18(2):174–8.

111. Al Rasel Bin Mahabub Zaman M, Hasan M, Rashid HA. Indication-based use of antibiotic in the treatment of patients attending at the primary health care facility. Cough. 2018;44(8):20–2.

112. Akm S, Shamsuddin A, Saifor Rahman M, Sultana N, Saiful Islam M, Kumar Shaha A, et al.
Current Trend of Antibiotic Practice in Paediatric Surgery in Bangladesh Current Trend of Antibiotic Practice in Paediatric Surgery in Bangladesh. IOSR J Dent Med Sci e-ISSN [Internet]. 2019;18(4):28–32.
Available from: www.iosrjournals.orgwww.iosrjournals.org

113. Haque M. Antimicrobial use, prescribing, and resistance in selected ten selected developing countries: A brief overview. Asian J Pharm Clin Res. 2017;10(8):37–45.

114. Biswas M, Roy MN, Manik MIN, Hossain MS, Tapu STA, Moniruzzaman M, et al. Self medicated antibiotics in Bangladesh: A cross-sectional health survey conducted in the Rajshahi City. BMC Public Health. 2014;14(1):1–7.

115. Chowdhury N, Rashedul Islam M, Mehedi Hasan M, Mahmudur Rouf M. Prevalence of self-medication of antibiotics among people in Bangladesh. Int J Pharm Teach Pract [Internet]. 2013;4(1):504–10.
Available from: https://www.iomcworld.org/articles/prevalence-of-selfmedication-of-antibiotics-among-people-in-bangladesh.pdf

116. Rana MM, Karim MR, Islam MR, Mondal MNI, Wadood MA. Knowledge and Practice about the Rational Use of Antibiotic among the Rural Adults in Rajshahi District , Bangladesh: A Community Clinic Based Study. 2018;7(Issn 22774424):259–71.

117. Antibiotics Overuse: Headed for a health calamity [Internet]. The Daily Star. 2022 [cited 26 May 2022]. Available from: https://www.thedailystar.net/health/health-alert/news/antibiotics-overuse-headed-health-calamity-2901166

118. Barai L, Saha MR, Rahman T, Khandaker T, Dutta S, Hasan R, Haq JA.

Antibiotic resistance: situation analysis in a tertiary care hospital of Bangladesh. Bangladesh Journal of Microbiology. 2017;34(1):15-9.

119. Chisti MJ, Harris JB, Carroll RW, Shahunja KM, Shahid AS, Moschovis PP, Schenkel SR, Hasibur Rahman AS, Shahrin L, Faruk T, Kabir F. Antibiotic-Resistant Bacteremia in Young Children Hospitalized With Pneumonia in Bangladesh Is Associated With a High Mortality Rate. InOpen Forum Infectious Diseases 2021 Jul (Vol. 8, No. 7, p. ofab260). US: Oxford University Press.

120. Dramatic rise in antibiotic-resistant infections in Bangladeshi kids [Internet]. Medicalnewstoday.com. 2022 [cited 26 May 2022].
Available from: https://www.medicalnewstoday.com/articles/antibiotic-resistant-infections-in-bangladeshi-kids-deemed-very-worrying

121. Shibl AM, Memish Z, Osoba A. Antibiotic resistance in developing countries. Journal of chemotherapy. 2001 Apr 1;13(sup1):40-4. 122. Sosa A. Antimicrobial Resistance in Developing Countries. New York, NY: Springer; 2010.

123. Asna SM, Haq JA, Rahman MM. Nalidixic acid-resistant Salmonella enterica serovar Typhi with decreased susceptibility to ciprofloxacin caused treatment failure: a report from Bangladesh. Japanese journal of infectious diseases. 2003 Feb 1;56(1):32-3.

124. Islam MA, Islam M, Hasan R, Hossain MI, Nabi A, Rahman M, et al. Environmental spread of New Delhi metallo-- lactamase-1-producing multidrug-resistant bacteria in Dhaka, Bangladesh. Appl Environ Microbiol. 2017;83(15).

125. Bhowmik P, Ahaduzzaman M, Hasan RB. a Cross Sectional Anthropo-Clinical Study on Antimicrobials Prescription Pattern in Goat Patients At Chittagong, Bangladesh. Bangladesh J Vet Med. 2018;15(2):119–26.

126. Hassan MM, Begum S, Faruq A Al, Alam M, Mahmud T, Islam A. Multidrug Resistant Salmonella Isolated from Street Foods in Chittagong, Bangladesh. Microbiol Res J Int. 2019;26(6):1–8.

127. Islam MM. Bacterial resistance to antibiotics: access, excess, and awareness in Bangladesh. Expert Rev Anti Infect Ther [Internet]. 2021;19(8):973–81. Available from: https://doi.org/10.1080/14787210.2021.1865804

128. Biswas M, Roy DN, Tajmim A, Rajib SS, Hossain M, Farzana F, Yasmen N. Prescription antibiotics for outpatients in Bangladesh: a cross-sectional health survey conducted in three cities. Annals of clinical microbiology and antimicrobials. 2014 Dec;13(1):1-7.

129. Sutradhar KB, Saha A, Huda NH, Uddin R. Irrational use of antibiotics and antibiotic resistance in southern rural Bangladesh: perspectives from both the physicians and patients. Annual Research Review in Biology. 2014 Jan 15:1421-30.

130. Ahmed I, Rabbi MB, Sultana S. Antibiotic resistance in Bangladesh: A systematic review. International Journal of Infectious Diseases. 2019 Mar 1;80:54-61.

131. Bilal H, Khan MN, Rehman T, Hameed MF, Yang X. Antibiotic resistance in Pakistan: a systematic review of past decade. BMC infectious diseases. 2021 Dec;21(1):1-9.

132. Kang CI, Song JH. Antimicrobial resistance in Asia: current epidemiology and clinical implications. Infection chemotherapy. 2013 Mar 1;45(1):22-31.

133. Yasmin F, Akhtar N, Hameed A. In vitro synergistic effect of ciprofloxacin with aminoglycosides against multidrug resistant-Pseudomonas aeruginosa. Pakistan Journal of Pharmaceutical Sciences. 2013 Sep 1;26(5):1041-5.

134. Qamar FN, Yousafzai MT, Khalid M, Kazi AM, Lohana H, Karim S, Khan A, Hotwani A, Qureshi S, Kabir F, Aziz F. Outbreak investigation of ceftriaxone-resistant Salmonella enterica serotype Typhi and its risk factors among the general population in Hyderabad, Pakistan: a matched case-control study. The Lancet Infectious Diseases. 2018 Dec 1;18(12):1368-76.

135. A cross-sectional study on knowledge, attitude and practices of medical doctors towards antibiotic prescribing patterns and resistance in Khyber Pakhtun Khawah, Pakistan. 2022.

136. Antimicrobial Resistance - National Institutes of Health Islamabad [Internet]. National Institutes of Health Islamabad. 2022 [cited 26 May 2022]. Available from: https://www.nih.org.pk/antimicrobial-resistance/

137. Nakkawita WM, Patabendige CG. Incidents of ventilator associated pneumonia in two intensive care units and a high dependency unit at National Hospital of Sri Lanka. Sri Lanka Coll Microbiol Bull. 2015;13:9-10.

138. Wickramasinghe D, Pathirage S and Chandrasiri NS. Identification of Acinetobacter spp. isolated in Teaching Hospital Anuradhapura in Sri Lanka by phenotypic tests and their antimicrobial susceptibility status. The Bulletin of the Sri Lanka College of Microbiologists. 2015; 13(1):51-55.

139. Liyanapathirana V, Thevanesam V. Combating Antimicrobial Resistance. 2022.

140. . Sanjeewanie HAD, Dassanayake M, Chandrasiri P, et al. The prevalence of carbapenamase producing Enterobacteriaceae isolated in two selected teaching hospitals in Sri Lanka. The Bulletin of the Sri Lanka College of Microbiologists. 2013; 11(1):23.

141. Hall JM, Corea E, Sanjeewani HA, Inglis TJ. Molecular mechanisms of -lactam resistance in carbapenemase-producing Klebsiella pneumoniae from Sri Lanka. Journal of medical microbiology. 2014 Aug 1;63(8):1087-92.

142. Perera V, Brahmanayake BSAJP, Basnayake P, et al. A retrospective study of methicillin-resistant Staphylococcus aureus (MRSA) infections in a private hospital in Sri Lanka. The Bulletin of the Sri Lanka College of Microbiologists. 2015; 13(1): 22

143. Perera V, Assellage P and De Silva P. Profile of extended spectrum lactamase (ESBL) producing enterobacteria isolated from clinical specimens in a private teaching hospital in Sri Lanka. The Bulletin of the Sri Lanka College of Microbiologists. 2014; 12(1):19-20

144. Pathirage MVSC, Wijewardena N, Perera R. Salmonella serotypes isolated or serotyped at the National Reference enteric laboratory and their antibiotic sensitivity pattern. The Bulletin of the Sri Lanka College of Microbiologists. 2014; 12(1):6

145. [Internet]. Daph.gov.lk. 2022 [cited 26 May 2022].
Available from: http://www.daph.gov.lk/web/images/content_image/publications /annual_reports/annual_report_2012.pdf

146. Herath E.M., Dandeniya W.S,. Samarasinghe A.G.S.I et al A preliminary investigation on methods of reducing antibiotic resistant bacteria in broiler litter in selected farms in mid country, Sri Lanka Tropical Agricultural Research 2015; 82

147. Rijal KR, Banjara MR, Dhungel B, Kafle S, Gautam K, Ghimire B, Ghimire P, Dhungel S, Adhikari N, Shrestha UT, Sunuwar DR. Use of antimicrobials and antimicrobial resistance in Nepal: A nationwide survey. Scientific reports. 2021 Jun 2;11(1):1-4.

148. Pokharel S, Raut S, Adhikari B. Tackling antimicrobial resistance in low-income and middle-income countries. BMJ Global Health. 2019 Nov 1;4(6):e002104.

149. Raut S, Rijal KR, Khatiwada S, Karna S, Khanal R, Adhikari J, Adhikari B. Trend and characteristics of Acinetobacter baumannii infections in patients attending Universal College of Medical Sciences, Bhairahawa, Western Nepal: a longitudinal study of 2018. Infection and Drug Resistance. 2020;13:1631.

150. Pokharel S, Adhikari B. Antimicrobial resistance and over the counter use of drugs in Nepal. Journal of Global Health. 2020 Jun;10(1).

151. Adhikari B, Pokharel S, Raut S, Adhikari J, Thapa S, Paudel K, Narayan GC, Neupane S, Neupane SR, Yadav R, Shrestha S. Why do people purchase antibiotics over-the-counter? A qualitative study with patients, clinicians and dispensers in central, eastern and western Nepal. BMJ global health. 2021 May 1;6(5):e005829.

152. Parajuli NP, Acharya SP, Mishra SK, Parajuli K, Rijal BP, Pokhrel BM. High burden of antimicrobial resistance among gram negative bacteria causing healthcare associated infections in a critical care unit of Nepal. Antimicrobial Resistance Infection Control. 2017 Dec;6(1):1-9.

153. Global Antibiotic Resistance Partnership (GARP)-India Working Group. Rationalizing antibiotic use to limit antibiotic resistance in India+. The Indian journal of medical research. 2011 Sep;134(3):281.

154. Laxminarayan R, Chaudhury RR. Antibiotic resistance in India: drivers and opportunities for action. PLoS medicine. 2016 Mar 2;13(3):e1001974.

155. World Health Organization. Community-based surveillance of antimicrobial use and resistance in resource-constrained settings: report on five pilot projects. World Health Organization; 2009.

156. Resistance Map - Center for Disease Dynamics, Economics Policy (CDDEP) [Internet]. Center for Disease Dynamics, Economics Policy (CDDEP). 2022 [cited 26 May 2022]. Available from: https://cddep.org/projects/resistance-map/

157. Datta S, Wattal C, Goel N, Oberoi JK, Raveendran R, Prasad K. A ten year analysis of multi-drug resistant blood stream infections caused by Escherichia coli Klebsiella pneumoniae in a tertiary care hospital. The Indian journal of medical research. 2012 Jun;135(6):907.

158. The State of the World's Antibiotics, 2015 - Center for Disease Dynamics, Economics Policy (CDDEP) [Internet]. Center for Disease Dynamics, Economics Policy (CDDEP). 2022 [cited 26 May 2022].
Available from: https://cddep.org/publications/state_worlds_antibiotics_2015/

159. Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, Laxminarayan R. Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. The Lancet infectious diseases. 2014 Aug 1;14(8):742-50.

160. Laxminarayan R, Matsoso P, Pant S, Brower C, Røttingen JA, Klugman K, Davies S. Access to effective antimicrobials: a worldwide challenge. The Lancet. 2016 Jan 9;387(10014):168-75.

161. Roy N, Madhiwalla N, Pai SA. Drug promotional practices in Mumbai: a qualitative study. Indian journal of medical ethics. 2007 Apr 1;4(2):57-61.

162. Kumar A, Biswal M, Dhaliwal N, Mahesh R, Appannanavar S, Gautam V et al. Point prevalence surveys of healthcare-associated infections and use of indwelling devices and antimicrobials over three years in a tertiary care hospital in India. Journal of Hospital Infection. 2014;86(4):272-274.

163. Hawser SP, Bouchillon SK, Hoban DJ, Badal RE, Hsueh PR, Paterson DL. Emergence of high levels of extended-spectrum--lactamase-producing gram-negative bacilli in the Asia-Pacific region: Data from the Study for Monitoring Antimicrobial Resistance Trends (SMART) program, 2007. Antimicrob Agents Chemother. 2009;53(8):3280–4

164. 5. Editorial Board. Clinical Microbiology Reviews. 2017;30(1).

165. Naimi H, Rasekh H, Noori A, Bahaduri M. Determination of antimicrobial susceptibility patterns in Staphylococcus aureus strains recovered from patients at two main health facilities in Kabul, Afghanistan. BMC Infectious Diseases. 2017;17(1).

166. Sutter DE, Bradshaw LU, Simkins LH, Summers AM, Atha M, Elwood RL, Robertson JL, Murray CK, Wortmann GW, Hospenthal DR. High incidence of multidrug-resistant gram-negative bacteria recovered from Afghan patients at a deployed US military hospital. Infection Control Hospital Epidemiology. 2011 Sep;32(9):854-60.

167. Vilaichone R korn, Aumpan N, Ratanachu-ek T, Uchida T, Tshering L, Mahachai V, et al. Population-based study of Helicobacter pylori infection and antibiotic resistance in Bhutan. Int J Infect Dis [Internet]. 2020;97:102–7. Available from: https://doi.org/10.1016/j.ijid.2020.05.077

168. Maharath A, Ahmed M. Bacterial Etiology of Bloodstream Infections and Antimicrobial Resistance Patterns from a Tertiary Care Hospital in Malé, Maldives. International Journal of Microbiology. 2021;2021:1-10.
169. Henriques Normark B, Normark S. Evolution and spread of antibiotic resistance. J Intern Med. 2002;252(2):91–106.

170. Baquero F, Negri M. Antibiotic-Selective Environments. 1998;27(Suppl 1):5–11.

171. Linkevicius M, Sandegren L, Andersson DI. Potential of tetracycline resistance proteins to evolve tigecycline resistance. Antimicrob Agents Chemother. 2016;60(2):789–96.

172. Bengtsson-Palme J, Kristiansson E, Larsson DGJ. Environmental factors influencing the development and spread of antibiotic resistance. FEMS Microbiol Rev. 2018;42(1):68–80.

173. Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet Infect Dis. 2019;19(1):56–66.

174. Baker-Austin C, Wright MS, Stepanauskas R, McArthur J V. Co-selection of antibiotic and metal resistance. Trends Microbiol. 2006;14(4):176–82.

175. Pal C, Asiani K, Arya S, Rensing C, Stekel DJ, Larsson DGJ, et al. Metal Resistance and Its Association With Antibiotic Resistance [Internet]. 1st ed. Vol. 70, Advances in Microbial Physiology. Elsevier Ltd.; 2017. 261–313 p. Available from: http://dx.doi.org/10.1016/bs.ampbs.2017.02.001

176. Beaber JW, Hochhut B, Waldor MK. SOS response promotes horizontal dis-

semination of antibiotic resistance genes. Nature. 2004;427(6969):72–4.

177. Gillings MR, Stokes HW. Are humans increasing bacterial evolvability? Trends Ecol Evol [Internet]. 2012;27(6):346–52.
Available from: http://dx.doi.org/10.1016/j.tree.2012.02.006

178. Gaze WH, Zhang L, Abdouslam NA, Hawkey PM, Calvo-Bado L, Royle J, et al. Impacts of anthropogenic activity on the ecology of class 1 integrons and integron-associated genes in the environment. ISME J [Internet]. 2011;5(8):1253–61. Available from: http://dx.doi.org/10.1038/ismej.2011.15

179. Maiques E, Úbeda C, Campoy S, Salvador N, Lasa Í, Novick RP, et al. -lactam antibiotics induce the SOS response and horizontal transfer of virulence factors in Staphylococcus aureus. J Bacteriol. 2006;188(7):2726–9.
180. Gillings MR. Integrons: Past, Present, and Future. Microbiol Mol Biol Rev. 2014;78(2):257–77.

181. Souque C, Escudero JA, MacLean RC. Integron activity accelerates the evolution of antibiotic resistance. Elife. 2021 Feb 26;10:e62474.

182. Gu D, Dong N, Zheng Z, Lin D, Huang M, Wang L, et al. A fatal outbreak of ST11 carbapenem-resistant hypervirulent Klebsiella pneumoniae in a Chinese hospital: a molecular epidemiological study. Lancet Infect Dis [Internet]. 2018;18(1):37–46. Available from: http://dx.doi.org/10.1016/S1473-3099(17)30489-9

183. Hughes D, Andersson DI. Evolutionary consequences of drug resistance: shared principles across diverse targets and organisms. Nature Reviews Genetics. 2015 Aug;16(8):459-71.