Evolution of Superbugs: A Lethal Bioterror

By

Md Abir Hossain
15346010

A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

Department of Pharmacy
Brac University
October 2019

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

Student's Full Name & Signature:

Md Abir Hossain
15346010

Approval

The project titled "Evolution of Superbugs: A Lethal Bioterror" submitted by Md Abir Hossain (15346010) of Summer, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on 10th October, 2019.

Examining Committee:

Supervisor:
(Member)

Md Abul Kalam Azad, PhD
Assistant Professor, Department of Pharmacy
Brac University

Program Coordinator:
(Member)

Dr. Hasina Yasmin
Professor, Department of Pharmacy
Brac University

Departmental Head:
(Chair)

Professor Dr. Eva Rahman Kabir
Chairperson, Department of Pharmacy
Brac University

Ethics Statement

This study comprises no human or animal trial.

Abstract

Over the past few decades, the increasing rate of antibiotic resistance (ABR) has led to the emergence of superbugs, a group of deadly pathogens with antibiotic resistance in their supreme level. About two million people die worldwide each year by the difficult-to-treat to incurable infections caused by them. Thus, superbugs are considered to be a global threat and named as lethal bioterror. In this study, the mechanism of antibiotic resistance, followed by the evolution of superbugs has been reviewed. The measures concerning the combating of antibiotic resistance, e.g., combination therapy, drug repurposing, novel antibiotic research etc., have been briefly discussed. Unless preventive measures are not established right from now on, antibiotics will lose their ability to cure infections, resulting in an epidemiological disaster.

Keywords: Antibiotic resistance; Superbugs; New antibiotic research; Drug repurposing; Combination therapy

Dedication

Dedicated to my parents and to all the victims who have died due to antibiotic resistance.

Acknowledgement

First of all, I am grateful to my Almighty Allah for the good health and wellbeing during the project which were vital to complete the project work on time.

It is a genuine pleasure to express my deep sense of thanks and gratitude to my supervisor, **Dr. Md Abul Kalam Azad** (Assistant Professor, Department of Pharmacy, Brac University) for giving me this golden opportunity to do this wonderful project. His prompt inspiration at every stage of my project work and timely suggestion with kindness, dedication, scientific approach, constant guidance, collaboration, enthusiasm make me enable to finalize the project.

I owe a deep sense of gratitude to our honorable chairperson, **Dr. Eva Rahman Kabir** (Chairperson, Department of Pharmacy, Brac University) whose support, encouragement, scholarly advice were the sustaining factors in carrying out the project successfully.

Also, I take this opportunity to express my gratitude to all the faculty members and lab officer for their help and support.

Last but not the least, I would like to express my greatest regards to my parents for their co-operation, continuous support, unconditional love throughout my life.

Table of Contents

List of Tables

List of Figures

List of Acronyms

MDR	Multi-Drug Resistant
MRSA	Methicillin Resistant *Staphylococcus aureus*
CA	Community-Acquired
AMR	Anti-Microbial Resistance
WBC	White Blood Cell
CDC	Centers for Disease Control and Prevention
WHO	World Health Organization
ICU	Intensive Care Unit
UTI	Urinary Tract Infection
MIT	Microbial Inhibition Test
ABR	Antibiotic Resistance
APUA	Alliance for the Prudent Use of Antibiotics
OMPs	Outer Membrane Proteins
FDA	Food and Drug Administration
CF	Cystic Fibrosis
NNIS	National Nosocomial Infection Surveillance
MYSTIC	Meropenem Yearly Susceptibility Test Information Collection
ESBLs	Extended Spectrum Beta Lactamases
KPC	*K. pneumoniae* Carbapenemase
NDM	New Delhi Metallo-Beta Lactamases
BSI	Bloodstream Infection
HAP	Hospital Acquired Pneumonia
VAP	Ventilated Acquired Pneumonia
NDA	New Drug Application

Chapter 1

Introduction

"Water, water, everywhere,

Nor any drop to drink."

The above memorable line from the poem "The Rime of the Ancient Mariner" by the famous poet Samuel Taylor Coleridge describes the tragic situation of a lost thirsty mariner in the ocean. There was water everywhere but not suitable to drink a drop because of being salty in nature (Fulmer, 1979). If we consider the situation metaphorically that depicts a situation where a child is infected with a simple but deadly pathogen against which no antibiotic is effective enough to defeat the killer pathogen, even though there are many available antibiotics. There might be nothing but a miracle to save the child from death. However, miracles happens rarely. The outcome would be the death of that child. Therefore, giving into any kind of pathogen might be regular event for mankind in any corner of the world, termed as superbug. In 1900, cholera, tuberculosis, mumps, diphtheria, typhoid fever, plague were the leading causes of death (Kumarasamy et al., 2010). Eight decades back, after the serendipitous discovery of penicillin, the frequency of infectious diseases was greatly reduced which was the driving cause of death within the USA (Yoshikawa, 2002). Let us imagine a situation, when antibiotics might be no more effective against bacterial pathogens due to development of resistance to antibiotics. Statistics shows approximately 700,000 people passed away due to the development of antibiotic resistance every year worldwide (Walsh, 2014). In context of Europe and America, antibiotic resistance kills approximately 50,000 people per year. Within 2050, antibiotic resistance might cause the death of 10 million people worldwide which would be greater than the mortality rate today due to cancer if proper steps is not taken as soon as possible (Walsh, 2014). This indicate that the consequence of

antimicrobial resistance would be alarming for the future generation as antimicrobial resistance might lead us to the pre antibiotic era. It is very difficult to tackle development of antibiotic resistance.

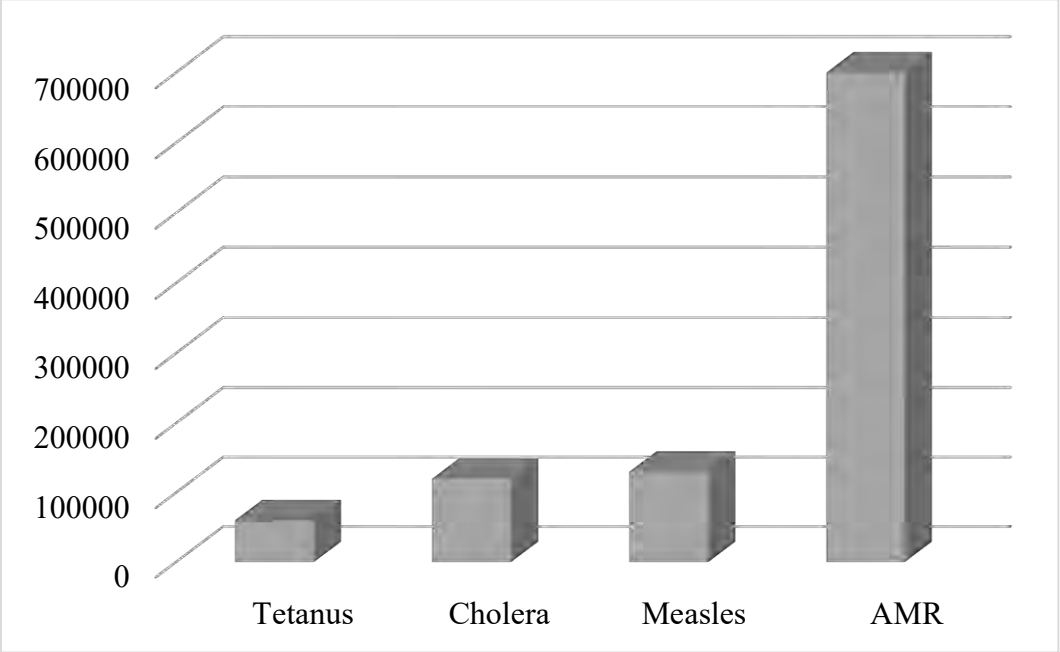

Figure 1: Comparison of major causes of death every year (Modified from Walsh, 2014).

Antibiotics are classes of drugs that are used by the body's natural defence to eliminate microorganisms as antibiotics are cytotoxic or cytostatic to the microorganisms (Zaman et al., 2017). All classes of antibiotics are designed according to the antibiotic structure which are found in the environmental microorganism, although many of the antibiotics are synthetic derivatives of the natural structure (Qiao, Ying, Singer, & Zhu, 2018). Antibiotics specifically target the disease causing microorganism and at the same time does not cause any harm to the host (Zaman et al., 2017). They function in a several ways including inhibition of bacterial cell synthesis, protein synthesis, or other specific actions (Paridah et al., 2016). To protect human being from harmful effects of microbes, undoubtedly antibiotics are one of the best option to human civilization (Zaman et al., 2017). 1930s to 1960s has been regarded to be the "golden era of antibiotics" (Aslam et al., 2018). The latest era of antibiotics began by 2 noble scientists named Alexander Fleming and Paul Ehrlich (Zaman et al., 2017).

Unfortunately, this era has been ended up due to the emerging resistance pathogens. Alexander Fleming alarmed about the potential resistance of penicillin when used for short periods of treatment (Zaman et al., 2017). Antibiotics are used to treat infections and prevent microbial growth in patients taking chemotherapy, who are suffering from long term diseases like diabetics, end- stage renal disease, or patients undergoing critical surgeries for example organ transplants, joint replacements (Ventola, 2015). The uses of antibiotics have surprisingly changed the fate of infected patients and improve their disease conditions and surgical procedure. The ability of antibiotics to cause numerous infections caused by microorganisms and reduces the childhood mortality rate and thus saving life of many people.

Now-a-days expansion of antibiotic resistance is a matter of concern. It is a threat in the effective way of inhibiting microbial growth and their prevention (Sabtu, Enoch, & Brown, 2015). It is the ability of pathogens to protect themselves from antimicrobial agents (Aslam et al., 2018). Commensal (non-pathogenic) or environmental microbiota may be the source of antibiotic resistance (Baquer & Martinez, 2014). Ream of metric tons of contemporary categories of antibiotics have been discovered which are used in many therapeutic purposes including serendipitous discovery of penicillin and the launching of first ever anti-resistance antibiotic called methicillin in 1959 expected to be 100% effective against the penicillinases (Aslam et al., 2018). Unfortunately, *methicillin resistant S. aureus* (MRSA) had been emerged and this acronym presently means *multidrug-resistant S. aureus.* Moreover, MRSA has ended up a major community-acquired (CA) pathogen, with upgraded harmfulness as it spreads out in the biosphere. A long-recognized clinic native, the toxin-producing *Clostridium difficile,* progressively found as the causes of serious intestinal diseases. Unfortunately, the increasing use (basically embezzle) of antibiotic is leading to antibiotic resistance due to which antibiotics are failed to response in the treatment of various infections (Webster & Li, 2018). Major causes of antibiotic resistance are overpopulation, increased

global migration, habits of self-treatment within the patients and among patients and use of non-selective antibiotics in various sectors such as agriculture and farming (Ahmed, Rabbi, & Sultana, 2019; Aslam et al., 2018). In the hospital, ward boy and nurses are extensively use antibiotics includes expanded-spectrum cephalosporin, the more up to date penicillin and fluoroquinolone that cause noteworthy exhaustion of the Gram-negative intestinal microflora, thus increasing the size of colony of *C. difficile*. To elaborate, nosocomial infections are due to the irrational use of antibiotics (Davies & Davies, 2010). Further, there is a very less scope of new drug discovery by pharmaceutical industries as there is a lower grade of economic condition as well as the regulatory requirements are getting harder to be met (Ventola, 2015). In bacterial genomes over 20000 potential resistance genes are found (Aslam et al., 2018). These resistance leads to economic impact, losses of many lives in developing countries.

Superbugs are special types of microbes that are resistance to multiple types of antibiotics which are commonly used to treat various types of infection caused by *Enterobacter spp, Acinetobacter, Citrobacter, Proteus, Burkholderia, Clostridium, Pseudomonas, Enterococcus, E. coli, Haemophilus, Klebsiella, Campylobacter, Salmonella, Serratia, S. aureus, Staphylococcus epidermidis, and Streptococcus pneumoniae. Staphylococcus aureus* is treated as the foremost infamous superbug (Nordmann, Naas, Fortineau, & Poirel, 2007). Superbugs are ubiquitous within the environment which is spread out vigorously during the civil unrest, famine, natural climates and undoubtedly due to the lacking of medical enactments. Superbugs are not known as pathogenic dangers, but they have been recognized to be the foremost threatening with regards the horribleness around the world. Considering the severity and mortality rate, superbugs are termed as lethal bioterror. Lethal bioterror are biological weapons including microorganisms causing infectious disease (bacteria), viruses and other biological products which leads to the death in animals, humans (Heitz, 2013).

According to the CDC report, superbugs are enlisted as lethal bioterror due to higher rate of morbidity and mortality. They reported that approximately 23000 American die because of multiple antibiotic resistance annually (Heitz, 2013; Walsh, 2014).

Considering the percentage of infections and result, *Acinetobacter baumannii* ought to be at the highest position of the superbug rankings (Palanichamy & Kaliappan, 2010). In 2017, WHO (World Health Organization) published the list of pathogen on the basis of priority or their ability to cause diseases where *Acinetobacter baumannii, Pseudomonus aeruginosa, Enterobacteriaceae* are placed in the critical priority pathogen category (Kieny & Tacconelli, 2017). These bacterial species are very dangerous and difficult to treat because of their gram (-) characteristics. Moreover, they are multidrug resistance. The risk factors that may causes infections are prolonged hospitalization, medical devices such as catheter, mechanical ventilation, hydrotherapy, patients with diabetes, severe burns, malignancies are the most common (Howard, O'donoghue, Feeney, & Sleator, 2012; Parkins, Somayaji, & Waters, 2018; Yang et al., 2013). Patients who are immunocompromised are at high risk to develop infection caused by *A. baumannii, P. aeruginosa* whereas *Enterobacteriaceae* attacks young and healthy individuals to cause infection (Parkins et al., 2018). These superbugs used various resistance mechanism to protect themselves from various antibiotics. *A. baumannii* uses active efflux, enzyme that causes hydrolysis of beta-lactam ring, modify aminoglycoside and chloramphenicol and alter the target which are responsible for the development of resistance (Dijkshoorn, Nemec, & Seifert, 2007). *P. aeruginosa* have intrinsic, acquired and adaptive mechanism to combat against various available antibiotics (Breidenstein, de la Fuente-Núñez, & Hancock, 2011). *Enterobacteriaceae* mainly form biofilm mediated by plasmid and produce enzymes that contributes to the resistance power (Cain, 2015). Treatment option for these superbugs are less as these are resistance to almost all the available antibiotics commonly used to treat them and patients need to stay at hospital for

long time which is very expensive and may leads to the development of nosocomial infections. This review paper mainly focus on three treatment strategies that may reduce the mortality or morbidity rates worldwide including new antibiotic research, drug repurposing or drug repositioning and combination therapy. Although it is very difficult and time consuming to discovery new antibiotics but to fight against these superbugs, a number of newer classes of antibiotics must be discovered (Bassetti & Righi, 2015; Isler & Doi, 2019). Pharmaceutical industries should take initiative to discover newer classes of antibiotics (Bassetti & Righi, 2015). Drug repurposing means to predict the new therapeutic indications or uses of FDA approved existing drug which are used to treat other diseases (Peyclit, Baron, & Rolain, 2019). Combination therapy is the treatment strategy where two or more antibiotics are combined to produce synergistic therapeutic effects (Kapoor & Murphy, 2018).

Aims and objectives of this review are given below:

1. To study the background of the evolution of superbugs, antimicrobial resistance mechanism and its pattern.

2. To explore the strategies to combat antibiotic resistance and superbugs.

Chapter 2

Methodology

This review is done based on the more recent, more relevant articles from high impact factor journals. The data used in this review paper has been collected by searching journal article from Google scholar, PubMed database, etc. While searching for the articles the key words that were used includes antibiotic resistance, superbugs, evolution of superbugs, evolution of *Acinetobacter baumannii,* evolution of *Pseudomonas aeruginosa*, evolution of *Enterobacteriaceae*, carbapenem resistant *Enterobacteriaceae,* epidemiology of *Acinetobacter baumannii*, antibiotic resistance mechanism, antibiotic mechanism, treatment of antibiotic resistance, combination therapy, drug repurposing, new antibiotic research. Searching these keywords lead to more than thousands of articles from the above mentioned journals. From these thousands of articles choice had been made to descend them to narrow them to 94 articles.

Chapter 3

Antibiotic

In modern era, antibiotic (anti-bacterial agent) is considered as powerful weapon to fight against various number of microbial infection because of which many people have lost their valuable life. Presently, it saves life of millions of people.

Antibiotics are classes of medicine that are casted in the treatment of pathogenic disease through killing bacteria or inhibiting (slowing down) their growth (Nordqvist & Carter, 2019; Ventola, 2015; Zaman et al., 2017). Normally, before the phase of multiplication of bacteria and causing symptoms of infection, body's immune system (defense system) combat to eliminate bacteria which is the general function of WBC. Unfortunately, when number of bacteria is excessive, body's natural defense cannot fight against these bacteria. In that case, antibiotics are the only option to treat these bacterial infection (Nordqvist & Carter, 2019).

3.1 History of Antibiotic Development

Historical evidence shows that herbs, honey and even animal feces had been used by ancient civilization in the treatment of various kind of infections. Mouldy bread as a topical agent had a good reputation to treat infection in Egypt, China, Greece and Rome and this medical treatment option was lasting over the years (1567-1640) (McDermott, Walker, & White, 2003). In addition, few of the antibiotic was available on that ancient times including the tittle of tetracycline from the buried human skeletons in Nubia though the source of tetracycline remains in riddle (D, 1980).

After this incident, Robert Koch (1843-1910) and Louis Pasteur (1822-1895) successfully entrenched the relationship within various bacteria and infectious disease. The spread of gonorrhea and syphilis spurred more experimentation, especially among the upper classes,

with possible treatments. Some of the heavy metals like bismuth, arsenic and mercury has been tested and for local or systematical use syringes was set forth. While symptoms have improved, the side effects and usage is seemed to have worst effects over the disease. (Gould, 2016; Playfair, 2004).

Pyocyanase was administered as the first antibiotics for human infection treatments. Rudolf Emmerich (1856–1914) and Oscar Lo¨w (1844–1941) found that the bandages of injured patients contained “green bacteria” which prohibited development of other microbes. Until Paul Ehrlich (1854–1915) began working on dyes' antibacterial impacts were not prominently observed. Salvarsan, a chemical containing arsenic, developed by Ehrlrich in 1909 to treat syphilis, was actually the first antimicrobial agent (Gould, 2016; Strebhardt & Ullrich, 2008). The first patient ever treated with an antibiotic in 1941 was Albert Alexander, a policeman in Oxford, England. He had been scratched by a rose while tending to his garden-a scratch that rapidly became infected with bacteria, possibly Staphylococcus aureus with a mixture of different Streptococci, and turned septic. The sepsis spread, made the patient's head swell with abscesses that required the expulsion of one eye; to put it plainly, he was nearly kicking the bucket. He was treated at the Radcliffe Infirmary by Drs. Howard Florey and Ernst Chain who were blending up concentrates of a mold called *Penicillium chrysogenum* and had blended an extremely little measure of penicillin. While having been found in 1938 by Alexander Fleming, that medication had never really been utilized to treat a human. It was viable in improving the police officer's condition. Sadly, insufficient penicillin had been orchestrated and at the point when the treatment halted, the sepsis thundered back and the patient in the end passed on (Fymat, 2017).

1925 to 1950 was considered as the golden era where high success was rated as natural products flourished in this era by the method of whole cell screens. With the development of sulfonamide and penicillin, the golden era began. (Brown & Wright, 2016). In 1928, after

about 50 years of research and examination by others, Sir Arthur Fleming serendipitously discovered penicillin that was considered as nontoxic and its intensity is very high in the treatment of bacterial infections. Sulfanilamide was first reports in 1933. In the U.S.A, sulfa drugs were being used in the treatment of UTI (urinary tract infection) and in the Japan for the treatment of diarrhea in 1937. Penicillin was first reported in 1938 and Rene Dubos discovered gramicidin in 1939, which is strong yet profoundly dangerous anti-toxin with emotional impacts against gram-positive microbes. In 1940, Selman Waksman discovered and after that named more than twenty potential antibiotic (except penicillin) later recognize 200 of them such as streptomycin, chloramphenicol, tetracycline, and erythromycin. In 1942, Sir Alexander Fleming, the pioneer of penicillin, cautioned about the spread out of the antibiotic resistance. In the middle of 1947, the antibiotic named Cephalosporin was discovered (Fymat, 2017).

1950 to 1975 was termed as the medicinal chemistry era where tweaking rose also by the method of whole cell screen and in the scaffolds of broad spectrum though the chemically modified species showed a very low success rate (Brown & Wright, 2016).

1975 to 2000 the resistance era where target based antibiotics were bought in the market. These antibiotics were made in broad spectrum but yet the success rate was low. However, it was the initiation of modern discovery of antibiotics especially broad-spectrum antibiotics (Brown & Wright, 2016). The antibiotic named carbapenem and fluoroquinolones were invented respectively in 1975 and 1990. Unfortunately, since 1990 no new classes of antibiotic has been discovered (Fymat, 2017).

2000 to 2025 the narrow spectrum era it is the futuristic discovery project for discovering the unknown. It has target of in vivo essentials and it has combinational approaches. Diagnostic development is also a concern of this era.

The predicted success shall be obtained within this span where the development and discovery of antibiotics occurred (Brown & Wright, 2016; Fymat, 2017).

Table 1: Representation of antibiotic drug discovery and development (Adapted from Brown & Wright, 2016).

1925-1950	Golden era	Products obtained from nature, whole-cell screens, tremendous success
1950-1975	Medicinal chemistry era	Synthetic modifications
1975-2000	Resistance era	Latest discovery of antibiotic, selective-based broad spectrum, favorable outcome low
2000-2025	Narrow-spectrum era	Unconventional discovery, combinational approaches, assay development, aimed success.

3.2 Major Antibiotics Currently in Use

It is significant that by 1974, more than 3,000 unique antibiotics and not less than 30,000 derivatives have been introduced. Introduction of new antibiotics proceeds to this date though at a slower pace while just somewhere in the range of forty or so is when all is said in done clinical practice at any one time. It might be noticed that in the last 30 years, no major new sorts of antibiotics have been generated (Fymat, 2017).

Table 2: Major antibiotics currently in use (Adapted and modified from Fymat, 2017).

Class	Major antimicrobial chemicals in use	Infection(s) treated
Aminoglycosides	Amikacin Gentamicin Kanamycin Streptomycin Vancomycin Tobramycin	Meningitis
Cephalosporins	Cefotaxime Cefatoxin	Upper respiratory system
Chloramphenicol-like products	Chloromycetin	Typhoid fever Bacterial meningitis
Erythromycins	Erythromycin	Upper respiratory system Whooping cough Diphtheria Penicillin-resistant staph
Penicillin	Amoxicillin Ampicillin Methicillin Penicillin G Penicillin K	Ear infection Skin staph Upper respiratory system Sore throat Tonsillitis
Streptomycin	Dihydro-streptomycin	Upper respiratory system

3.3 Mechanism of Actions of Antibiotics

One of the most dominant characteristics of antibiotics is that their metabolic pathway can easily be detected on the basis of their mode of action. There are a number of modes of action which antibiotics uses for identifying bacterial cells avoiding human cells.

3.3.1 Interference with Microbial Ability to Make Cell Walls

If cell wall is not rigid then it becomes very easy for antibiotics to invade the cell by rupturing the cell wall. Though some bacteria manages to construct an outer layer replacing cell walls. The mucopeptide which gives the cell its rigidity is the antibiotics target. Few examples include narrow spectrum antibiotics such as Penicillin and its derivatives, Cephalosporin. These two has the capability of inactivating peptidase which gives the cell wall its rigidity. They play a significant role of being nontoxic in normal dosage as they don't target human cell walls or membranes (Fymat, 2017).

3.3.2 Inhibition of Protein Synthesis

Protein synthesis is a complex procedure in a bacterium where majority of the broad spectrum antibiotics inhibit the synthesis of protein by interfering with the processes at the 50S or 30S subunit of the 70S bacterial ribosome. This mode of action includes broad spectrum antibiotics such as Chloramphenicol, Tetracycline, Polymyxin (B and E forms), and Erythromycin (Nierhaus, Brimacombe, & Wittmann, 2006).

3.3.3 Interference with Membrane Synthesis

Bacterial cell membrane act as a target for many antibacterial agents. There are two different types of bacteria which are gram (+) and gram (-) being different from each other in their characteristics surface membrane. Surface membrane of gram (-) bacteria are rich in lipopolysaccharide, the target for many antibiotics. The antibiotics typically binds over

superficial membrane as a result inhibits synthesis of the membrane and closing the direction of matters among cell exterior and cell envelope, causing bacterial destruction.

In Gram (+) bacteria, the main bacterial fatty part, phosphatidylglycerol can be synthetically changed through bacterial enzyme for changing over the fat from anion to zwitterion structure. The mentioned procedure prompts rising in the antibiotic resistance. Inhibitors of this enzyme would cause the bacterial toxicity. Here both aerobic and anaerobic bacteria are included such as cephalosporin (Epand, Walker, Epand, & Magarvey, 2016).

3.3.4 Interference with the Genetic Synthesis (DNA or RNA)

An example of this pathway is Aminoglycosides that have neomycin, kanamycin, gentamycin and tobramycin effect, have broad spectrum antibacterial effect which cause wrong reading of mRNA and as a consequence, cause translation error. In addition, antibiotics can inhibit the replication and transcription process. That is how it inhibits bacterial growth (Kaufman, 2011).

3.3.5 Inhibition of the Folic Acid Synthesis

Folic acid is considered as the synthetic form of folate which is used by the bacteria for the synthesis of nucleic acid that is necessary for the formation of DNA. One of the antibiotic named trimethoprim is used to inhibit the dihydrofolate reductase, an enzyme act as a catalyst which accelerate the last stage of the synthesis of folic acid, resulting in inhibition of folic acid synthesis (Kaufman, 2011; Paridah et al., 2016).

3.3.6 Combination Modes

In general, trimethoprim act as a bacteriostatic rather than bactericidal. However, when trimethoprim combined with sulfamethoxazole, it gives synergistic effect, functioned as a bactericidal (Shin et al., 2014).

Chapter 4

Antibiotic Resistance

Antibiotic resistance have become the major concern in the health care system and increasingly dominant nowadays especially in the developing and developed countries which may cause death of many people (Chokshi, Sifri, & Cennimo, 2019). These antibiotic resistances can leads to the introduction of superbugs. Let us imagine a condition when a person is admitted to a hospital due to bacterial infections and doctor cannot treat this bacterial infection even though he is applying multiple antibiotics because of the emergence of superbugs. According to CDC, approximately two million people become sick due to this emerging superbugs, which is really alarming situation for the global health care system that cannot be ignored (Miller, 2015).

The term “antibiotic resistance” is commonly used to explain the phenomenon of microbes that are less susceptible to antibiotic, microbial agent used to kill or slow down their growth (Chandler, 2019). Antibiotic resistance basically the ability of pathogen to develop or improve various characteristics that render the medication (anti-microbial agent) less effective (Chandler, 2019).

4.1 Bacteria Responsible for Antibiotic Resistance

Table 3: Antibiotic resistances shown by major bacterial species (Modified from Chandler, 2019).

Bacterial species	**Bacterial species shown resistant to**
E. coli	Ampicillin, Imipenem, Meropenem, Amoxiclav, Ciprofloxacin, Co-trimoxazole, Cefotaxime, ceftazidime, ceftriaxone
Klebsiella spp.	Ampicillin, Amoxiclav, Ciprofloxacin, Co-trimoxazole, Cefotaxime, ceftazidime, ceftriaxone, Imipenem, Meropenem
Pseudomonas spp.	Co-trimoxazole, Ciprofloxacin, Imipenem, Meropenem
Enterococcus spp.	Co-trimoxazole, Ciprofloxacin, Vancomycin
Streptococcus pneumonia	Penicillin , Ampicillin
Staphylococcus aureus	Penicillin , Ampicillin, Co-trimoxazole, Amoxicillin Vancomycin, Imipenem
Salmonella spp.	Cefixime, Ceftriaxone
Shigella spp.	Ciprofloxacin, Mecillinam
Acinetobacter spp.	Resistant to all above antibiotics

4.2 Sources of Antibiotic Resistance

To strain the precipitates the antibiotic residues in milk and eggs, microbial inhibition test (MIT) and thin layer chromatography has been used and to get the estimated antibiotic concentration, ultra-high performance liquid chromatography has been used.

Due to the irrational administration of antibiotics as the treatment of mastitis, injection and other diseases, antibiotic residues are found above the maximum approved level. Importantly,

in case of human health, the existence of antibiotic residues in foods of animal produces harmful effect in human whoever takes that animal product. Diseases like cancer, allergic reaction etc. may be developed and finally, antibiotic resistance has been developed which leads to the failure of antibiotic treatment in upcoming future(Chowdhury et al., 2015).

From the study, it has been found that antibiotic residues in commercial dairy product is higher than local. For example, tetracycline, amoxicillin and ciprofloxacin are found in higher concentration in commercial farms than local. Direct boiling of the dairy farm product (either commercial or local) may help to reduce the antibiotic residues but not sufficiently (Chowdhury et al., 2015).

In the treatment of diseases of animals such as in cow, chicken, antibiotic are used frequently. Mainly, in the treatment of mastitis, antibiotic named amoxicillin are used. Around the world, a number of countries follow regulation of drug use in foods. In accordance with U.S official, the maximum level of amoxicillin is 10 μg/kg in milk(Chowdhury et al., 2015).

As antibiotic residues higher than tolerance level leads to allergic reaction, produces toxic effect and other health complications, so the use of antibiotic in dairy product should be effectively monitored and controlled to minimize the health hazard.

4.3 Timeline of Antibiotic Resistance Response (1945-2018)

Over the last 75 years a significant evolution has been made by the resistance of antibiotics. This response in divided into five eras:

Era I: Staph concerns and persisting optimism, 1945–1963

In 1945 alexander Fleming's award acceptance speech has somewhat mentioned about the potential for clinically relevant antibiotic resistance. After 6 years, urges for antibiotic chain were placed by Allen trooper and Howard Holley. However, high chance

of resistance growth is possible by antibiotics such as ethril (a combination of penicillin and itself). (Podolsky, 2018).

Era II: plasmids and the specter of "Superbugs", 1963–1981

In the 1960s and 1970s, there were no potential activity showed by antibiotic resistance especially in the international concern. In 1969 somewhere in Great Britain swann report, it was mentioned that a lot of antibiotics are used for agricultural growth promotion. Though this fact given by Swann was banned. During this span of time within US huge attention was diverted towards the usage of antibiotic resistance. (Podolsky, 2018)

Era III: APUA and the shared global problem of antibiotic resistance, 1981–1992

In the 1970s plasmid-mediated resistance were highlighted with respect to their species and national boundaries. Levy used this term which generated the concept of APUA (Alliance for the Prudent Use of Antibiotics) which was an international degree concerned to educating the health profession and public for knowing the proper usage of antibiotics, sales and labeling, standardized and coordinated police investigation of antibiotic resistance. Nevertheless, in 1980s the Great Britain and America notices very little attention of people towards the outburst of antibiotic resistance. (Podolsky, 2018).

Era IV: emerging infections and the "End of antibiotics", 1992–2013

In the mid-1990s, leadership of antibiotic resistance moved towards Europe whereas US was too busy concentrating on the raising of governmental funding to work for the increasing infections by antibiotic resistance. By then it was completely hold that antibiotic resistance has not only become a worldwide threat but also it was carrying multi-sectorial interventions. Even after getting to know about this drawback of antibiotic resistance reformers were more focused on the existing funds and as lean to forestall the top of antibiotics. (Podolsky, 2018).

Era V: "As important as climate change", 2013–present

The emerging effect of antibiotic resistance was becoming such as "a virtue turning to a curse." The potential use of antibiotics was a forehand for general people getting used to it and developing a curse within their bodies. Devies, chose to see hoe antibiotic resistances reacts to temperature changes which lead to the futuristic measures of the patterns of growing resistance. In 2015, US unleashed its own national setup which they set for Antibiotic-Resistant microorganism by increasing their communications among US agency for their trade, collaborations and international counterparts. Afterwards in 2016 UN called for a high level meeting of its general assembly, which was meant for the creation of awareness among people regarding antibiotic resistance and heath concerns. The main focus was to keep pace of using antibiotics and its overuse. (Podolsky, 2018).

4.4 Mechanism of Action of Antibiotic Resistance

4.4.1 Biofilms: Responsible for Antibiotic Resistance

Adherence of the bacteria to the natural, or synthetic medical equipment or harms tissue envelop surrounding in the protein and hydrated matrix of polysaccharides and forms a thin area termed as biofilm. Bacteria which are resistant as a form of biofilm may involve in the production of long-term infection. For an example, bacteria which are attached to the medical devices may contribute to the chronicity of infection. The resistance mechanism of bacteria in the biofilm are quite different from the known transposons, plasmids and mutations (Stewart & Costerton, 2013).

Capsules or Glycocalyx

Glycocalyx is one of the most important parts of the bacterial biofilms. The glycocalyx matrix helps in the resistance aroused against the antimicrobial agents. This layer of

glycocalyx collects about 25% of the antimicrobial components. The matrix of the glycocalyx has an adsorption site which serves two functions such as the transportation of the biocides and the exoenzyme attachment. The versatility of the exoenzyme helps in the protection of the of the particular agent's movement along with providing a source to the substrate for metabolic degradation which further causes the slow action of the vulnerable drugs (Stewart & Costerton, 2013).

Enzyme-Mediated Resistance

Bactericide is potentially transformed to its nontoxic form with the help of enzymes which results in the resistance of the biofilms. Some of the bacteria responsible for the degradation of this toxic substance are as follows- aromatic, phenolic and other heavy metals (nickel, cadmium, mercury, antimony, silver, copper, zinc, lead, cobalt, *etc*. (Stewart & Costerton, 2013)

The role of biofilm in the antibiotic resistance is very significant. It works by preventing the diffusion of antibiotics into the bacterial cell by glycocalyx, the physiology of the bacteria and appropriate environmental condition where biofilm resides (Stewart & Costerton, 2013).

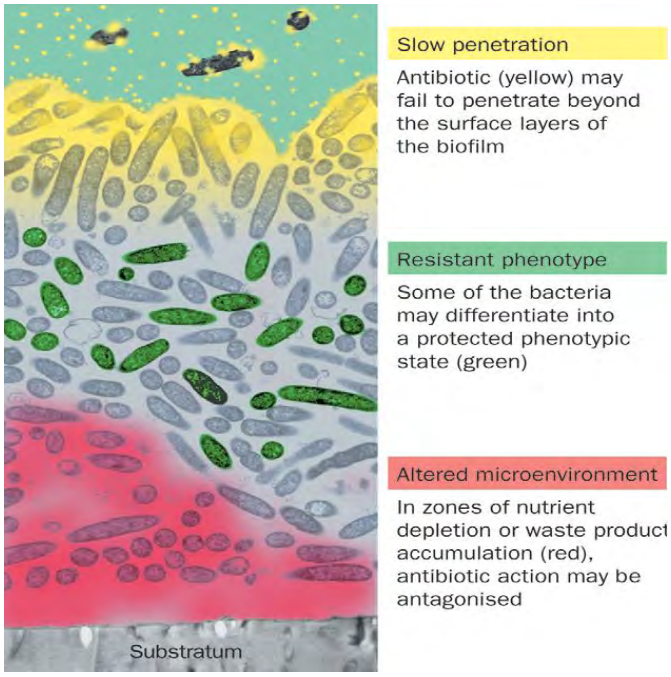

Figure 2: Pathway of resistance by biofilms. (Adapted from Stewart & Costerton, 2013).

4.4.2 Genetic Mechanism of Transmission

Antimicrobial resistance to antibiotics could be one of the following-natural or acquired and the possible way of transmission can be horizontal or vertical. Natural antibiotic resistance is less commonly occurs and it is occurred due to the spontaneous gene mutation which maintains a significant part in the development of antibiotic resistance. Susceptible bacteria can be affected or can develop resistance by the genetic mutation or receiving antibiotic resistance gene from the alternate bacteria. In addition, these resistance gene may be present in the particular fragment of DNA called transposon. With the help of the transposon the resistance gene moves from one plasmid to another. After genetic mutation occurs, a massive change in the bacterial DNA takes place and the gene is shifted from a bacterial plasmid to other by several paths including conjugation, transformation and transduction (Alanis, 2005).

Conjugation

Conjugation is one of the major path for the transmission of genetic material containing the resistance gene in the DNA fragment. In this process, bacterial plasmid can replicate independently of the chromosome. Once the infected bacteria come closer to another bacteria, a bridge like structure is formed among the bacteria called pilus which allows the pathway of DNA splinter (Alanis, 2005).

Transformation

Transformation is another way to transfer resistance gene from the resistance bacterium to the sensitive bacterium. Here, direct transmission of DNA (also known as naked DNA) occurs from the bacteria which have died and broken into the surrounding environment. The recipient bacteria simply accept the free DNA which has advantageous resistance gene into the cytoplasm by inserting it in their own DNA(Alanis, 2005).

Transduction

By this mechanism, genetic material are shifted from the susceptible bacteria to the another bacteria with the help of a carrier (vector). Most often viruses named ‘‘bacteriophages’’ (or simply ‘‘phage’’) which infects the bacteria and essentially takes over the bacterial genetic processes to produce more phage. After the death, lysis or breaking of bacteria, these new phage bacteria start to infect other bacteria by introducing the resistance gene (Alanis, 2005).

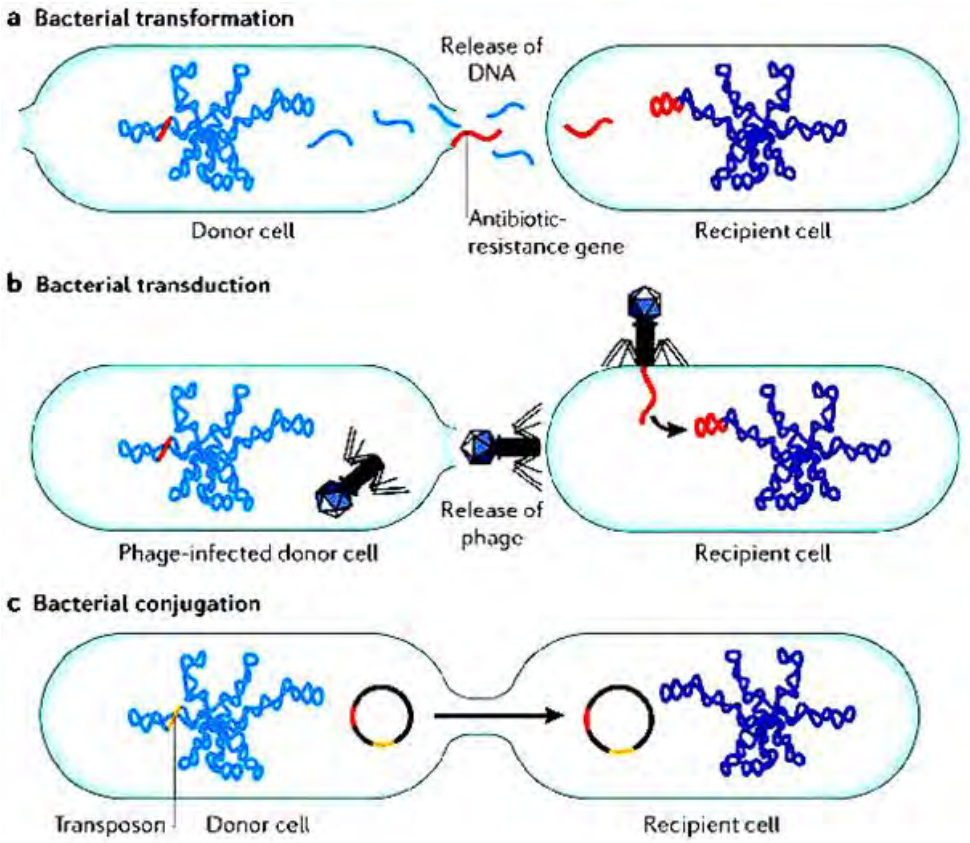

Figure 3: Main Mechanism of Horizontal Gene Transfer (HGT) (a-Bacterial transformation; b-Bacterial transduction; C-Bacterial conjugation) (Adapted from Manage, 2018).

4.4.3 Antibiotic Active Efflux

Antibiotic resistance can be developed by a mechanism known as antibiotic active efflux. Efflux pump is a channel which actively exports the antimicrobial agents and other components which are harmful for the bacteria. In this process, antimicrobial agents are enters into the cell of bacteria by a channel called porin. After that antimicrobial agents are pumped back out of the bacteria by the efflux pump before it reaches the therapeutic concentration inside the bacterium. Thus, by using active efflux pump, bacteria develop antibiotic resistance (Alanis, 2005).

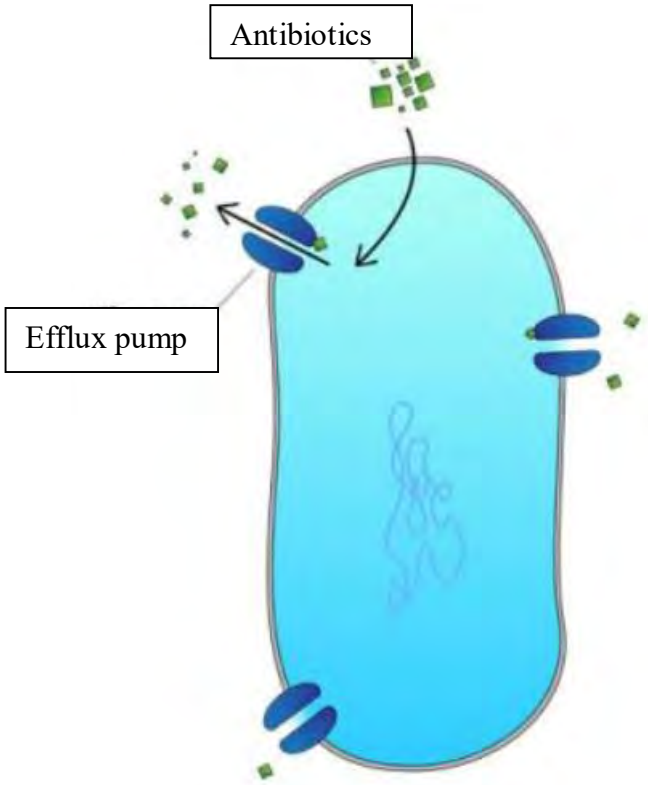

Figure 4: Mechanism of antibiotic resistance by active efflux pump (Modified from Bishai, 2015).

4.5 Role of Antibiotic Resistance in Bangladesh

One of the South Asian countries, Bangladesh has been predicted with higher rate of ABR (Antibiotic resistance) which is a global threat. For the prevailing resistance in this country the first line medications were not even applied (ahmed, 2018). This has been a failure for therapeutic efficacies. The main target was to come up with a standard for the futuristic functions along with creating new policies and guidelines for adapting the supreme way of decreasing the increasing antibiotic resistance. In a survey done in Bangladesh, it was observed that out of there are no documents of data of ABR in 58 districts. This survey was done in 64 districts in Bangladesh. (ahmed, 2018). The efficiency of the antibiotics working against UTI seemed to have a very lower percentage. However this survey was done in Bangladesh its inference was considered worldwide. The resistance strains does not really

remain confined to discrete spaces, if there is abundance of resistance in any region then it can act as a source of reservoir through which it is possible that the strains travel to other parts of the place by spreading anonymously. This spreading is possible by carriers such as animals, humans, water, agricultural products, air, etc. Unfortunately, some of the south Asian countries such as Bangladesh are acting as a very promising source of drug resistant infection (Sultana, 2018). Very soon this condition shall raise the alarming effect of antibiotic use in Bangladesh. Thereby, it must be eradicated and so rigid laws shall be implemented on the usage of antibiotics and professionals should let people know about the proper use of antibiotics. Self-medication of antibiotics should be completely banned and no antibiotics shall be given without authentic prescription.

Chapter 5

Superbugs

There are certain types of bacterial species which are resistant to two or more classes of antibiotic which means these antibiotics are ineffective to treat that special type of bacteria. As a result, millions of people has died due to the evolution and development of these certain types of bacterial species, Superbugs, all over the world (Nordmann et al., 2007).

The term “superbugs” attribute to the special type of bacterial species that are resistant in nature to multiple class of antibiotics which are generally used to terminate or inhibit bacterial growth and to cure infectious disease. Sometimes they are considered as multidrug resistant bacteria because of having the multiple resistance mechanism against multiple antibiotics class (Bravo, Ruiz-cruz, Alkorta, & Espinosa, 2018; Miller, 2015).

5.1 List of Superbugs

Two years back, in 2017, WHO published a list containing bacterial species that are multiple antibiotic resistant in nature and are big threats for the human being. These types of bacteria are powerful in the sense that they can make the antibiotic ineffective by transferring gene material from susceptible bacteria continuously. WHO categorize them in three segments on the basis of their frequency of causing death, frequency of developing multiple antibiotic resistance, available treatment option means available antibiotic to treat them (Kieny & Tacconelli, 2017).

Three categories are named as:

1. Critical priority pathogen

2. High priority pathogen

3. Medium priority pathogen

In the critical priority pathogen category, there are all the multidrug resistant bacteria that are found in nursing home, hospital setting even in the ICU and responsible to cause deadly infections e.g., pneumonia (Kieny & Tacconelli, 2017).

Whereas, in the high and medium priority pathogen category, there are other bacteria which are developing multidrug resistance and responsible for common diseases.

Table 4: Priority pathogen catalogue by WHO (Modified from Kieny & Tacconelli, 2017).

Category	**Bacterial Species**	**Resistant to**
1. Critical Priority Pathogen	1. *Acinetobacter baumannii* 2. *Pseudomonas aeruginosa* 3. *Enterobacteriaceae*	1. Carbapenem-resistant 2. Carbapenem-resistant 3. Carbapenem-resistant ESBL producing
2. High Priority Pathogen	*1. Enterococcus faecium* *2. Staphylococcus aureus* *3. Helicobacter pylori* *4.Campylobacter spp* *5.Neisseria gonorrhoeae*	1. Vancomycin-resistant 2. Methicillin-resistant 3. Clarithromycin-resistant 4. Fluoroquinolone-resistant 5. Cephalosporin-resistant Fluoroquinolone-resistant
3. Medium Priority Pathogen	*1. Shigella spp* *2. Streptococcus pneumonia* *3. Haemophilus influenzae*	1. Fluoroquinolone-resistant 2. Penicillin-non-susceptible 3. Ampicillin-resistant

This review paper mainly focus on risk factors, resistance mechanism and evolution of critical priority pathogens including *A. baumannii, P. aeruginosa and Enterobacteriaceae.*

5.2. *Acinetobacter baumannii*

A. baumannii is basically a gram (-) coccobacilli in nature which is named as nosocomial, opportunistic bacteria because of their occurrence in the hospital and nursing home among the patients suffering from infections because of their prolonged stay in the hospital premises (approximately 90 days) (Howard et al., 2012). This type of bacteria form colony in the skin of the host and attack the host (Howard et al., 2012). *Acinetobacter baumannii* is considered to be sensitive to most of the first-line antibiotics in 1970s (Hawkey et al., 2018). Unfortunately, after 1970s for the first time resistance to first-line antibiotics has been emerged including sulfonamides, aminoglycosides and tetracycline (Hawkey et al., 2018). Moreover, within the period 1980-1990, resistance to fluoroquinolones, third-generation cephalosporin has been developed (Antunes, Visca, & Towner, 2014; Yang et al., 2013). Carbapenem, an antibiotic, thought to be effective against MDR pathogen (Antunes et al., 2014). Sadly, *A. baumannii* become resistance to carbapenem, become superbug. Treatment option available for infection caused by *A. baumannii* are polymyxins (colistin) and tigecycline (Hua et al., 2017). The Infectious Diseases Society of America categorized it as one of the most important pathogen responsible for MDR, considering its mortality rate which is around 35% (Antunes et al., 2014). It can cause wide range of infections such as pneumonia, bacteremia (bacteria present in blood), UTI, bloodstream infection, meningitis which is alarming situation for both community acquired and nosocomial infection (Yang et al., 2013).

5.2.1 Risk Factors of *A. baumannii* Infection

There are certain risk factors for patients that contribute to development of *A. baumannii* infection given below-

1. Patients who are admitted into ICU due to severe illness for prolonged period of time as they are immmunocompromised,

2. Patients who need medical devices such as sutures, catheters and ventilators,

3. Patients undergoing antimicrobial therapy, hydrotherapy (used to treat burn), dialysis within their past 80-90 days.

4. Patients who are suffering from pneumonia and needed mechanical ventilators

The above mentioned patients are at high risk of developing *A. baumannii* infection (Dijkshoorn et al., 2007; Howard et al., 2012).

5.2.2 Antibiotic Resistance Mechanism of *A. baumannii*

A. baumannii uses various mechanism to develop resistance against a number of antibiotics. Commonly used resistance mechanisms are β-lactam hydrolysis, target alteration, active efflux, chloramphenicol modification, aminoglycoside modification, changes in outer membrane proteins which contribute to the development of resistance and exerts resistance to cephalosporins, aminopenicillins, tobramycin, gentamycin, amikacin, kanamycin, streptomycin, chloramphenicol, aminoglycosides, quinololones, tetracycline carbapenem etc. antibiotics.

Table 5: Resistance mechanism of A. baumannii (Modified from Dijkshoorn et al., 2007).

Antibiotic Resistance Mechanism	Target Drug
β-lactam hydrolysis	Cephalosporins, Penicillins, Aminopenicillins, Carboxypenicillins, β-lactams, such as extended-spectrum cephalosporins, Cefotaxime, Oxacillins, Carbapenems
Aminoglycoside modification	Tobramycin, Gentamicin, Amikacin, Kanamycin, Streptomycin
Chloramphenicol modification	Chloramphenicol
Target alteration	Quinolones, Aminoglycosides
Active efflux	Broad (Aminoglycosides and quinolones), Minocycline, Tetracycline
Changes in outer-membrane proteins (OMPs)	Carbapenems

5.2.3 Evolution of *A. baumannii* as Superbug

Before 1970s, *A. baumannii* thought to be susceptible to most classes of antibiotics. At that time, first-line antibiotics has been applied to treat infection caused by *A. baumannii*. But, in 1970s, for the first time *A. baumannii* has been identified as resistant to first-line antibiotics including aminoglycoside, tetracycline, sulfonamides (Hawkey et al., 2018; Yang et al., 2013).

After that, during the period of 1980-1990s, resistant to third-generation cephalosporin has been emerged for the first time (Hawkey et al., 2018; Yang et al., 2013). Third generation cephalosporin are antibiotic that are effective against a wide range of bacterial infection.

Carbapenem (such as imipenem and meropenem) is an antibiotic that are effective against multidrug resistant bacteria. It is a matter of sorrow that recently carbapenem is not working in the treatment of the infection caused by *A. bauamnnii* (Hua et al., 2017; Ku et al., 2015). Within the period of 2001-2003, sensitivity to carbapenem began to decrease steadily. Comparatively, sensitivity to carbapenem was 88.1% between 2001-2003 which was greatly reduced to less than 25% within 2010-2012 (Qureshi et al., 2015). Moreover, there is no or slightly sensitivity difference between meropenem and imipenem. During the time of 2007-2009, *A. baumannii* shows less sensitivity to meropenem compared to imipenem (Antunes et al., 2014; Ku et al., 2015).

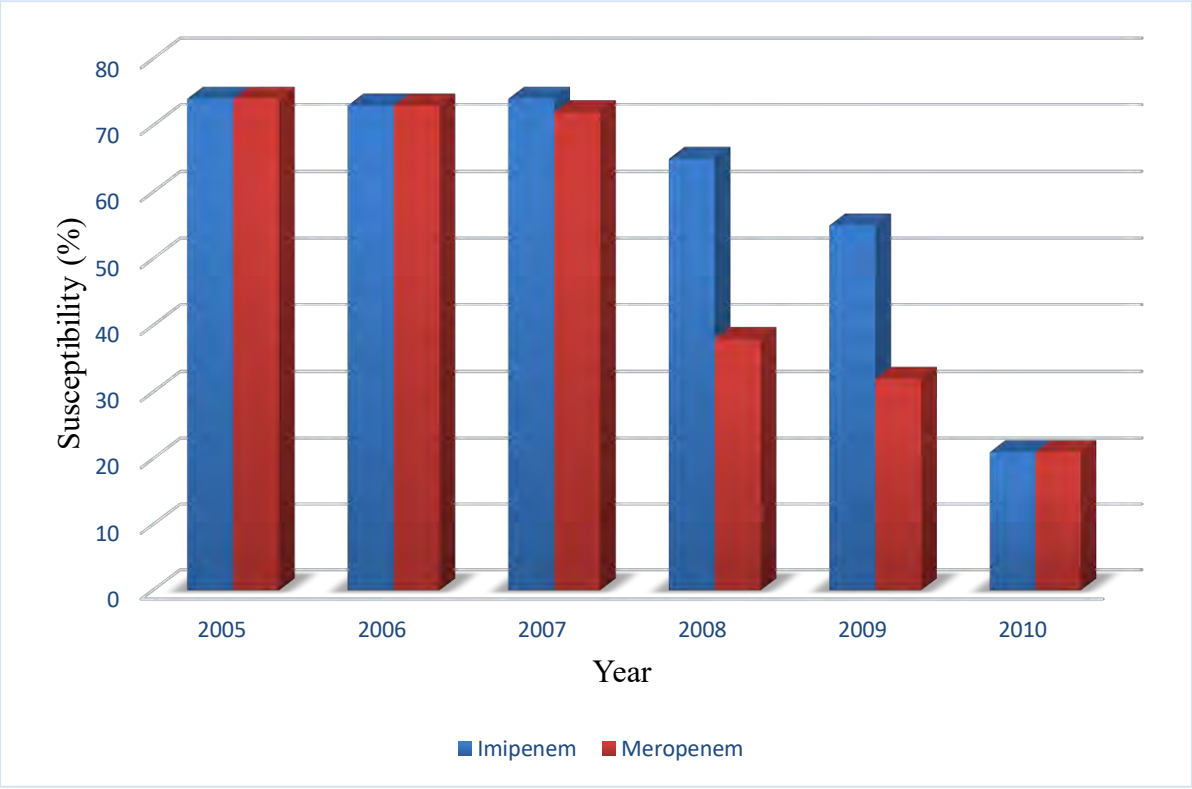

Figure 5: Comparison of sensitivity pattern to Imipenem and Meropenem (2005-2010) (Modified from Ku et al., 2015).

From then till now, resistance to carbapenem has gradually increased. To overcome this problem, tigecycline had been introduced by altering the chemical structure of tetracycline to

improve broad spectrum activity. It was approved for the first time by FDA (Food and Drug Administration) on June, 2005 (Greer, 2006).

Table 6: Sensitivity pattern of A. baumannii to various available antibiotics (2015-2017) (Modified from Huang et al., 2019).

Antibiotic Name	Resistance (in percentage)		
	2015	2016	2017
Ampicillin	100	100	100
Ceftaetan	100	100	100
Aztreonam	100	94.7	100
Piperacillin tazobactam	73.7	78.9	81.8
Amikacin	63.2	68.4	72.7
Tobramycin	78.9	84.2	95.5
Sulfamethoxazole	73.7	78.9	86.4
Ampicillin sulbactam	57.9	73.7	81.8
Levofloxacin	63.2	73.7	86.4
Imipenem	63.2	78.9	90.9
Cefepime	73.7	78.9	90.9
Furazolidin	100	94.7	100
Cefatriaxone	73.7	84.2	95.5
Gentamicin	68.4	78.9	95.5
Ciprofloxacin	68.4	78.9	95.5
Ceftazidime	73.7	78.9	95.5

A. baumannii remain susceptible to tigecycline about 80% (Greer, 2006; Qureshi et al., 2015). Although sensitivity to polymyxin (such as colistin) has been stable and it is 100% susceptible, but its application is limited due to the excessive side effects of colistin (Gogou

et al., 2011). To determine the sensitivity pattern of *A. baumannii* to various class of antibiotics, a study had been performed in the general ICU of Suizhou Central Hospital for the period of 2015-2017 where *A. baumannii* showed 100% resistance to ceftaetan and ampicillin respectively. Resistance rate to other antibiotics were above 65% (Huang et al., 2019).

Finally, WHO Classified *A. baumannii* as a superbug in 2017, considering its ability to protect themselves from multiple antibiotics (Kieny & Tacconelli, 2017)

The situation might lead to an epidemiological disaster. It could be happened that *A. baumannii* would be 100% resistance to other antibiotics as the resistance rate is continuously increasing day by day.

5.3 *Pseudomonas aeruginosa*

Pseudomonus aeruginosa, an omnipresent, rod shaped opportunistic gram (-ve) bacteria. It is termed as opportunistic bacteria because of its nature to attack the host during weakened immunity (Yayan, Ghebremedhin, & Rasche, 2015). This bacteria attacks healthy host very rarely (Jenny & Kingsbury, 2018). Sometimes it is called as nosocomial infection considering its prevalence to hospital environment (Lister, Wolter, & Hanson, 2009). *P. aeruginosa* can be found in water, soil. They have the power to adjust with different environmental conditions and needed minimal nutritional supply to survive. *P. aeruginosa* causes a wide variety of infections including pneumonia, bacteremia, UTI infections, and lung infections in CF (cystic fibrosis) patient (Yayan et al., 2015). Infection could be transmitted in hospital settings by supportive personnel, nurse, antiseptics, disinfectants, medical devices such as catheter, mechanical ventilations. Being nosocomial infection, it is can affect chronically ill patient and causes death especially in patient with hospital acquired pneumonia (Jenny & Kingsbury, 2018; Yayan et al., 2015). A report by National Nosocomial Infection

Surveillance System (NNIS) showed that between the year of 1992-1997, P. aeruginosa causes 21% pneumonia, 3% bacteremia, 10% UTI in USA. Whereas, in Europe it causes 30% pneumonia, 10% bacteremia and 19% of UTI which is greater than infection occurs in USA (Lister et al., 2009). This is due to the geographical position.

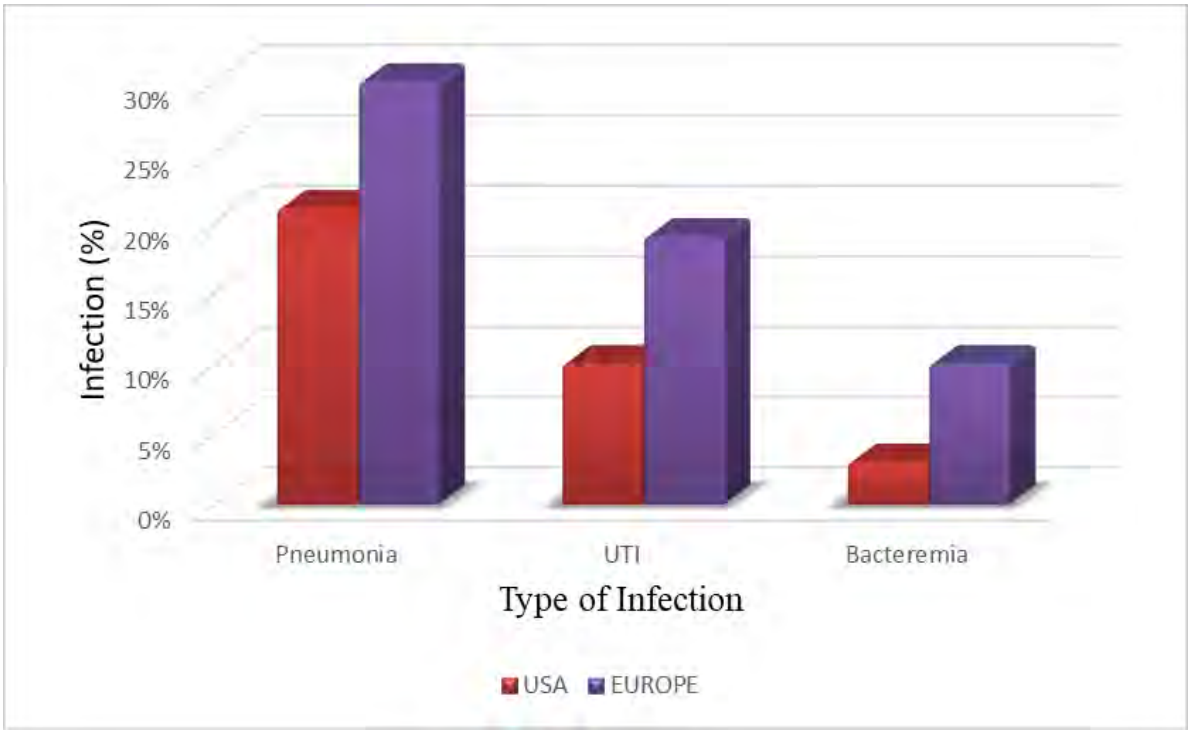

Figure 6: Percentage of infection caused by P. aeruginosa in USA and Europe (Modified from Lister et al., 2009).

In 2017, *P. aeruginosa* classified as superbug by WHO considering its resistance to multiple classes of antibiotics and its resistance rate.

5.3.1 Risk Factors for Patients Infected by *P. aeruginosa*

There are a number of risk factors for patients that contribute to the infection which are caused by *P. aeruginosa.*

1. Patients with weakened immune system and prolonged hospitalization are at risk for infection (McCarthy, 2015; Parkins et al., 2018).

2. Patients with lung infections (cystic fibrosis) are at high risk to develop infection. It is important to mention that patient with cystic fibrosis has persistent lung infection (Jiang et al., 2017).

3. Patients with caners, severe burns and neutropenia are at risk to contribute infection (Feng et al., 2017; McCarthy, 2015).

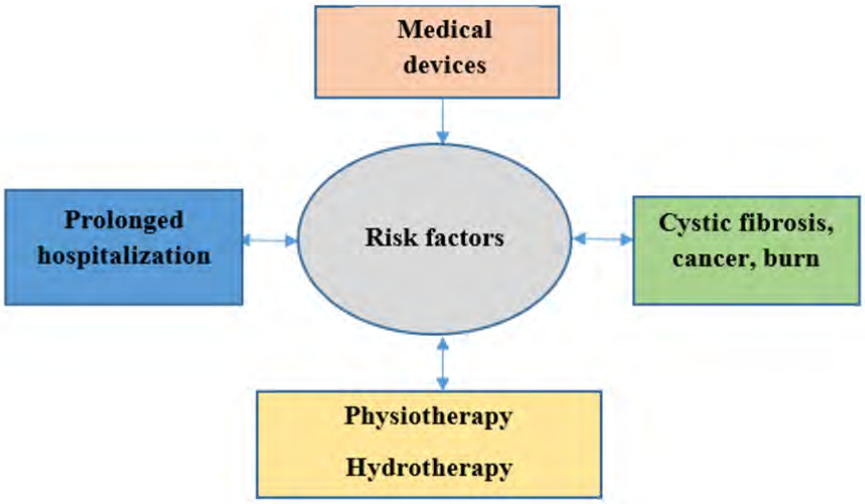

Figure 7: Major risk factors for patients infected by P. aeruginosa (Modified from McCarthy, 2015).

4. Medical devices such as central vascular catheter, urinary catheter and ventilation machine are also the risk factors for patients infected by *P. aeruginosa* (Feng et al., 2017; McCarthy, 2015).

5.3.2 Resistance Mechanism of *P. aeruginosa*

Being a gram (-) bacteria, *P. aeruginosa* has two membranes in its structure which are inner membrane (also called cell membrane) and outer membrane. By using different types of mechanism it exhibits resistance to almost all classes of available antibiotics. They are intrinsic mechanism, acquired mechanism and adaptive mechanism (Pachori, Gothalwal, & Gandhi, 2019).

In intrinsic mechanism, *P. aeruginosa* uses its normal physiology to show resistance. Here, any modification of the physiological structure is not necessary. Interestingly, target is not present for drug in them and low outer membrane permeability, narrow porins in the outer membrane does not allow antibiotics to enter into the cell. Moreover, it produces β- lactamase which causes development of resistance. As a consequences, *P. aeruginosa* shows resistance to β-lactam (monobactam, carbapenem, cephalosporin), quinolone (ciprofloxacin), macrolide (azithromycin, Chloramphenicol) (Breidenstein et al., 2011).

In acquired mechanism, *P. aeruginosa* changes it normal physiology to become resistance including either horizontal gene material transfer or mutation which leads to reduce uptake of antibiotics and overexpression of efflux pump to develop resistance mechanism. In addition, hyperproduction of β-lactamase is also responsible for the development of resistance mechanism. Using this mechanism it shows resistance to aminoglycoside, β-lactam, tetracycline, sulfonamide, imipenem, fluoroquinolone, tobramycin, cefepime (Breidenstein et al., 2011).

Table 7: Overview of various types of resistance mechanism shown by P. aeruginosa (Modified from Breidenstein et al., 2011).

Type of Resistance	Mechanism	Target Antibiotics
Intrinsic	Lower outer membrane permeability, β-lactamase production, overexpression of efflux pump	β-lactam antibiotics (monobactam, carbapenem,), quinolone (ciprofloxacin), macrolide (azithromycin, chloramphenicol).
Acquired	Horizontal transfer of gene material, Mutation leading to decrease in uptake and efflux pump overexpression, β-lactamase hyperproduction	Aminoglycoside, β-lactam (Penicillin, cephalosporin, carbapenem), tetracycline, sulfonamide, tobramycin, cefepime, imipenem.
Adaptive	Changes in gene expression, Efflux pump overexpression,	Tetracycline, polymyxin, colistin, ceftazidime, penicillin, ceefotaxime, fourth generation cephalosporin, cefepime

In adaptive mechanism, *P. aeruginosa* exhibits changes in gene expression including β-lactam, overexpression of efflux pump. They may form biofilm which trigger many factors causes overexpression of efflux pumps that pump out many antibiotic from the cell.

Antibiotic that become resistance due to this mechanism are tetracycline, polymyxin, ceftazidime, penicillin, cefotaxime, fourth generation cephalosporin (Strateva & Yordanov, 2009).

5.3.3 Evolution of *P. aeruginosa* as a Superbug

P. aeruginosa is considered as critical priority pathogen in the list of pathogen published by WHO (Lister et al., 2009). It is very dangerous for patient with weakened immunity. It can be isolated from various sources such as water, soil, vegetable (Lucca et al., 2018). Before 1882, *P. aeruginosa* cannot be isolated in its pure form (Lister et al., 2009). Surprisingly, in 1882, Carle Gessard for the first time isolate the pure culture of this *P. aeruginosa* (Lister et al., 2009). Within 1889-1894, several reports marked *P. aeruginosa* responsible for purulent in patient with wounds. Once it was thought to be susceptible to all available antibiotics (Lister et al., 2009). Unfortunately, during 1986-1998, NNIS (National Nosocomial Infection Surveillance) system reported it as fifth most rapidly isolated hospital acquired infection, second most causative agent of ventilated acquired pneumonia (14-16%), third most leading causes of UTI infection in USA. Sadly, very rapidly it had shown resistance to a number of antibiotics commonly used to treat these infectious diseases (Lister et al., 2009). In 2002, Meropenem Yearly Susceptibility Test Information Collection (MYSTIC), a worldwide multicenter surveillance, had exhibit resistance pattern of various available antibiotics where cefepime showed 24.1%, ciprofloxacin showed 36.2%, ceftazidime, meropenem, imipenem, gentamycin, tobramycin showed 55.8%, 33.1%, 36.1%, 52.4%, 55.4% respectively (Mendes, Oplustil, Sakagami, Turner, & Kiffer, 2005).

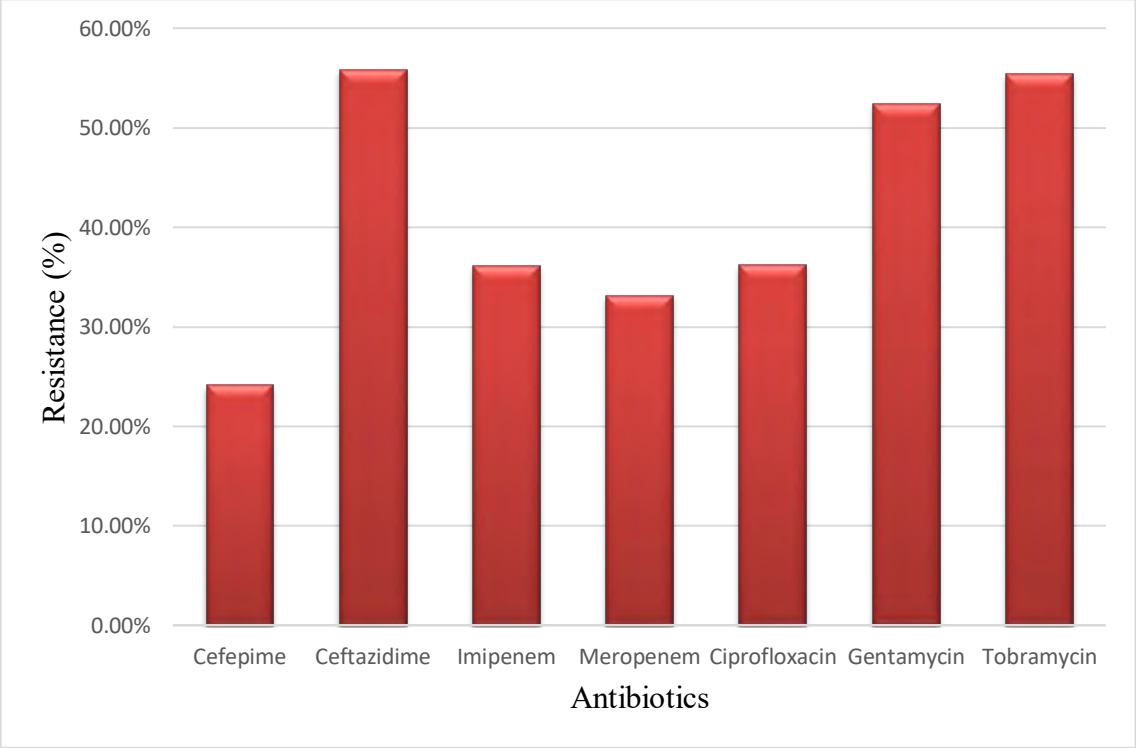

Figure 8: Resistance pattern of P. aeruginosa in 2002 by MYSTIC (Modified from Mendes et al., 2005).

Resistance rate to various available antibiotics were increased overtime. To prove that, another experiment has been performed in Ruijin hospital, Shanghai, China from during the time from 2007-2012 which had shown gradual increase in resistance rate of *P. aeruginosa.* This study had been performed by using Kirby-Bauer disk diffusion method, a method used to determine bacterial sensitivity to antimicrobial agents. The test is performed for some of the anti-gram (-) antibiotics including amikacin, ceftazidime, imipenem, meropenem, ciprofloxacin (Dou, Huan, Guo, Zhou, & Shi, 2017).

Table 8: P. aeruginosa exhibits resistance to anti-gram negative antibiotics by Kirby-Bauer disk diffusion method (Modified from Dou et al., 2017).

Antibiotic	Resistance in percentage					
	2007	2008	2009	2010	2011	2012
Amikacin	42.7	22.5	45.7	61.8	73.3	88.9
Ceftazidime	26.9	22.5	60.9	20.8	32.6	14.3
Imipenem	42.3	45	58.7	74	77.9	87
Meropenem	38.5	57.5	54.3	74	72.9	88.4
Ciprofloxacin	53.8	52.5	58.70	14.3	18.6	17.1

Resistance of *P. aeruginosa* not only increased in human but also increased in animals especially among dogs and cats. It is proved by performing an experiment in Colombia in 2013, by isolating samples from dogs which showed 100% resistance to cephalosporin, penicillin, lincosamide, sulfonamide, chloramphenicol and 90% resistance to macrolide, 80% resistance to tetracycline, 90% resistance to meropenem (Bernal-Rosas, Osorio-Muñoz, & Torres-García, 2015).

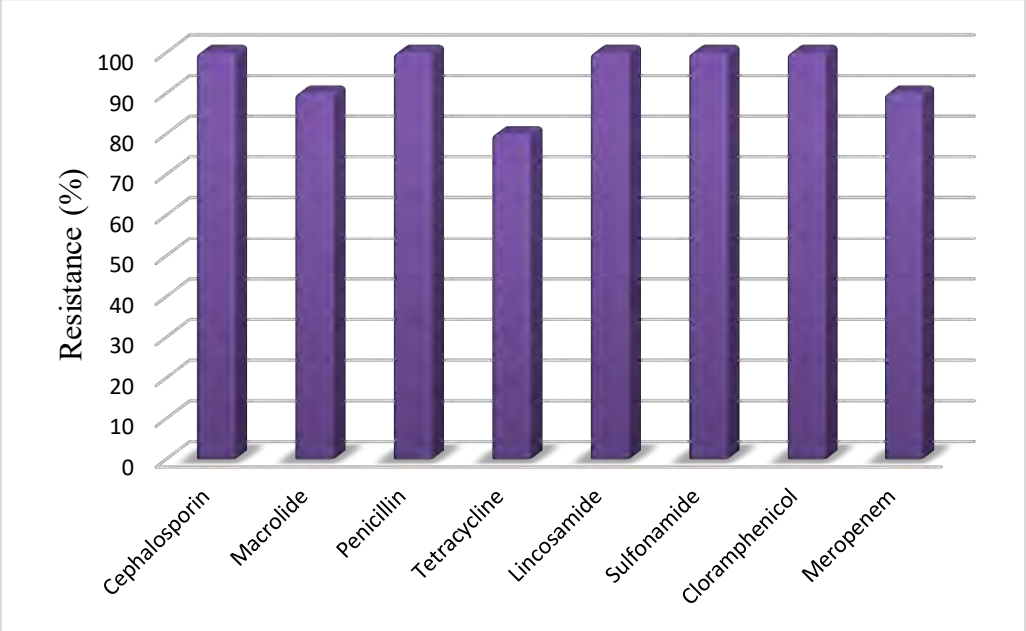

Figure 9: Resistance pattern of P. aeruginosa in dog (2013) (Modified from Bernal-Rosas et al., 2015).

Another study had been performed in Tehran, Iran showed the resistance pattern in patients with burn injuries. *P. aeruginosa* strains were collected from the patients who had burn injuries. Samples were collected from 2012-2015, which had shown gradual increase in resistance to various available antibiotics. *P. aeruginosa* showed 100% resistance to some of the antibiotics including piperacillin, cefazolin, ceftriaxone, tigecycline. In 2015, *P. aeruginosa* had shown almost 100% resistance to other types of antibiotics such as gentamicin (95%), amikacin (95%), imipenem (96%), cefepime (96%), meropenem (96%), ciprofloxacin (97%), ceftazidime (95%), azthreonam (97%), ticarcillin (99%), doripenem (95%) (Tarashi, Goudarzi, Erfanimanesh, Pormohammad, & Hashemi, 2016).

Table 9: Overview of comparison of resistance of P. aeruginosa to burn patient (2012-2015) (Modified from Tarashi et al., 2016).

Antibiotic	Resistance in percentage			
	2012	2013	2014	2015
Gentamicin	49	72.34	95	95
Amikacin	79	80	83.8	95
Imipenem	83	78.7	93.5	96
Cefepime	87	68.08	85.5	96
Meropenem	83	74.46	87.1	96
Ciprofloxacin	88	68.08	88.7	97
Ceftazidime	83	72.34	66.1	95
Azthreonam	84	82.97	62.9	97
Ticarcillin	-	-	95.1	99
Doripenem	-	-	88.7	95
Piperacillin	67	72.6	82.4	100
Cefazolin	72	89.4	84.4	100
Ceftriaxon	68.9	79.6	88.3	100
Tigecycline	57.5	86.1	87.6	100

From the table it can be depicted that, in 2015 *Psedomonos aeruginosa* grew 95-100% resistance against the above mentioned antibiotics. Who knows in the future whether this bacteria will grow 100% resistance for all the available antibiotics or not.

5.4 *Enterobacteriaceae*

Carbapenem resistant *Enterobacteriaceae* is a bacterial including *Klebsiella, E.coli, Salmonella, Shigella* that are resistance to carbapenem, a broad spectrum antibiotic that are effective against multidrug resistance bacteria, due to the production of carbapenemase which is enzyme that hydrolyze carbapenem (Singh-Moodley & Perovic, 2016). *Enterobacteriaceae* are resistance to various available classes of antibiotics including β-lactam, aminoglycoside, fluoroquinolone, polymyxin using various mechanism such as lower outer membrane permeability, efflux pump, enzyme that hydrolyze the antibiotics (Codjoe & Donkor, 2017).

Thus, treatment option for *Enterobacteriaceae* has become limited to colistin and tigecycline (Codjoe & Donkor, 2017). We will discuss one species of *Enterobacteriaceae* which is *Klebsiella pneumoniae. Klebsiella pneumoniae* is a rod shaped, gram (-) opportunistic bacteria that is responsible for various infection such as pneumonia, bloodstream infection, bacteremia specially in the patients with diabetes, malignancies (Lee et al., 2017). Two decades back, during 1980s-1990s, "hypervirulent" *Klebsiella pneumoniae* (hvKP) had been emerged for the first time in southeast Asia and Taiwan with severe community acquired infection in young and healthy individuals (Lee et al., 2017). *K. pneumoniae* uses various resistance mechanism to exhibit its virulence effects (Bengoechea & Sa Pessoa, 2019). The two main mechanism are formation of biofilm and production of enzyme which has been described in upcoming part of this paper (Livorsi et al., 2018).

5.4.1 Risk Factors of *K. pneumoniae* Infection

There are some most common risk factor for patient which may contribute to the *K. pneumoniae* infection, including-

1) Prolonged stay in the hospital (patient with immune compromised condition) (Gasink, Edelstein, Lautenbach, Synnestvedt, & Fishman, 2009)

2) Use of medical devices including catheter (central, urinary catheter)

3) Malignancies (Gastrointestinal, bile, liver malignancy) (Gasink et al., 2009)

4) Patient with solid organ transplantation history, diabetes mellitus (Gasink et al., 2009).

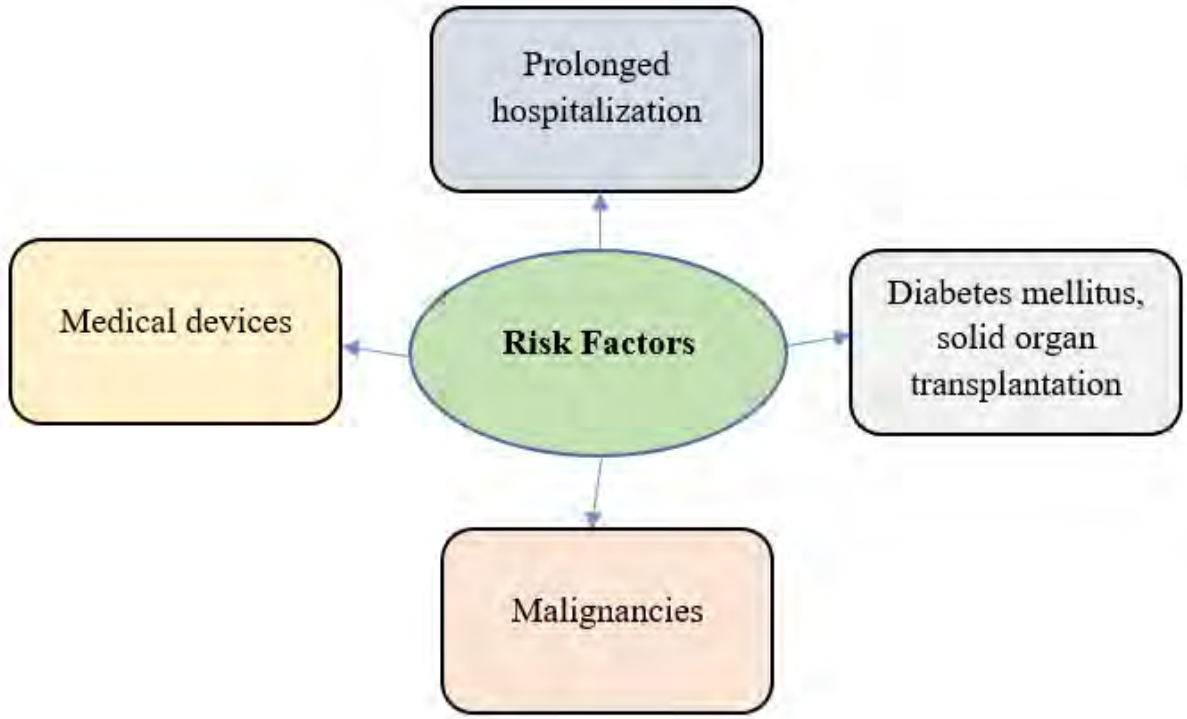

Figure 10: Most common risk factors for patients infected with K. pneumoniae (Modified from Gasink et al., 2009).

5.4.2 Resistance Mechanism of *Klebsiella pneumoniae*

K. pneumonia is considered as multidrug resistance (MDR) bacteria due to the expression of resistance over almost all available antibiotics (Eichenberger & Thaden, 2019). Two major mechanism used *by K. pneomoniae* are the enzyme production that basically hydrolyzed the antibiotics used to treat infection caused by this bacteria and biofilm formation that protect the bacteria from the antibiotics (Cain, 2015). Other mechanism is also involved but these are not thoroughly researched. *K. pneumoniae* shows resistance to almost all available antibiotics including beta lactam, aminoglycoside, fluoroquinolones, sulfamethoxazole, trimethoprim and so on (Cain, 2015; Eichenberger & Thaden, 2019).

Enzyme: *K. pneumoniae* produces certain enzymes that are encoded on plasmid and *K. pneumoniae* can easily take to deactivate the drug molecule. The enzymes include Extended Spectrum Beta-Lactamases (ESBLs), *K. pneumoniae* Carbapenemases (KPCs), New Delhi

Metallo-Beta Lactamases (NDMs). It has been found that ESBLs carry certain type of gene which improve the resistance ability against beta lactam antibiotics and aminoglycosides. Metallo-Beta Lactamases (MBLs) normally uses hydrolysis mechanism that means hydrolyze beta lactam rings in which it uses divalent Zn^{2+} cation which is most common type that leads to the nucleophilic attraction of β-lactam rings and cause resistance to fluoroquinolones, sulfamethoxazole. *K. pneumoniae* carbapenemase (KPC) also hydrolyze β-lactam rings and exhibit resistance to penicillin, third and fourth generation cephalosporin, aztreonam, carbapenem (Cain, 2015; Eichenberger & Thaden, 2019).

Biofilm formation: *K. pneumoniae* also form biofilm which protect the KPC from the antibiotic attack as it reduces the power of the antibiotics to penetrate the *K. pneumoniae* outer membrane. This biofilm formation Showed resistance to aminoglycoside (Cain, 2015).

Other mechanism: A study that had been performed in France found that *K. pneumoniae* strains had resistance mechanism through efflux pump by using this mechanism it showed resistance to chloramphenicol, quinolone, macrolide, nalidixic acid and erythromycin (Cain, 2015; Eichenberger & Thaden, 2019).

5.4.3 Evolution of *K. pneumoniae* as a Superbug

Two decades back, for the first time, in between 1980s-1990s, hypervirulent *K. pneumoniae* had been emerged in the Southeast Asia and Taiwan (Lee et al., 2017). The main difference between classic *K. pneumoniae* and hypervirulent *K. pneumoniae* (hvKP) in their pathogenicity where hvKP causes severe infection than classic *K. pneumoniae* in people including young and immnunocompromised people. After the first observation of hvKP in the mid-1980s, endemic and sporadic spread of hvKP has been observed (Lee et al., 2017). Endemic means a disease that occurs continuously amongst the people of certain area or region. On the other hand, sporadic means a disease that occurs occasionally or irregularly

(Heidary, Nasir, Dabiri, & Tarashi, 2018). In 1996, a report had been shown that endemic spread of hvKP had been observed in Taiwan, China, Iran and South Korea whereas sporadic spread of hvKP had been observed in United states and Japan (Lee et al., 2017).

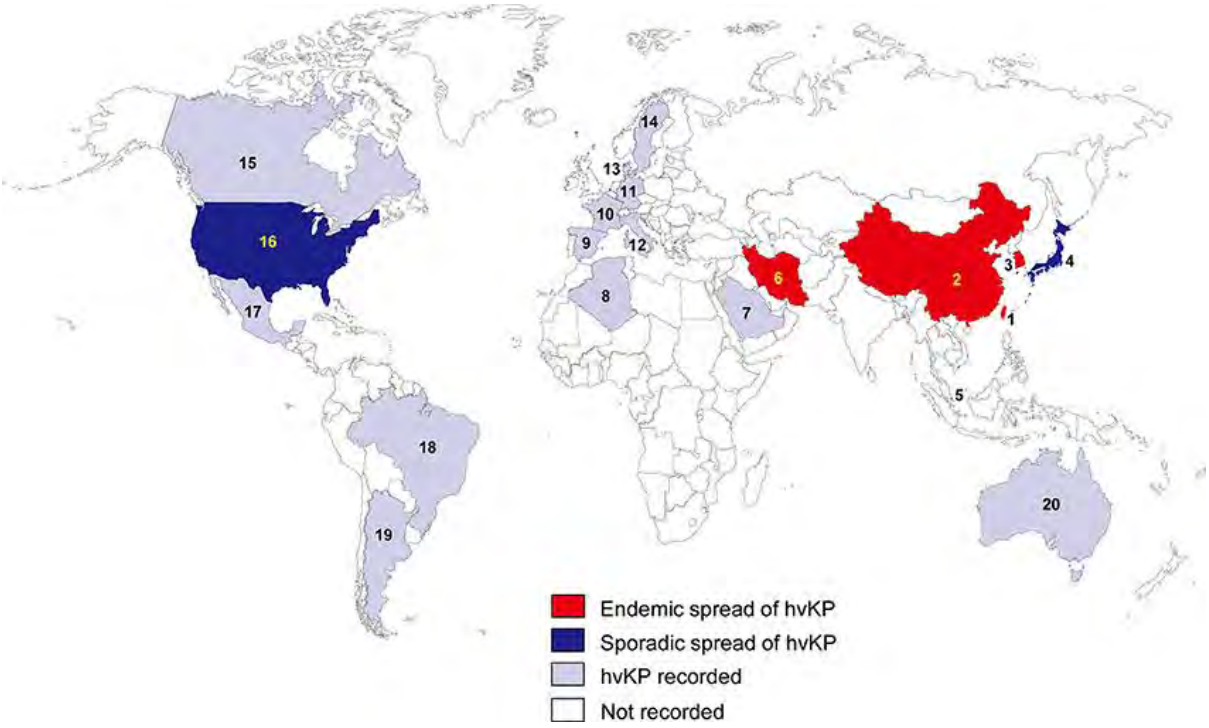

Figure 11: Representation of endemic and sporadic spread of hvKP where 1. Taiwan, 2. China, 3. South Korea, 4. Japan, 6. Iran, 16. United States (Adapted from Lee et al., 2017).

The prevalence of *K. pneumoniae* was increased continuously. The resistance rate was also increased. Most of the K. pneumoniae are ESBLs producing which means *K. pneumoniae* produces enzyme that is extended spectrum beta lactamase. Study performed at two hospitals in Brazil in 2006 showed that *K. pneumoniae* strains with ESBLs producing exhibit higher resistance rate compared to non-ESBLs producing strains (Pimenta & Alves, 2008).

Table 10: In vitro resistance rate of Non-ESBLs and ESBLs producing strains of K. pneumoniae in two hospitals in Brazil, 2006 (Modified from Pimenta & Alves, 2008).

Antibiotic	Non-ESBLs strains (N=30)	ESBLs strains (N=24)
Ampicillin	66.70%	100%
Ampicillin/sulbactam	6.70%	87.50%
Piperacillin	26.70%	87.50%
Cefalotin	20.0%	91.70%
Cefpodoxime	-	100%
Tobramycin	6.70%	70.80%
Trimethoprim	33.30%	100%

Another report had been published in 2007 in Peshawar which showed the resistance and susceptibility pattern of *K. pneumoniae* for available antibiotics (Ullah, Malik, & Ahmed, 2009).

From the report it has been observed that ampicillin (AMP) was 100% resistance and 00.0% susceptible, amoxicillin+clavulanic acid (AMC), cephradine (CE), cefaclor (CEC), cefpirome (CPO), piperacillin+tazobactam (TZP), meropenem (MEP), imipenem (IPM), ciprofloxacin (CIP), gentamicin (CN), doxycycline (DO), co- trimoxazole was 36%, 80.43%, 80.43%, 67.39%, 39.13%, 6.52%, 13.64%, 52.17%, 80.43%, 82.61%, 93.48% resistance respectively (Ullah et al., 2009).

Table 11: Susceptibility and resistance pattern of isolated K. pneumoniae in Peshawar, 2007 (Modified from Ullah et al., 2009).

Antibiotic	**N=(92)**	
	R (%)	**S (%)**
AMP	100	00.00
AMC	36.09	17.39
CE	80.43	15.22
CEC	80.43	19.57
CPO	67.39	30.43
TZP	39.13	45.65
MEP	6.52	93.48
IPM	13.64	86.96
CIP	52.17	41.30
CN	80.43	17.39
DO	82.61	15.22
SXT	93.48	06.52

Four years back, in 2015, an analysis had been done to evaluate the increased resistance pattern of *K. pneumoniae* in Shanghai Children's Medical Center, one of the largest pediatric hospital in China with 800 beds facility for patients. In this analysis 41 *K. pneumoniae* strains had been collected and analyzed by testing colony formation, biochemical test and the test is done for almost all the available antibiotics (Zhang, Chen, Xu, Huang, & Wang, 2018).

Table 12: Antibiotic resistance pattern of K. pneumoniae for available antibiotics in China, 2015 (Modified from Zhang et al., 2018).

Antibiotic	Resistance (%)
Ertapenem	100
Imipenem	100
Ceftazidime	100
Piperacillin/tazobactam	100
Nitrofurantoin	58.50
Tobramycin	29.30
Ceftriaxone	100
Ampicillin	100
Cefazolin	100
Gentamicin	43.90
Levofloxacin	24.40
Ampicillin/sulbactam	100
Cefotetan	100
Aztreonam	100

From the above table it has been observed that ertapenem, imipenem, ceftazidime, piperacillin/tazobactam, ceftriaxone, ampicillin, cefazolin, ampicillin/sulbactam, cefotetan, aztreonam did not work against *K. pneumoniae*. Other antibiotics also ineffective to combat *K. pneumoniae* though the resistance rate was not 100% for few antibiotics (Zhang et al., 2018). In future, *K. pneumoniae* could exhibit 100% resistance to all available antibiotic if necessary steps will not be taken.

Chapter 6

Combatting of Superbugs

Superbugs are threats for current and future generation. Superbugs can be treated by new antibiotic research, drug repurposing and combination therapy which has great success rate to cure various infections caused by these superbugs.

6.1 New Antibiotic Research

As days are passing the resistance of bacteria is increasing with the increase in number of antibiotics. There might be new drugs working against resistance bacteria. However it is time consuming process but it might work (Mirshojaei, 2015). Therefore, new antibiotics are being introduced to fight back this situation by conducting various researches. New compounds are formulated to treat gram negative bacteria (Bravo et al., 2018).

6.1.1 Aminoglycosides

Aminoglycosides, a class of antibiotic with broad spectrum activity against multidrug resistance bacteria. Recently new neoglycoside, named plazomicin has been approved by FDA which is usually used to treat gram negative bacteria such as *Acinetobacter baumannii*, *P. aeruginosa*, *Enterobacteriaceae* by inhibiting their protein synthesis (Isler & Doi, 2019). In addition, plazomicin are stable against most of the aminoglycoside modifying enzyme (Villegas & Lyon, 2018). Study have been found that it gives synergistic effect in combination with imipenem, cafepime and side effects are less than other aminoglycoside. Plazomicin is basically used in the treatment of bacterial bloodstream infection (BSI) caused by Enterobacteriaceae (Bassetti, Vena, Croxatto, Righi, & Guery, 2018).

6.1.2 β-lactamase Inhibitor

β-lactamase inhibitor are inhibitors which shield the beta-lactam ring from being hydrolyzed. They exhibit broad spectrum activity against ESBLs producing bacteria specially gram negative bacteria (Bassetti et al., 2018; Isler & Doi, 2019). Relebactam, vaborbactam are β-lactamase inhibitor that is under investigation. Relebactam is effective against ESBLs, KPC, and phase 2 clinical trial is applied in the treatment of UTIs (Bassetti & Righi, 2015). On the other hand, vaborbactam, formerly known as RPX7009 have the ability to restore carbapenem activity (Bassetti & Righi, 2015). Investigation on vaborbactam found that they can restore carbapenem activity ranging from 2-98% (Bassetti & Righi, 2015; Bassetti et al., 2018). Currently, phase 3 clinical trial are used in the treatment of various infection including urinary tract infections, hospital acquired pneumonia (HAP) and ventilated acquired pneumonia (VAP) (Isler & Doi, 2019).

6.1.3 Carbapenem

Carbapenem are considered to be most effective antibiotic against gram negative bacteria including *A. baumannii*, *P. aeruginosa*, *Enterobacteriaceae* (Villegas & Lyon, 2018). Doripenem, biapenem shows their activity against *P. aeruginosa*, *A. baumannii*. Doripenem exerts its activity against colistin resistance *Enterobacteriaceae* and used to treat UTIs, HAP, and VAP. Biapenem had been approved in 2002 in United States and active against *P. aeruginosa* and *A. baumannii* (Isler & Doi, 2019). On the other hand, panipenem shows effectiveness against *K. pneumoniae* but not against *P. aeruginosa* (Kontopidou et al., 2014). This antibiotic is currently in clinical trial phase 2 and approved for the treatment of UTIs, surgical infection in Japan, Korea, and China. Combination of another novel antibiotic, tebipenem/pivoxil is also in clinical trial phase 2, approved for use in the treatment of respiratory tract infections in Japan (Bassetti et al., 2018; Isler & Doi, 2019).

6.1.4 Cephalosporin

Cephalosporin, a class of antibiotic commonly used to treat bacterial infection losses its effectiveness due to the emergence of bacterial resistance pattern (Bassetti & Righi, 2015; Isler & Doi, 2019). Thus, investigation is still going on for developing new compound of this class. Ceftozolane and tazobactam combination shows activity against *Pseudomodes* and efficacy against some ESBL and AmpC producing bacteria (Isler & Doi, 2019). In addition, new members of cephalosporin are ceftobiprole medocaril, ceftaroline fosamil which has broad spectrum activity against gram negative bacteria (Isler & Doi, 2019).

6.1.5 Quinolones

Fluoroquinolones have broad spectrum activity which is decreasing day by day because of the evolution of superbugs. So, it creates a need for the discovery of new compound of this class to combat MDR. Delafloxacin and finafloxacin are new compounds that are active against gram (-) bacteria producing β-lactamase (Piddock, 2012). Their unique chemical structure helps to improve their antibacterial activity (Bassetti et al., 2018; Isler & Doi, 2019).

6.1.6 Tetracycline

Eravacycline, a novel fluorocycline has successfully completed clinical trial phase 2 and used for the treatment of UTIs. It shows effectiveness against *Enterobacteriaceae* and *A. baumannii* and efficacy is better than tigecycline. Cure rate of eravacycline is more than 90%. Further preparation has been taken for clinical trial phase 3 to evaluate its activity towards bacterial infection (Bassetti et al., 2018; Isler & Doi, 2019).

6.2 Drug Repurposing

A very recent term known as ‘drug repurposing’ has become a centre of attraction of research for the scientists (Subramani, Narayanasamy, & Feussner, 2017). To demonstrate the synergistic effect of drugs this phenomenon has been established (Baker, Payne, Rappuoli, &

De Gregorio, 2018). Drug repurposing also known as drug repositioning can be define d as the drug development strategies where FDA approved existing drugs are used for new therapeutic indications (Farha & Brown, 2019). This is a great scope for broadening the therapeutic effect of any drug. Basically, such kind of reuse of drugs brings greater effect to the therapeutic efficacy of existing drugs (Bello, Ayanda, Aworunse, & Olukanmi, 2018). These modifications are done depending on the chemical structures, biochemical pathways, and mode of action of the drugs. It has been termed as a 'blessing to the drug development processes.' Drugs which are produced by such re-usage are a bridge between threat and possibility (Subramani et al., 2017).

6.2.1 Benefits of Drug Repurposing

There is a wide range of benefits of repurposed drugs. First of all drug repurposing directly leads to new drug development and drug discovery. It consumes years after year to develop a new drug whereas reusing the existing drug consumes lesser time for a new drug to be produced (Farha & Brown, 2019). Secondly, drug repurposing escapes all sorts of pre-clinical and clinical trials (Subramani et al., 2017). For example, when a new drug is discovered it has to undergo preclinical trials on animal model, then approval from FDA/NDA followed by clinical trials of 4 phases and finally if approved by all then gaining the marketplace. All these procedures take about 10-17 years (Farha & Brown, 2019). But in case of repurposed drugs they have already gone through the preclinical trials and FDA approval. These drugs directly enter the phase 2 of clinical trials where its success being approved by NDA is released in the marketplace. This process takes only 3-12 years that is a very short span of time unlike new drugs to release in the market. Thirdly, this field is very useful for small scale laboratories and academic purposes. They are also very effective for pharmaceutical industries. Finally, such drugs are very economic and effective for greater use (Farha & Brown, 2019).

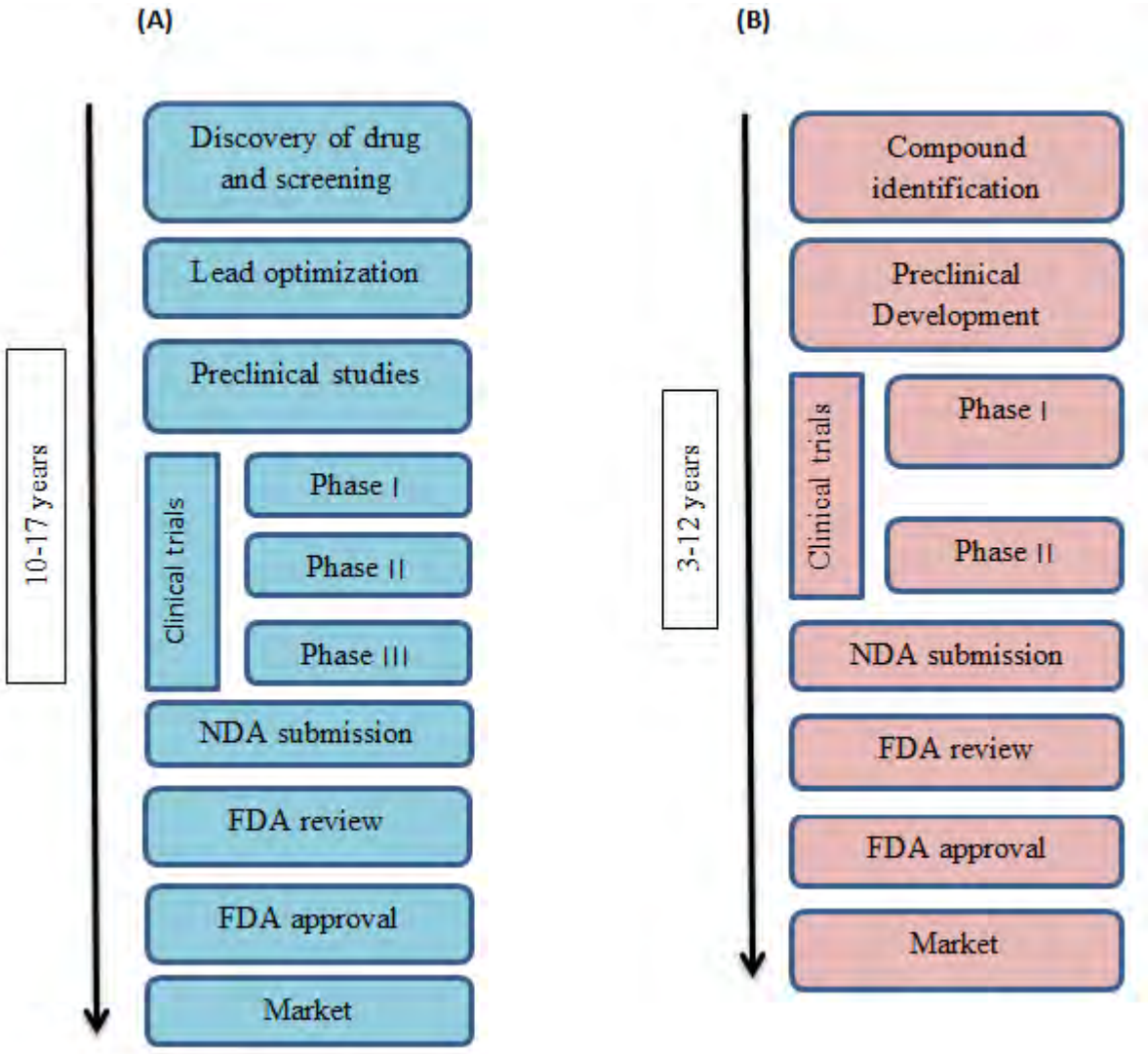

Figure 12: Comparison of drug regulatory process of licensing for conventional compound (A) and repurposed drug (B) (Adapted and modified from Farha & Brown, 2019).

6.2.2 Procedure of Drug Repurposing

The procedure of drug repurposing might follow a strategy of three steps such as the computational method, phenotypic assay and serendipity. After the processing is done the drug must undergo its clinical evaluation.

Computational method: This is a technological approach where databases and activities are analyzed with vast information from the internet and in a number of collaboration of

networks. This method assembles all the data collected regarding a single activity which are very complex for humans to gather. It provides a hypothetical biological pathways of different substances. Though this is a very quick process to get hold of newly occurring diseases (Zaman et al., 2017).

Phenotypic assays: This is an activity based assay of molecules which is done by accumulating various field containing information. It is a laboratory based procedure, here bacteria is inoculated in broths to observe their activities. Comparatively advantages process as experiment is based on physiological activity (Bassetti & Righi, 2015).

Serendipity: An accidental approach. When coincidently new therapeutic indications of existing drugs has been observed. For example, antibacterial drugs sulfamide coincidently showed anti diabetic property. Still to avoid side effects it is better to recheck the drugs upon discovered (Farha & Brown, 2019; Subramani et al., 2017).

Results of repurposing carbepenem and colistin resistant bacteria-

Carbepenem is termed to be the most powerful antibiotic of all time but unfortunately almost all bacteria has become resistant to it.

Table 13: A list of drugs used as antibacterial agents for multi-drug resistance gram (-) bacteria (Modified from Peyclit et al., 2019).

Compound	FDA approved use	Activity (Alone or combination)	Bacteria tested	Resistance phenotype
Zidovudine	Antiretroviral	Alone	*K. pneumoniae*	Colistin
		Colistin	*K. pneumonia*	Colistin
			P. aeruginosa	
			A. baumannii	
		Tigecycline	*K. pneumoniae*	Carbapenems
Niclosamide	Anthelmintic	Colistin	*A. baumannii*	Colistin
			K. pneumoniae	
Pentamidine	Antiprotozoal	Rifampicin	*K. pneumoniae*	Carbapenems
Ciclopirox	Antifungal	Alone	*K. pneumoniae*	Carbapenems
			A. baumannii	
5-fluorouracil	Antineoplastic	Zidovudine	*A. baumannii*	Carbapenem
Mitotane	Antineoplastic	Polymyxin B	*P. aeruginosa*	Carbapenem
			K. pneumoniae	Polymyxin
			A. baumannii	
Sertraline	Antidepressant	Polymyxin B	*P. aeruginosa*	Colistin
			K. pneumoniae	Carbapenem
Citalopram			*A. baumannii*	Colistin

6.3 Combination Therapy

One of the advancements in the pharmaceutical field is combinational therapy. This antibiotic therapy is for the treatment of bacteria having a gram negative cell wall that is an outer protective layer. The combinational antibiotic therapy shows more affectivity than monotherapy (Kapoor & Murphy, 2018). It can interact with broad spectrum antibiotics which shortens the resistance growth by over powering the synergistic effects. To combat multi-drug resistant species combination therapy is taken into consideration though there was lack of evidences (Peyclit et al., 2019).

In case of gram negative bacteria the combination therapy the pathway follows two separate antibiotics where both the antibiotics works against the bacteria and combining both can alter the effect to solve critical conditions which a monotherapy cannot provide. Moreover the most positive side of this therapy is that it is used right at the peak where the resistance starts to grow and applying the therapy digests this growth. But as a matter of fact as there are not enough verifications to prove the definitive combination therapy (Mendes et al., 2005).

Some of the combinations of antibiotics include the following:

- For infection of *Enterobacteriaceae* = colistion + tigecycline or aminoglycosides + carbapenem + colistin + fosfomycin + rifamopin or tigecycline (Faiz & Ariful, 2011).
- For infections of *Acinetobactor spp.* = aminoglycosides + ampicilling /sulbactam + carbapenem+ colistin/rifampin (Kapoor & Murphy, 2018).
- For infections of *Pseudomonas spp.* = beta lactam +aminoglycoside/fluoroquinoline (Kapoor & Murphy, 2018).

Table 14: Comparison of monotherapy and combination therapy outcome used for the treatment of gram (-) bacterial infection (Modified from Bassetti & Righi, 2015).

Regimen	Pathogens	Outcome
Monotherapy (tigecycline, aminoglycoside, colistin,) vs Combination therapy	*Klebsiella* KPC (ICU)	Mortality- tigecycline 31.3%, aminoglycoside 22.7%, colistin 23.1% when given individually.
		Mortality- colistin+ aminoglycoside 11.7%, tigecycline+ aminoglycoside 18.1%, colistin+ tigecycline 44.4%
Monotherapy vs Combination therapy of colistin or tigecycline+carbapenem	*Klebsiella* KPC	Mortality 57.8% vs 13.3%
Monotherapy vs Combination therapy of colistin+tigecycline or gentamicin+tigecycline	*Klebsiella* KPC, *P. aeruginosa*	Mortality 47% vs 0%
Monotherapy of colistin vs combination of colistin	*A. baumannii*	Mortality 72% vs 52%

The process by which the synergistic effect is achieved occurs in a number of ways for different antibiotics. For example, colistin, a drug of choice which is effectively used in

different combinations, basically increases the permeability of the outer membrane of gram negative bacteria. A gram negative bacterium which has achieved resistance has a lower permeability in its cell wall which repels anything to cross the membrane. Monotherapeutic drug colistin cannot invade this non-permeable layer, so a combination with it increases the permeability of the cell wall of the gram negative bacteria. So the combination easily invades the resistant bacteria and destroys it by inhibiting further resistance (Tängdén, 2014).

In a monotherapy to treat *klebsiella* KPC infection the mortality rate for certain drugs colistin is 23.1%, aminoglycoside is 22.7%, tigecycline is 31.3% whereas combination of this decreases the rate of mortality to tigecycline + aminoglycosides 18.1%, colistin+ aminoglycosides 11.7%, colistin + tigecycline 44.4%, colistin/tigecycline + carbapenem 13.3%. Again in XDR *A. baumanii* shows mortality 52% for monotherapy of colistin and 80% for combination of colistin + carbapenem/ aminoglycosides, colistin + rifampicin shows 61% mortality rate (Tängdén, 2014).

Chapter 7

Conclusion and Future Aspects

The term “superbugs” which is the evolution from small range of antibiotic resistance to a massive threat is the most dreadful emerging warning worldwide. The issues started with overuse of antibiotics where we take antibiotics even if for a normal fever, do not maintain the dose frequency, remain exposed to infections for a long period (nosocomial infections), etc. such reasons made susceptible bacteria which were resistant to certain antibiotics to become resistant to all antibiotics achieving the title of “superbugs.” As the plight is expanding, we must step forward in bringing it to an end. A number of solutions might include a generalised comment of the healthcare experts on the prescription regarding the threats of antibiotic usage. This shall create apprehension among people to avoid excessive use of antibiotics. Again a correlation can be built up between the industry people and the doctors to create a balance between the restriction and requirements. The hospital authorities can check and audit the usage rate of antibiotics monthly for bringing a control in the use of antibiotics. In the educational institutions students shall be given proper knowledge of this alarming situation. In addition to these, some salient steps are to be established for a permanent explication of the superbugs. One of the consequential technique can be “drug repurposing” which will mend the action of the resistant bacteria. This will help in finding solutions for resistance keeping the number of antibiotics constant. It modifies the reuse of existing licensed drug for better efficiency. Another technique can be “combination therapy” where different modalities are fused together for greater therapeutic effect. Furthermore, substantial research work is to be done in finding the patterns of the resistance caused by superbugs and blocking their way to more resistance for the future.

References

Ahmed, I., Rabbi, M. B., & Sultana, S. (2019). Antibiotic resistance in Bangladesh: A systematic review. *International Journal of Infectious Diseases*, *80*, 54–61. https://doi.org/10.1016/j.ijid.2018.12.017

Alanis, A. J. (2005). Resistance to antibiotics: Are we in the post-antibiotic era? *Archives of Medical Research*, *36*(6), 697–705. https://doi.org/10.1016/j.arcmed.2005.06.009

Antunes, L. C. S., Visca, P., & Towner, K. J. (2014). Acinetobacter baumannii: Evolution of a global pathogen. *Pathogens and Disease*, *71*(3), 292–301. https://doi.org/10.1111/2049-632X.12125

Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., … Baloch, Z. (2018). Antibiotic resistance: a rundown of a global crisis. *Infection and Drug Resistance*, *11*, 1645–1658. https://doi.org/10.2147/IDR.S173867

Baker, S. J., Payne, D. J., Rappuoli, R., & De Gregorio, E. (2018). Technologies to address antimicrobial resistance. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(51), 12887–12895. https://doi.org/10.1073/pnas.1717160115

Baquer, J. L., & Martinez, F. (2014). Emergence and spread of antibiotic resistance: Setting a parameter space. *Upsala Journal of Medical Sciences*, *119*(2), 68–77. https://doi.org/10.3109/03009734.2014.901444

Bassetti, M., & Righi, E. (2015). New antibiotics and antimicrobial combination therapy for the treatment of gram-negative bacterial infections. *Current Opinion in Critical Care*, *21*(5), 402–411. https://doi.org/10.1097/MCC.0000000000000235

Bassetti, M., Vena, A., Croxatto, A., Righi, E., & Guery, B. (2018). How to manage

Pseudomonas aeruginosa infections. *Drugs in Context*, *7*, 1–18. https://doi.org/10.7573/dic.212527

Bello, O. A., Ayanda, O. I., Aworunse, O. S., & Olukanmi, B. I. (2018). *Pharmacognosy Reviews*. *1*(2), 57–72. https://doi.org/10.4103/phrev.phrev

Bengoechea, J. A., & Sa Pessoa, J. (2019). Klebsiella pneumoniae infection biology: Living to counteract host defences. *FEMS Microbiology Reviews*, *43*(2), 123–144. https://doi.org/10.1093/femsre/fuy043

Bernal-Rosas, Y., Osorio-Muñoz, K., & Torres-García, O. (2015). Pseudomonas aeruginosa: an emerging nosocomial trouble in veterinary. *Revista MVZ Córdoba*, *20*, 4937. https://doi.org/10.21897/rmvz.9

Bishai, W. R. (2015). *Efflux Pump Inhibitors : Increase Activity with the Same or Lower Drug Dosing*.

Bravo, A., Ruiz-cruz, S., Alkorta, I., & Espinosa, M. (2018). Mini review When Humans Met Superbugs : Strategies to Tackle Bacterial Resistances to Antibiotics What Is So Different About Was ist so anders am Neuroenhancement ? *De Gruyter*, 216–226.

Breidenstein, E. B. M., de la Fuente-Núñez, C., & Hancock, R. E. W. (2011). Pseudomonas aeruginosa: All roads lead to resistance. *Trends in Microbiology*, *19*(8), 419–426. https://doi.org/10.1016/j.tim.2011.04.005

Brown, E. D., & Wright, G. D. (2016). Antibacterial drug discovery in the resistance era. *Nature*, *529*(7586), 336–343. https://doi.org/10.1038/nature17042

Cain, R. (2015). Antimicrobial Resistance in Klebsiella pneumoniae: Mechanisms and Clinical Impact and Developments. *Biom 250*, 3.

Chandler, C. I. R. (2019). Current accounts of antimicrobial resistance: stabilisation, individualisation and antibiotics as infrastructure. *Palgrave Communications*, *5*(1), 15–17. https://doi.org/10.1057/s41599-019-0263-4

Chokshi, A., Sifri, Z., & Cennimo, D. (2019). Global Contributors to Antibiotic Resistance. *Journal of Global Infectious Diseases.*, 1–2.

Chowdhury, S., Hassan, M. M., Alam, M., Sattar, S., Bari, M. S., Saifuddin, A. K. M., & Hoque, M. A. (2015). Antibiotic residues in milk and eggs of commercial and local farms at Chittagong. *Veterinary World*, *8*(4), 467–471. https://doi.org/10.14202/vetworld.2015.467-471

Codjoe, F., & Donkor, E. (2017). Carbapenem Resistance: A Review. *Medical Sciences*, *6*(1), 1. https://doi.org/10.3390/medsci6010001

D, S. N. A. (1980). *Tetracycline-Labeled Human Bone from Ancient*. *367*(January), 94–95.

Davies, J., & Davies, D. (2010). Origins and Evolution of Antibiotic Resistance. *Microbiology and Molecular Biology Reviews*, *74*(3), 417–433. https://doi.org/10.1128/mmbr.00016-10

Dijkshoorn, L., Nemec, A., & Seifert, H. (2007). An increasing threat in hospitals: Multidrug-resistant Acinetobacter baumannii. *Nature Reviews Microbiology*, *5*(12), 939–951. https://doi.org/10.1038/nrmicro1789

Dou, Y., Huan, J., Guo, F., Zhou, Z., & Shi, Y. (2017). Pseudomonas aeruginosa prevalence, antibiotic resistance and antimicrobial use in Chinese burn wards from 2007 to 2014. *Journal of International Medical Research*, *45*(3), 1124–1137. https://doi.org/10.1177/0300060517703573

Eichenberger, E. M., & Thaden, J. T. (2019). Epidemiology and mechanisms of resistance of extensively drug resistant gram-negative bacteria. *Antibiotics*, *8*(2). https://doi.org/10.3390/antibiotics8020037

Epand, R. M., Walker, C., Epand, R. F., & Magarvey, N. A. (2016). Molecular mechanisms of membrane targeting antibiotics. *Biochimica et Biophysica Acta - Biomembranes*, *1858*(5), 980–987. https://doi.org/10.1016/j.bbamem.2015.10.018

Faiz, M. A., & Ariful, B. (2011). Antimicrobial resistance: Bangladesh experience. (Special Issue: Antimicrobial resistance in South-East Asia.). *Regional Health Forum*, *15*(1), 1–8.

Farha, M. A., & Brown, E. D. (2019). Drug repurposing for antimicrobial discovery. *Nature Microbiology*, *4*(4), 565–577. https://doi.org/10.1038/s41564-019-0357-1

Feng, W., Sun, F., Wang, Q., Xiong, W., Qiu, X., Dai, X., & Xia, P. (2017). Epidemiology and resistance characteristics of Pseudomonas aeruginosa isolates from the respiratory department of a hospital in China. *Journal of Global Antimicrobial Resistance*, *8*, 142–147. https://doi.org/10.1016/j.jgar.2016.11.012

Fulmer, 0. BRYAN. (1979). The Ancient Mariner and the Wandering Jew. In *University of North Carolina Press* (Vol. 66).

Fymat, A. L. (2017). Antibiotics and Antibiotic Resistance. *Biomedical Journal of Scientific & Technical Research*, *1*(1). https://doi.org/10.26717/bjstr.2017.01.000117

Gasink, L. B., Edelstein, P. H., Lautenbach, E., Synnestvedt, M., & Fishman, N. O. (2009). Risk Factors and Clinical Impact of Klebsiella pneumoniae Carbapenemase-Producing K. pneumoniae . *Infection Control & Hospital Epidemiology*, *30*(12), 1180–1185.

https://doi.org/10.1086/648451

Gogou, V., Pournaras, S., Giannouli, M., Voulgari, E., Piperaki, E. T., Zarrilli, R., & Tsakris, A. (2011). Evolution of multidrug-resistant Acinetobacter baumannii clonal lineages: A 10 year study in Greece (2000-09). *Journal of Antimicrobial Chemotherapy*, *66*(12), 2767–2772. https://doi.org/10.1093/jac/dkr390

Gould, K. (2016). Antibiotics: From prehistory to the present day. *Journal of Antimicrobial Chemotherapy*, *71*(3), 572–575. https://doi.org/10.1093/jac/dkv484

Greer, N. D. (2006). Tigecycline (Tygacil): The First in the Glycylcycline Class of Antibiotics. *Baylor University Medical Center Proceedings*, *19*(2), 155–161. https://doi.org/10.1080/08998280.2006.11928154

Hawkey, J., Ascher, D. B., Judd, L. M., Wick, R. R., Kostoulias, X., Cleland, H., … Holt, K. E. (2018). Evolution of carbapenem resistance in Acinetobacter baumannii during a prolonged infection. *Microbial Genomics*, *4*(3). https://doi.org/10.1099/mgen.0.000165

Heidary, M., Nasir, M. J., Dabiri, H., & Tarashi, S. (2018). Prevalence of drug-resistant Klebsiella pneumoniae in Iran: A review article. *Iranian Journal of Public Health*, *47*(3), 317–326.

Heitz, D. (2013). Deadly Bioterror Threats: Six Real Risks. *Healthline*, 9–12.

Howard, A., O'donoghue, M., Feeney, A., & Sleator, R. D. (2012). An emerging opportunistic pathogen. *Virulence*, *3*(3), 243–250. https://doi.org/10.4161/viru.19700

Hua, X., Zhou, Z., Yang, Q., Shi, Q., Xu, Q., Wang, J., … Yu, Y. (2017). Evolution of Acinetobacter baumannii in vivo: International Clone II, more resistance to ceftazidime, Mutation In ptk. *Frontiers in Microbiology*, *8*(JUL), 1–13.

https://doi.org/10.3389/fmicb.2017.01256

Huang, Y., Zhou, Q., Wang, W., Huang, Q., Liao, J., Li, J., … Tu, M. (2019). Acinetobacter baumannii ventilator-associated pneumonia: Clinical efficacy of combined antimicrobial therapy and in vitro drug sensitivity test results. *Frontiers in Pharmacology*, *10*(FEB). https://doi.org/10.3389/fphar.2019.00092

Isler, B., & Doi, Y. (2019). *New Treatment Options against Carbapenem-Resistant Acinetobacter baumannii Infections*. (December 2018), 1–17.

Jenny, M., & Kingsbury, J. (2018). Properties and Prevention: A Review ofPseudomonas aeruginosa. *Journal of Biology and Medical Research*, *2*(3), 1–8. Retrieved from http://www.imedpub.com/articles/properties-and-prevention-a-review-ofpseudomonas-aeruginosa.php?aid=23355

Jiang, H., Cao, L., Qu, L., Qu, T., Liu, G., Wang, R., … Chen, J. (2017). Evolution of Drug-resistant Acinetobacter baumannii after DCD Renal Transplantation. *Scientific Reports*, *7*(1), 1–9. https://doi.org/10.1038/s41598-017-01683-7

Kapoor, P., & Murphy, P. (2018). Combination antibiotics against Pseudomonas aeruginosa, representing common and rare cystic fibrosis strains from different Irish clinics. *Heliyon*, *4*(3), e00562. https://doi.org/10.1016/j.heliyon.2018.e00562

Kaufman, G. (2011). Antibiotics: mode of action and mechanisms of resistance. *Nursing Standard (Royal College of Nursing (Great Britain) : 1987)*, *25*(42), 49–55.

Kieny, M.-P., & Tacconelli, E. (2017). WHO publishes list of bacteria for which new antibiotics are urgently needed.

Kontopidou, F., Giamarellou, H., Katerelos, P., Maragos, A., Kioumis, I., Trikka-Graphakos,

E., … Heleni, H. (2014). Infections caused by carbapenem-resistant Klebsiella pneumoniae among patients in intensive care units in Greece: A multi-centre study on clinical outcome and therapeutic options. *Clinical Microbiology and Infection*, *20*(2), O117–O123. https://doi.org/10.1111/1469-0691.12341

Ku, W. W., Kung, C. H., Lee, C. H., Tseng, C. P., Wu, P. F., Kuo, S. C., … Fung, C. P. (2015). Evolution of carbapenem resistance in Acinetobacter baumannii: An 18-year longitudinal study from a medical center innorthern Taiwan. *Journal of Microbiology, Immunology and Infection*, *48*(1), 57–64. https://doi.org/10.1016/j.jmii.2013.07.005

Kumarasamy, K. K., Toleman, M. A., Walsh, T. R., Bagaria, J., Butt, F., Balakrishnan, R., … Woodford, N. (2010). Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. *The Lancet Infectious Diseases*, *10*(9), 597–602. https://doi.org/10.1016/S1473-3099(10)70143-2

Lee, C. R., Lee, J. H., Park, K. S., Jeon, J. H., Kim, Y. B., Cha, C. J., … Lee, S. H. (2017). Antimicrobial resistance of hypervirulent Klebsiella pneumoniae: Epidemiology, hypervirulence-associated determinants, and resistance mechanisms. *Frontiers in Cellular and Infection Microbiology*, *7*(NOV). https://doi.org/10.3389/fcimb.2017.00483

Lister, P. D., Wolter, D. J., & Hanson, N. D. (2009). Antibacterial-resistant Pseudomonas aeruginosa: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clinical Microbiology Reviews*, *22*(4), 582–610. https://doi.org/10.1128/CMR.00040-09

Livorsi, D. J., Chorazy, M. L., Schweizer, M. L., Balkenende, E. C., Blevins, A. E., Nair, R., … Perencevich, E. N. (2018). A systematic review of the epidemiology of carbapenem-

resistant Enterobacteriaceae in the United States. *Antimicrobial Resistance and Infection Control*, *7*(1), 1–9. https://doi.org/10.1186/s13756-018-0346-9

Lucca, F., Guarnieri, M., Ros, M., Muffato, G., Rigoli, R., & Da Dalt, L. (2018). Antibiotic resistance evolution of pseudomonas aeruginosa in cystic fibrosis patients (2010-2013). *Clinical Respiratory Journal*, *12*(7), 2189–2196. https://doi.org/10.1111/crj.12787

Manage, P. M. (2018). Heavy use of antibiotics in aquaculture: Emerging human and animal health problems – A review. *Sri Lanka Journal of Aquatic Sciences*, *23*(1), 13. https://doi.org/10.4038/sljas.v23i1.7543

McCarthy, K. (2015). Pseudomonas aeruginosa: Evolution of antimicrobial resistance and implications for therapy. *Seminars in Respiratory and Critical Care Medicine*, *36*(1), 44–55. https://doi.org/10.1055/s-0034-1396907

McDermott, P. F., Walker, R. D., & White, D. G. (2003). Antimicrobials an Resistance Mechanisms. *International Journal of Toxicology*, *22*, 135–143. https://doi.org/10.1080/10915810390198410

Mendes, C., Oplustil, C., Sakagami, E., Turner, P., & Kiffer, C. (2005). Antimicrobial susceptibility in intensive care units: MYSTIC Program Brazil 2002. *Brazilian Journal of Infectious Diseases*, *9*(1), 44–51. https://doi.org/10.1590/S1413-86702005000100008

Miller, K. (2015). Superbugs: What They Are and How You Get Them. *WebMD*, 2015. Retrieved from https://www.webmd.com/a-to-z-guides/news/20150417/superbugs-what-they-are#1

Mirshojaei, S. F. (2015). Advances in infectious foci imaging using 99mTc radiolabelled antibiotics. *Journal of Radioanalytical and Nuclear Chemistry*, *304*(3), 975–988.

https://doi.org/10.1007/s10967-015-4003-y

Nierhaus, K. H., Brimacombe, R., & Wittmann, H. G. (2006). Inhibition of protein biosynthesis by antibiotics. *Current Topics in Infectious Diseases and Clinical Microbiology*, *2*(13), 29–40.

Nordmann, P., Naas, T., Fortineau, N., & Poirel, L. (2007). Superbugs in the coming new decade; multidrug resistance and prospects for treatment of Staphylococcus aureus, Enterococcus spp. and Pseudomonas aeruginosa in 2010. *Current Opinion in Microbiology*, *10*(5), 436–440. https://doi.org/10.1016/j.mib.2007.07.004

Nordqvist, C., & Carter, A. (2019). What to know about antibiotics. *Αγαη*, *8*(2), 2019. https://doi.org/10.22201/fq.18708404e.2004.3.66178

Pachori, P., Gothalwal, R., & Gandhi, P. (2019). Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. *Genes and Diseases*, *6*(2), 109–119. https://doi.org/10.1016/j.gendis.2019.04.001

Palanichamy, K., & Kaliappan, K. P. (2010). Discovery and syntheses of “superbug challengers”-platensimycin and platencin. *Chemistry - An Asian Journal*, *5*(4), 668–703. https://doi.org/10.1002/asia.200900423

Paridah, M. ., Moradbak, A., Mohamed, A. ., Owolabi, F. abdulwahab taiwo, Asniza, M., & Abdul Khalid, S. H. . (2016). The Action Mechanisms of Antibiotics and Antibiotic Resistance. *Intech*, *i*(tourism), 13. https://doi.org/http://dx.doi.org/10.5772/57353

Parkins, M. D., Somayaji, R., & Waters, V. J. (2018). Epidemiology, Biology, and Impact of Clonal Pseudomonas aeruginosa Infections in Cystic Fibrosis. *Clinical Microbiology Reviews*, *31*(4), 1–38. https://doi.org/10.1128/CMR.00019-18

Peyclit, L., Baron, S. A., & Rolain, J. M. (2019). Drug repurposing to fight colistin and carbapenem-resistant bacteria. *Frontiers in Cellular and Infection Microbiology*, *9*(JUN), 1–11. https://doi.org/10.3389/fcimb.2019.00193

Piddock, L. J. V. (2012). The crisis of no new antibiotics-what is the way forward? *The Lancet Infectious Diseases*, *12*(3), 249–253. https://doi.org/10.1016/S1473-3099(11)70316-4

Pimenta, F. C., & Alves, R. (2008). *Extended-Spectrum B -Lactamases Producing*. 608–612.

Playfair, J. (2004). Living With Germs : On Infectious Diseases. *Oxford: Oxford University Press*.

Podolsky, S. H. (2018). The evolving response to antibiotic resistance (1945–2018). *Palgrave Communications*, *4*(1). https://doi.org/10.1057/s41599-018-0181-x

Qiao, M., Ying, G. G., Singer, A. C., & Zhu, Y. G. (2018). Review of antibiotic resistance in China and its environment. *Environment International*, *110*(October), 160–172. https://doi.org/10.1016/j.envint.2017.10.016

Qureshi, Z. A., Hittle, L. E., O'Hara, J. A., Rivera, J. I., Syed, A., Shields, R. K., … Doi, Y. (2015). Colistin-resistant acinetobacter baumannii: Beyond carbapenem resistance. *Clinical Infectious Diseases*, *60*(9), 1295–1303. https://doi.org/10.1093/cid/civ048

Sabtu, N., Enoch, D. A., & Brown, N. M. (2015). Antibiotic resistance: What, why, where, when and how? *British Medical Bulletin*, *116*(1), 105–113. https://doi.org/10.1093/bmb/ldv041

Shin, H. W., Lim, J., Kim, S., Kim, J., Kwon, G. C., & Koo, S. H. (2014). Characterization of trimethoprim-sulfamethoxazole resistance genes and their relatedness to class 1 integron

and insertion sequence common region in gram-negative bacilli. *Journal of Microbiology and Biotechnology*, *25*(1), 137–142. https://doi.org/10.4014/jmb.1409.09041

Singh-Moodley, A., & Perovic, O. (2016). Antimicrobial susceptibility testing in predicting the presence of carbapenemase genes in Enterobacteriaceae in South Africa. *BMC Infectious Diseases*, *16*(1), 1–10. https://doi.org/10.1186/s12879-016-1858-7

Stewart, P. S., & Costerton, J. W. (2013). Antibiotic resistance of bacteria in biofilms. *THE LANCET*, *358*, 135–138. https://doi.org/10.1016/S0140-6736(01)05321-1

Strateva, T., & Yordanov, D. (2009). Pseudomonas aeruginosa - A phenomenon of bacterial resistance. *Journal of Medical Microbiology*, *58*(9), 1133–1148. https://doi.org/10.1099/jmm.0.009142-0

Strebhardt, K., & Ullrich, A. (2008). *Paul Ehrlichs magic bullet concept*. *8*(june), 473–480.

Subramani, R., Narayanasamy, M., & Feussner, K. D. (2017). Plant-derived antimicrobials to fight against multi-drug-resistant human pathogens. *3 Biotech*, *7*(3), 1–15. https://doi.org/10.1007/s13205-017-0848-9

Tängdén, T. (2014). Combination antibiotic therapy for multidrug-resistant Gram-negative bacteria. *Upsala Journal of Medical Sciences*, *119*(2), 149–153. https://doi.org/10.3109/03009734.2014.899279

Tarashi, S., Goudarzi, H., Erfanimanesh, S., Pormohammad, A., & Hashemi, A. (2016). Phenotypic and molecular detection of metallo-beta-lactamase genes among imipenem resistant pseudomonas aeruginosa and acinetobacter baumannii strains isolated from patients with burn injuries. *Archives of Clinical Infectious Diseases*, *11*(4).

https://doi.org/10.5812/archcid.39036

Ullah, F., Malik, S. A., & Ahmed, J. (2009). Antimicrobial susceptibility pattern and ESBL prevalence in Klebsiella pneumoniae from urinary tract infections in the North-West of Pakistan. *African Journal of Microbiology Research*, *3*(11), 676–680.

Ventola, C. L. (2015). The antibiotic resistance crisis: causes and threats. *P&T Journal*, *40*(4), 277–283. https://doi.org/Article

Villegas, M. V., & Lyon, S. (2018). Gram-negative infections: Evolving treatments with expanding options. *Future Science OA*, *4*(9), 2–5. https://doi.org/10.4155/fsoa-2018-0071

Walsh, F. (2014). Superbugs to kill "more than cancer" by 2050. *BBC News*, (December 2014), 1–14. Retrieved from http://www.bbc.com/news/health-30416844

Webster, T. J., & Li, B. (2018). Bacteria Antibiotic Resistance: New Challenges and Opportunities for Impland-associated Orthopaedic infections. *Journal of Orthopaedic Research*, *36*(1), 22–32. https://doi.org/10.1002/jor.23656.Bacteria

Yang, Y. S., Lee, Y. T., Huang, T. W., Sun, J. R., Kuo, S. C., Yang, C. H., … Chang, F. Y. (2013). Acinetobacter baumannii nosocomial pneumonia: Is the outcome more favorable in non-ventilated than ventilated patients? *BMC Infectious Diseases*, *13*(1), 1. https://doi.org/10.1186/1471-2334-13-142

Yayan, J., Ghebremedhin, B., & Rasche, K. (2015). Antibiotic resistance of pseudomonas aeruginosa in pneumonia at a single university hospital center in Germany over a 10-Year Period. *PLoS ONE*, *10*(10), 1–20. https://doi.org/10.1371/journal.pone.0139836

Yoshikawa, T. T. (2002). Antimicrobial Resistance and Aging: Beginning of the End of the

Antibiotic Era? *Journal of the American Geriatrics Society*, *50*, 226–229. https://doi.org/10.1046/j.1532-5415.50.7s.2.x

Zaman, S. Bin, Hussain, M. A., Nye, R., Mehta, V., Mamun, K. T., & Hossain, N. (2017). A Review on Antibiotic Resistance: Alarm Bells are Ringing. *Cureus*, *9*(6). https://doi.org/10.7759/cureus.1403

Zhang, X., Chen, D., Xu, G., Huang, W., & Wang, X. (2018). Molecular epidemiology and drug resistant mechanism in carbapenem-resistant Klebsiella pneumoniae isolated from pediatric patients in Shanghai, China. *PLoS ONE*, *13*(3), 1–10. https://doi.org/10.1371/journal.pone.0194000