Antibiotic Susceptibility Pattern of *Staphylococcus Aureus* in Dhaka, Bangladesh: A Cross Sectional Study of Antibiograms

By

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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 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 have acknowledged all main sources of help.

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Approval

The thesis/project titled "Antibiotic Susceptibility Pattern of Staphylococcus Aureus in Dhaka, Bangladesh: A Cross Sectional Study of Antibiograms" submitted by Faijul Amin (15146066) of Spring, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on October.

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Ethics Statement

No animals were used to conduct this study. Antibiogram data from different hospitals diagnostic centers were collected by maintaining privacy of the patient and organization.

Abstract

Antibiotic resistance has become a major issue across the world. This study conducted to find out resistance pattern of *S. aureus* in Bangladesh. The study implicated that the bacteria were highly resistant against cefaclor, cefixime, cefradine, chloramphenicol, clindamycin. However, it was very sensitive to amikacin, ampicillin, doxycycline, fusidic acid, imipenem, norfloxacin. In case of cefuroxime, amoxiclav, tigecycline, vancomycin there were no clear results from our study. Hopefully, this study will increase awareness of antibiotic usage, promote rational usage and pave the way for future studies.

Keywords: Antibiotic resistance; *Staphylococcus aureus*

Dedication

Dedicated to My Family

Acknowledgement

This research could not have been completed without the support of many people who are gratefully acknowledged here.

First and foremost, my special gratitude is due to my supervisor, Md. Tanvir Kabir, Senior Lecturer, Department of Pharmacy, Brac University. He has been content to let me shape this work as I thought best, yet his criticisms and suggestions have always been available and have always proved stimulating. His constant effort and encouragement towards my research-based project allowed me to grow as a research scientist. His skills and experience in the field of pharmacology helped to conduct this study smoothly. He continually and persuasively conveyed a spirit of adventure in regard to research and an excitement in regard to teaching. I would like to express my appreciation to our chairperson Professor Dr. Eva Rahman Kabir, Department of Pharmacy, Brac University, who I am deeply thankful for her valuable contribution.

Last but not the least, I would like to give a special gratitude to my parents for their constant invaluable support and prayers which have enabled me to dream bigger and pursue something which can only be realistic after passing hurdles and my friends who have supported me always.

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List of Acronyms

ABC	ATP Binding Cassette
AME	Aminoglycoside Modifying Enzyme
BLAZ	Beta Lactamase
HGT	Horizontal Gene Transfer
MATE	Multidrug And Toxin Extrusion
MDR	Multi Drug Resistant
MFS	Major Facilitator Superfamily
MGE	Mobile Genetic Elements
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin Resistant *Staphylococcus aureus*
PBP	Penicillin Binding Protein
RND	Resistance Nodulation Division
SMR	Small Multidrug Resistance
SPSS	Statistical Package for the Social Sciences
TET	Ten Eleven Translocation
Tn552	Transposase for transposon Tn552
TP	Transpeptidase
UTI	Urinary Tract Infection
VISA	Vancomycin Intermediate *Staphylococcus aureus*
VRSA	Vancomycin Resistant *Staphylococcus aureus*

Chapter 1

Introduction

In the 19th century, diseases caused by bacterial infections cause high number of death in the world. 47 years was the average life expectancy. Even in the era of industrial revolution diseases like cholera, pneumonia, and tuberculosis were highly prevalent.

What changed all that and marked a new era of medicine was the innovation of penicillin by Sir Alexander Fleming. This marked the beginning of antibiotic era. This golden time period in the mid-20th century saw the emergence of many new antibiotics which helped to reduce the death caused by bacterial infections very significantly (Adedeji, 2016). As it can be seen in Figure 1 the leading cause of death has changed drastically from 1900 to 1997. People mostly died from infectious diseases like pneumonia, tuberculosis in the beginning of 20th century. By the end of the century people mostly died from non-infectious diseases like heart conditions, cancer etc.

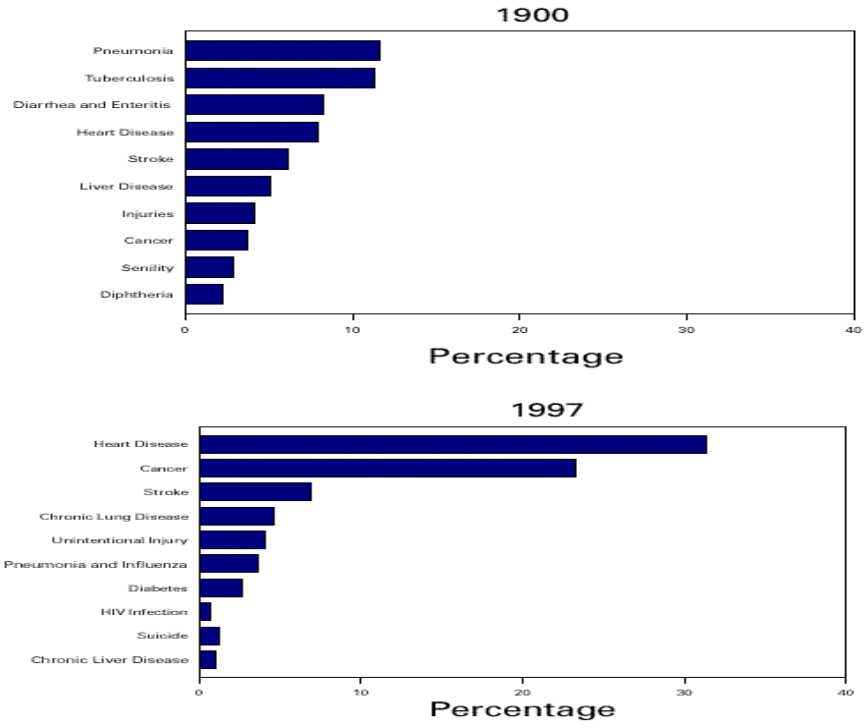

Figure 1: Leading cause of death being changed over last 100 years (Adedeji, 2016).

Without antibiotics we would be set back by decades. However, due to resistance development the very existence of antibiotics is in grave danger (Microbiology Society, 2018).

1.1 Definition of antibiotics

Antibiotics are referred to any substance which kills bacteria, inhibits growth and stops replication. They are developed to specifically target bacterial infections in the body. Any substance that inhibits the growth and replication of a bacterium or kills it outright can be called an antibiotic. This makes it distinct from other antimicrobials. Most of the antibiotics used today are developed in laboratories. However, a number of them have been isolated from the environment (Microbiology Society, 2018).

1.2 Types of antibiotics

1. **Penicillins**: It is widely used to treat a number of infections such as chest, skin and urinary tract infections. Examples include, penicillin V potassium, amoxicillin (Figure 2). Microorganisms can cause enzymatic destructions, alteration of target sites to develop resistance against penicillins.

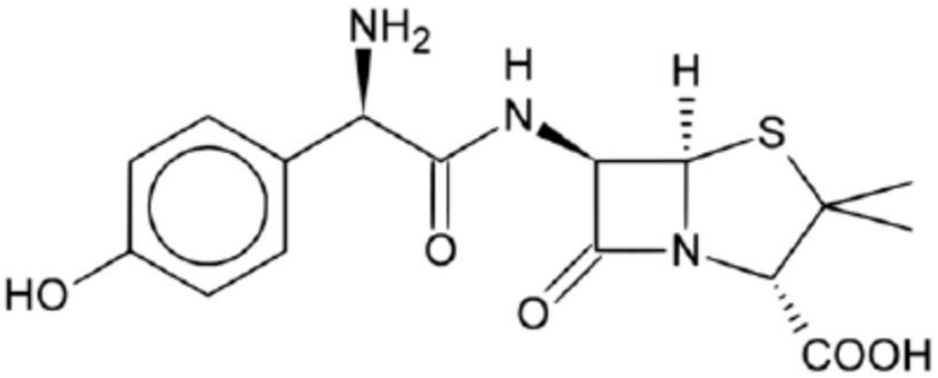

Figure 2: Structure of amoxicillin (Shen et al., 2016).

2. **Cephalosporins:** Like penicillins, cephalosporins can be used to treat verities of infections. Examples include ear infections, urinary tract infections (UTIs) etc. They are

also highly effective against septicemia. Cephalosporins are spread through five generations. Examples include, cefdinir, cefuroxime, ceftaroline (Figure 3) etc. Bactria use β- lactamase inhibition, reduction in permeability of cephalosporins to obtain resistance.

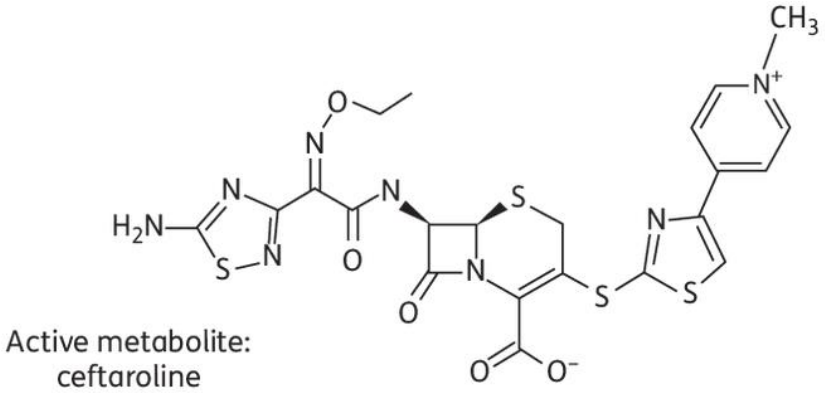

Figure 3: Active metabolite of Ceftaroline fosamil prodrug which active against methicillin-resistant *Staphylococcus aureus* (Biek, Critchley, Riccobene, & Thye, 2010)

3. Aminoglycosides: Tend to only be used in hospital to treat very serious illnesses such as septicemia, as they can cause serious side effects, including hearing loss and kidney damage; they're usually given by injection, but may be given as drops for some ear or eye infections. Gentamycin (Figure 4) and tobramycin are common examples. Many bacteria use enzymatic modification and alteration of target site to acquire resistance.

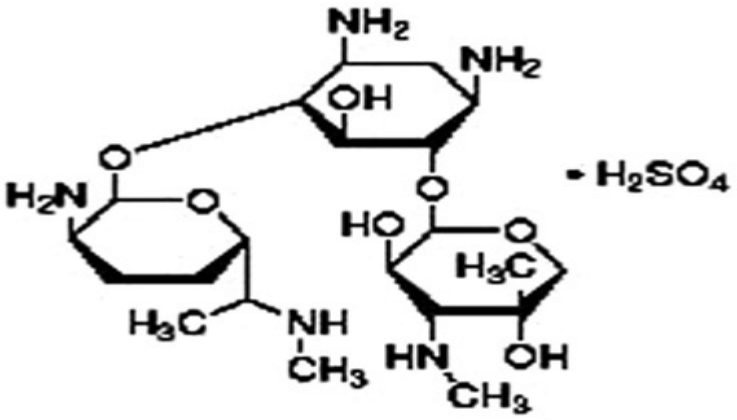

Figure 4: Structure of gentamycin sulfate (Ahangari, Salouti, Heidari, Kazemizadeh, & Safari, 2013).

4. Tetracyclines: Tetracyclines are broad-spectrum antibiotics against many bacteria and treat conditions such as acne, UTIs, intestinal tract infections, eye infections, sexually transmitted diseases, periodontitis (gum disease), and other bacterial infections. Examples include doxycycline (Figure 5). Doxycycline can induce bacterial resistance by antibiotic inactivation method.

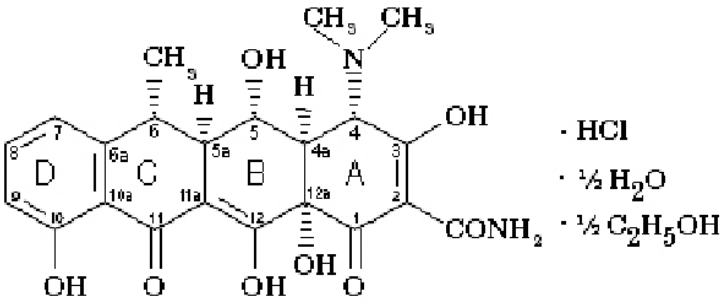

Figure 5: Structure of doxycycline hycalte (Kumar, Mohamed Kamil, Shri Prasad, Gayathri, & Shabeer, 2013).

5. Macrolides: They can be particularly useful for treating lung and chest infections, community-acquired pneumonia, pertussis or an alternative for people with a penicillin allergy, or to treat penicillin-resistant strains of bacteria. Azithromycin (Figure 6), clarithromycin is common in this class. These class of drugs can cause bacterial resistance through alteration of target site and enzymatic changes.

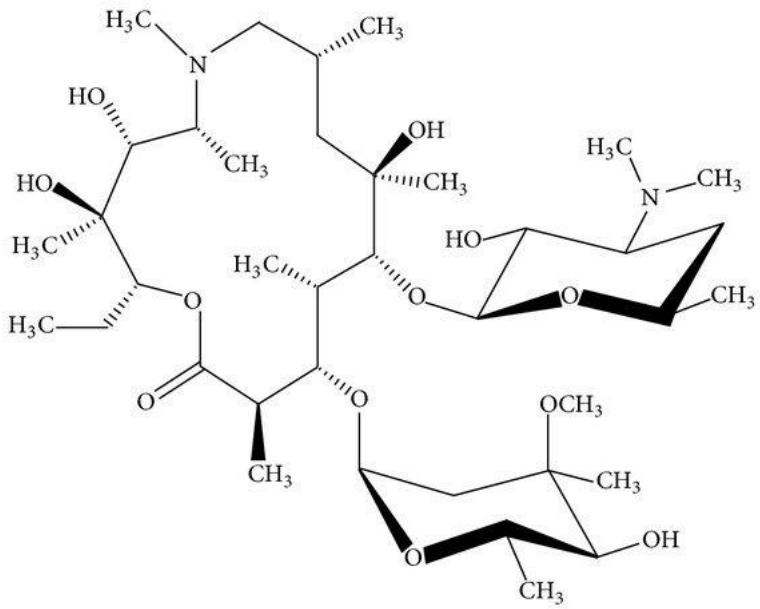

Figure 6: Structure of azithromycin (Rivulgo et al., 2013).

6. Fluoroquinolones: These are broad- spectrum synthetic antibiotics and are also referred as quinolones. They mainly used in serious urinary tract infections, pneumonia. They are also effective in anthrax, plague, bacterial prostatitis. Examples include, ciprofloxacin as shown in Figure 7. Bacteria can develop resistance against these drugs through reducing the uptake of antibiotic inside the bacterial cell.

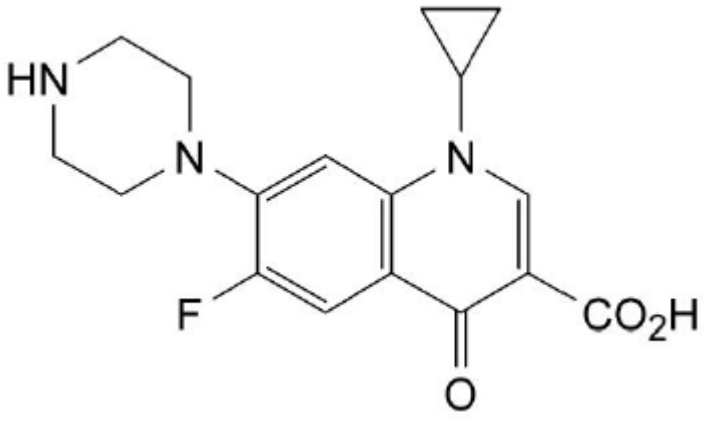

Figure 7: Structure of ciprofloxacin (Musa, Odunola, Yakasai, & Olayinka, 2012).

7. Glycopeptide: Methicillin-resistant *Staphylococcus aureus* (MRSA) caused diseases can be treated with this class of antibiotic. This can be useful in diarrhea, skin diseases.

Furthermore, it can be used to treat beta-lactam resistant bacterial infections such as endocarditis caused by enterococcal invasion. Vancomycin (Figure 8) teicoplanin, telavancin, ramoplanin and decaplanin,

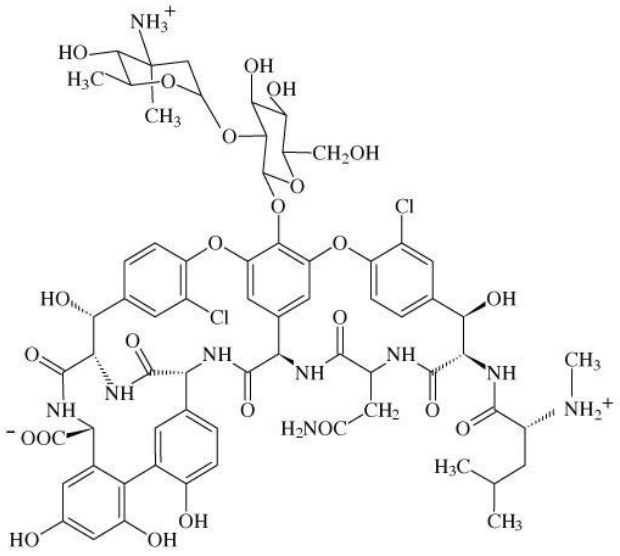

Figure 8: Structure of vancomycin (Schäfer, Schneider, & Shieldrick, 1996).

8. Lincosamide: This is also a broad- spectrum antibiotic used in both gram-positive and gram-negative bacterial infections. They are particularly effective against anaerobes. Complicated diseases such as lower respiratory tract infection, bone infections, pelvic inflammatory disease, joint infections can be treated with this class of antibiotics. Example: lincomycin (Figure 9).

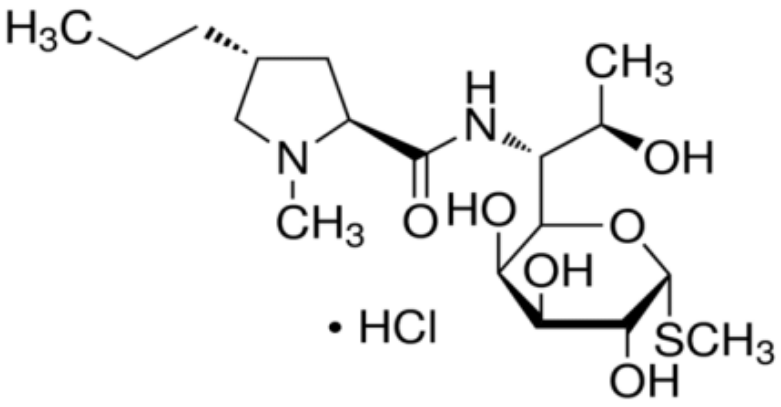

Figure 9: Structure of lincomycin (Beavis, Parsons, & Salfield, 1976).

9. Sulfonamides: Bacterial resistance is most common in this class of antibiotics. However, they can be effective against a variety of gram-negative and gram-positive bacterial infections. Drugs like sulfamethoxazole-trimethoprim, sulfisoxazole can be used to treat pneumonia, urinary tract infections, ear infections etc. These antibiotics can cause bacterial resistance through antibiotic deactivation (National Health Services, 2016).

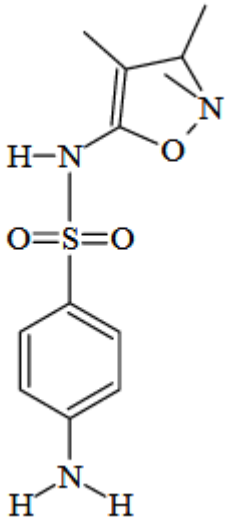

Figure 10: Structure of sulfisoxazole (Karthikeyan, Paramasivama, Jeeva, & Raja, 2015).

1.3 Antibiotic resistance

It is the capacity of bacteria to work against antibiotics. A property of bacteria that confers the capacity to inactivate or exclude antibiotics, or a mechanism that blocks the inhibitory or killing effects of antibiotics is known as antibiotic resistance. Resistance occurs when bacteria undergoes certain mutations in their genome and subsequently become capable of fighting against antibiotics (Microbiology Society, 2018).

For example, *S. aureus* develops resistance can synthesize certain proteins. These proteins increase attachment of the bacteria to tissues to increase its toxicity. Mutations can also enable them release biofilms making them more effective against antibiotics (Gordon & Lowy, 2008).

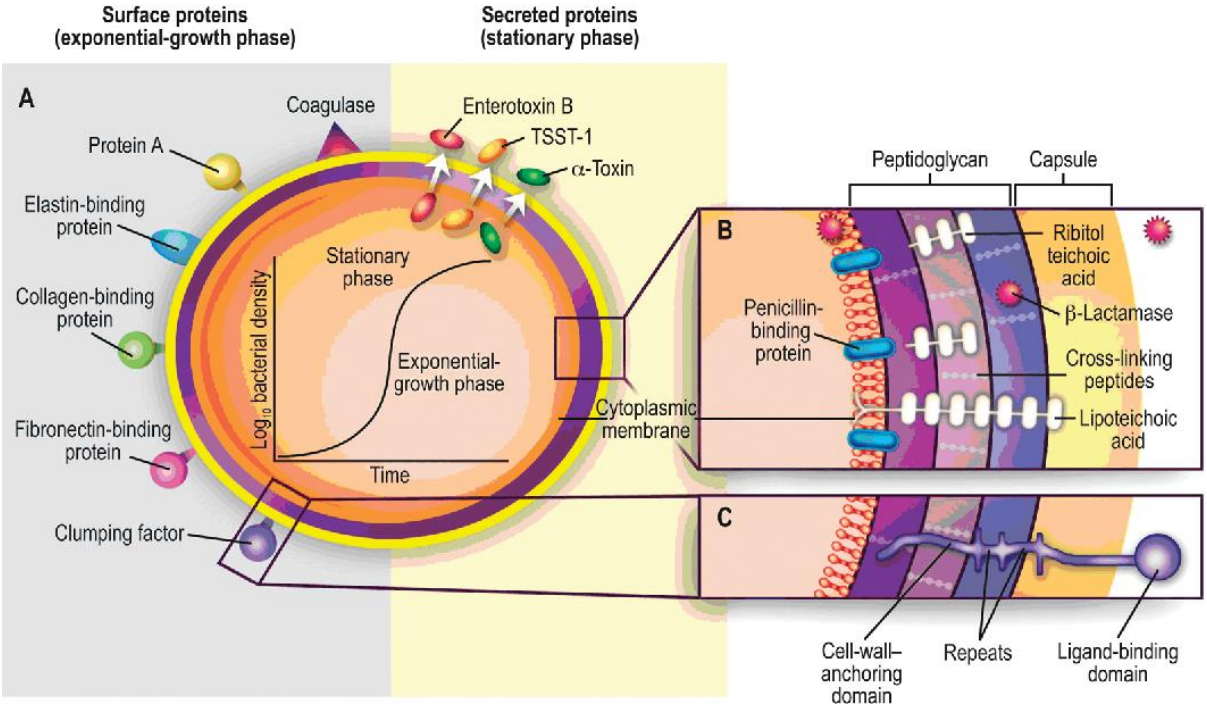

Figure 11: Mutations in resistant S. aureus (Gordon & Lowy, 2008).

1.4 Types of antibiotic resistance

1.4.1 Intrinsic resistance

It is a naturally occurring process. This resistance existed before antibiotic medications and is still occurs in all bacterial species. It is controlled through outer membrane and active efflux. It is the innate ability of a type of bacteria species to resist the action of an antibiotic as a consequence of the bacteria's structural or functional characteristics. In contrast to acquired resistance, intrinsic resistance is normal for bacteria of a given type (Cox & Wright, 2013). *M. pneumoniae, M. genitalium* forms mycoplasma in which there is no cell wall as shown in Figure 12. Thus the bacteria can save itself from antibiotics such as penicillins which mainly targets the cell wall synthesis (Acharya, 2017).

Figure 12: Mycoplasma formation in bacteria (Acharya, 2017).

1.4.2 Acquired resistance

Acquired resistance refers to the resistance of microbe which was previously absent. Mutations and other alterations can be involved in this process. Getting resistant genes from foreign bodies is also a possibility. Characteristics of acquired resistance are found only in some strains or subpopulations bacterial species. Mutation and horizontal gene transfer are the two main factors here as can be seen in Figure 13 (Vladimir & Luca, 2016).

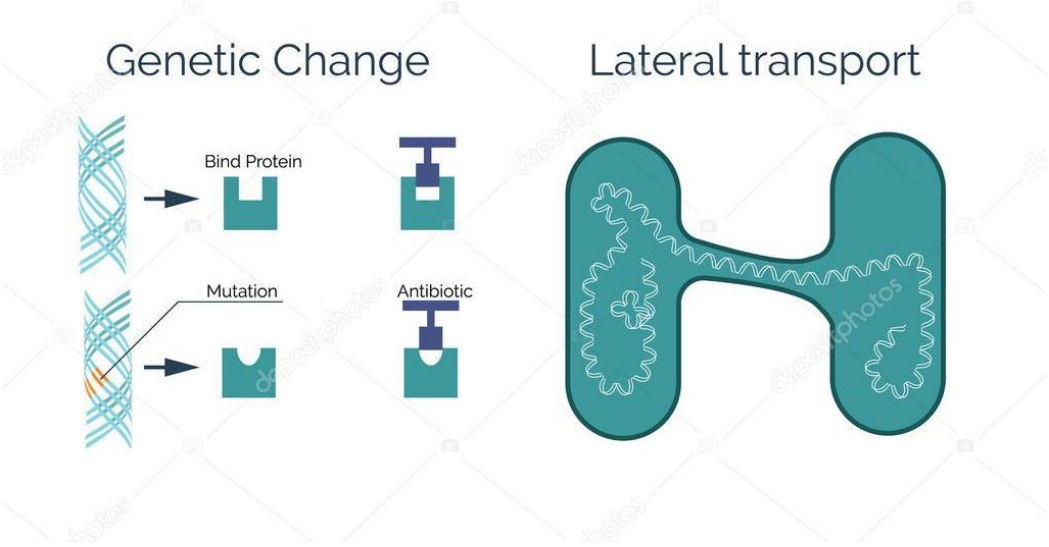

Figure 13: Factors of acquired resistance (Kovalenko, 2018)

1.5 Development of antibiotic resistance

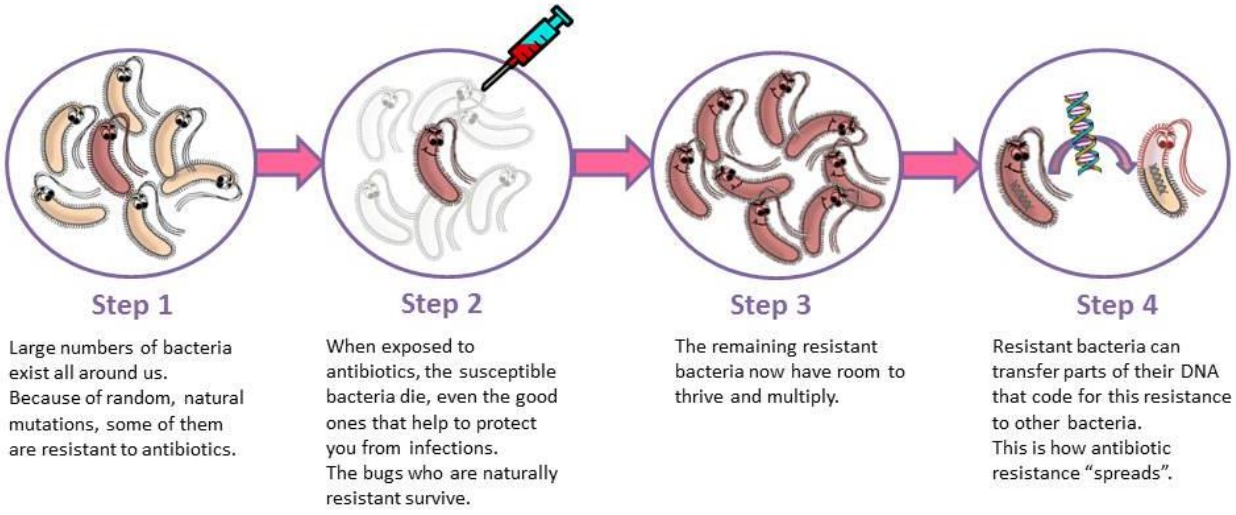

Figure 14: Process of antibiotic resistance (WHO, 2018)

Antibiotic resistance occurs when specific bacteria undergo mutation. Mutation can give microorganism certain advantages of the antibiotics. Hence, unlike other bacteria they are not killed a when antibiotic is administered. When the normal bacteria are reduced in number the mutated bacteria can divide and make colonies in the body (WHO, 2018).

1.6 Resistance spread of antibiotics

In Figure 15, it can be seen that the antibiotic resistance can reach to major population by contaminated food sources. Animals used for food production can be introduced to high and improper usage of antibiotics. This can lead to emergence of resistant bacteria in the animals which can eventually reach humans. Furthermore, the antibiotics remaining in the animals can also reach humans and develop resistance inside human body. These resistant microorganisms can be transmitted from person to person. Antibiotics and resistant bacteria can also be introduced in hospitals and from patients carrying those (NAID, 2018).

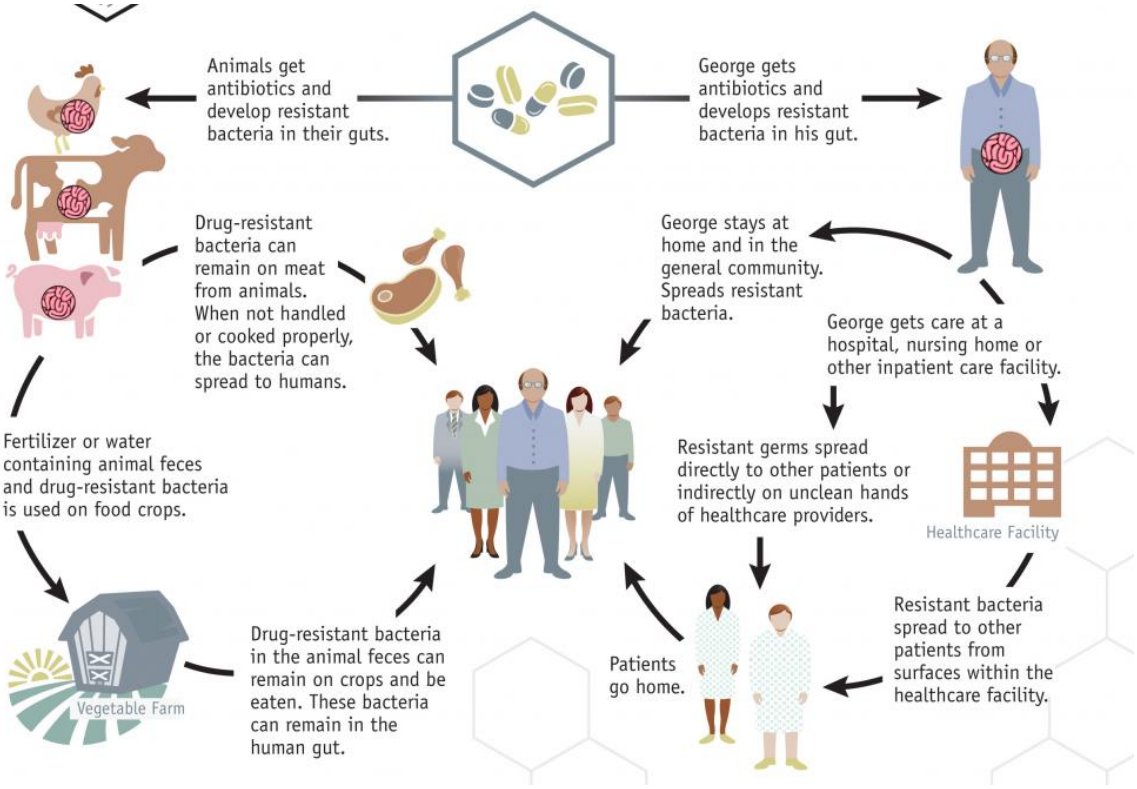

Figure 15: Spreading of resistance (Holly M. Whetstone, 2014)

1.7 Factors of antibiotic resistance

1.7.1 Too much usage

Antibiotic resistance has increased with the passing of time due to irrational usage. Studies have established a clear relation between resistance and consumption. Bacteria can inherit genes from other bacteria through horizontal gene transfer. Sir Alexander Fleming himself had some suspicions and he warned about antibiotic abuse. Mutation is another way of developing resistance. Antibiotics may kill the non-resistant substances but leave resistant bacteria which will reproduce. Despite several studies and warnings regarding the usage of antibiotics, they are still over used all over the world. In Bangladesh antibiotics can be

acquired without proper prescriptions. This makes it cheap, easily accessible which contributes to the overall resistance of antibiotics.

1.7.2 Inappropriate Prescribing

Incorrectly prescribed antibiotics have a questionable therapeutic advantage and expose patients to future antibiotic therapy complications. Concentrations of subinhibitory and subtherapeutic antibiotics can encourage the growth of antibiotic resistance by promoting genetic alterations such as gene expression modifications, HGT, and mutagenesis. Enhanced mutagenesis and HGT encourage and spread antibiotic resistance. Low concentrations of antibiotics in bacteria such as *Pseudomonas aeruginosa* have been shown to contribute to strain diversification. In Bacteroides fragilis, it has also been shown that subinhibitory levels of piperacillin and/or tazobactam cause wide proteomic changes.

1.7.3 Extensive Agricultural Use

Antibiotics used in livestock are ingested by humans when they eat food. It was first observed 35 years ago when elevated levels of antibiotic resistance were discovered in the intestinal flora of both farm animals and farmers. More recently, molecular detection methods have demonstrated that resistant bacteria in farm animals reach consumers through meat products. This happens through the following series of occurrences. Firstly, antibiotic use in food-producing animals kills or suppresses sensitive bacteria, enabling antibiotic-resistant bacteria to flourish. Secondly, resistant bacteria are transferred to humans through the supply of food. These bacteria can cause diseases in humans that can have negative health effects.

1.7.4 Lack of new antibiotics

Antibiotic innovation for the pharmaceutical industry is not anymore regarded a financially sound investment. The pharmaceutical industry's development of modern antibiotics, a

strategy that had been efficient in the fight against resistant bacteria in the past, was largely hampered by financial and regulatory barriers. The antibiotic field was abandoned by 15 of the 18 largest pharmaceutical companies. The amount and variety of study teams have also been significantly decreased by mergers between pharmaceutical companies. As a consequence of financing reductions due to the economic crisis and antibiotic study in universities has been reduced.

Furthermore, lab personnel and experts working with bacterial infections have recommended that the use of antibiotics should be restricted. Therefore, once a fresh antibiotic is marketed, doctors often keep this latest agent in reserve for only the worst instances due to fear of encouraging drug resistance, rather than prescribing it immediately, and they continue to prescribe older agents that have demonstrated similar effectiveness. Consequently, to tackle severe diseases, new antibiotics are often used when no other medications are working. This tradition results in lack of new antibiotics.

1.7.5 Regulatory Barriers

Obtaining regulatory approval is often a barrier even for those businesses that are optimistic about pursuing the discovery of new antibiotics. Between 1983 and 2007, there was a significant reduction in the amount of new approvals for antibiotics. Difficulties observed in seeking regulatory approval include: bureaucracy, lack of clarity, country-specific variations in clinical trial demands, changes in legislative and licensing regulations, and ineffective communication channels (Ventola, 2015).

1.8 Mechanisms of antibiotic resistance

Bacteria can achieve resistance against antibiotics is many ways. Bacteria can live in different hostile salutations can adapt accordingly. As can be seen from Figure 16 bacteria can achieve

resistance through enzymatic modification, decrease of permeability, alteration of target sites and controlling the efflux pump etc.

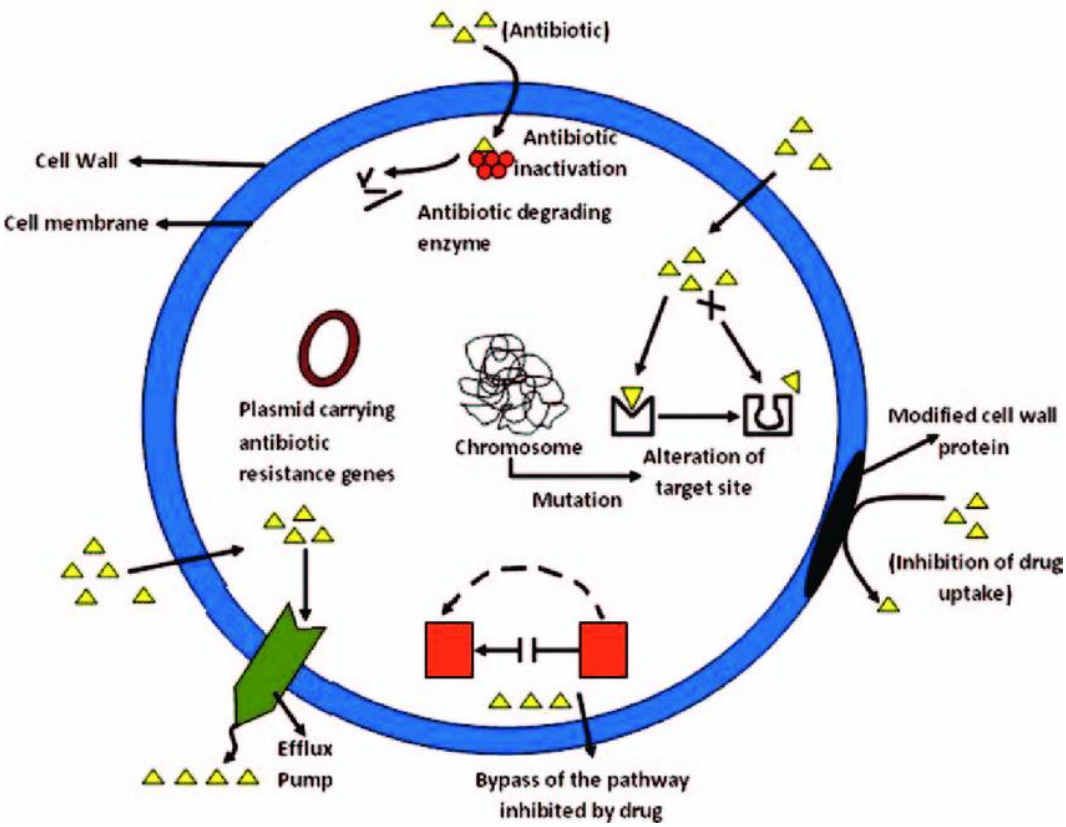

Figure 16: Different mechanism of antibiotic resistance (Singh, Smitha, & Singh, 2014).

1.8.1 Chemical alterations of the antibiotic

Enzymes produced in both gram-positive and gram-negative bacteria have the ability to cause chemical alterations in the drug molecule. However, antibiotics can still produce their effect by inhibiting protein synthesis. Many types of modifying enzymes have been described such as acetylation, adenylation and phosphorylation. Examples include alteration of aminoglycoside molecule by aminoglycoside modifying enzymes (AMEs).

1.8.2 Drug molecule destruction

In this scenario enzymes released from the bacteria completely destroys the drug molecule. For example, β- lactamases are the enzymes which cause β-lactam resistance. The amide

bond of β- lactam antibiotic is broken resulting in breaking down of the molecule (shown in Figure 17).

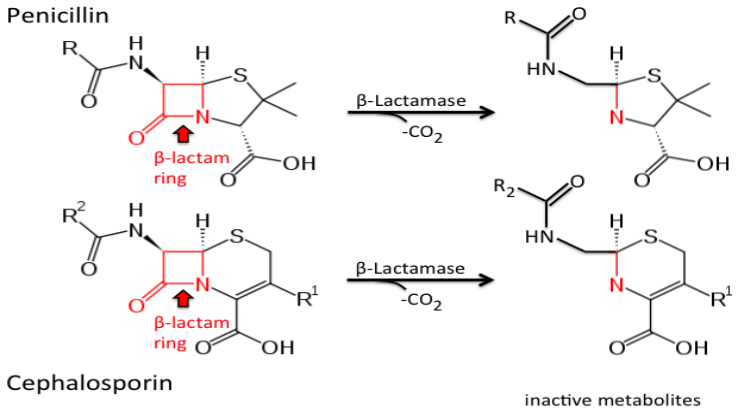

Figure 17: Enzymes breaking β-lactam antibiotics (Drusano, 2004)

The effects of β- lactamases are well known since 1940. Genes referred as bla are responsible for the encoding of β- lactamases. More than a thousand β-lactamases have been discovered and new enzymes are being discovered very frequently.

1.8.3 Efflux Pumps

Antimicrobial resistance can also be achieved by producing complicated bacterial machinery that can extrude a toxic compound out of the cell. By definition an efflux-system is capable of pumping tetracycline from the *E. coli* cytoplasm. In both gram negative and gram-positive bacteria many kinds of efflux pumps have since been identified. These systems may have broad substrate specificity which are usually found in MDR bacteria or may be substrate-specific. or, mechanism of resistance affects a wide range of antimicrobial classes including protein synthesis inhibitors, fluoroquinolones, β-lactams, carbapenems and polymyxins. The genes encoding efflux pumps can be located in MGEs (as initially described for the *tet* gene) or in the chromosome. Importantly, chromosomally encoded pumps can explain the inherent resistance of some bacterial species to a particular antibiotic. The process can be seen in Figure 18.

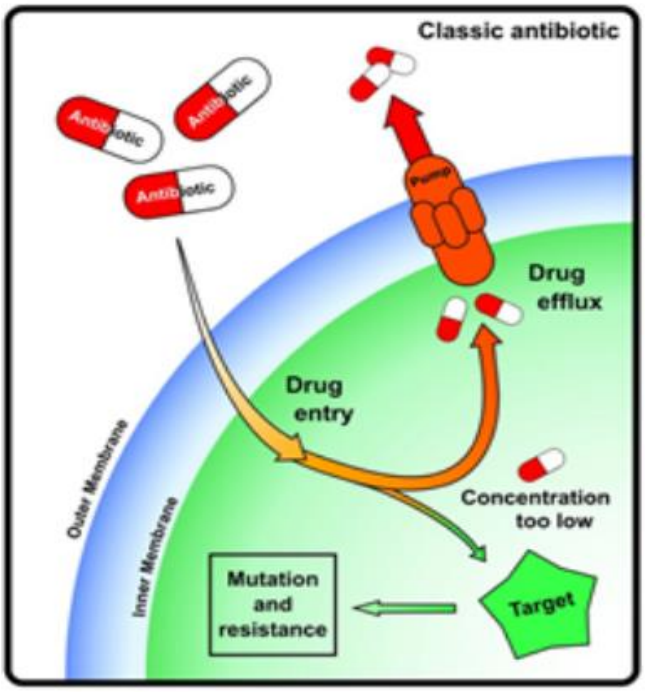

Figure 18: Efflux pumps in preventing drugs to reach target site (Franco, Altagracia Martínez, Sánchez Rodríguez, & Wertheimer, 2009)

There are 5 major families of efflux pumps, including the major facilitator superfamily (MFS), the small multidrug resistance family (SMR), the resistance-nodulation-cell-division family (RND), the ATP-binding cassette family (ABC), and the multidrug and toxic compound extrusion family (MATE). These families differ in terms of structural conformation, energy source, range of substrates they are able to extrude and in the type of bacterial organisms in which they are distributed.

1.8.4 Changes in Target Sites

A popular approach for the development of antimicrobial resistance in bacteria is to prevent the antibiotic from functioning by interacting with the target area. In order to accomplish this, bacteria have developed distinct strategies, including the defense of the target evicting the antibiotic to reach its binding site and altering the target resulting in a reduced sensitivity to the antibiotic molecule (Franco et al., 2009). A demonstration can be seen in Figure 19, the

bacteria changed its site of attachment through mutations to prevent the antibiotic from binding to the bacteria. As a result, the effectiveness of the antibiotic is destroyed.

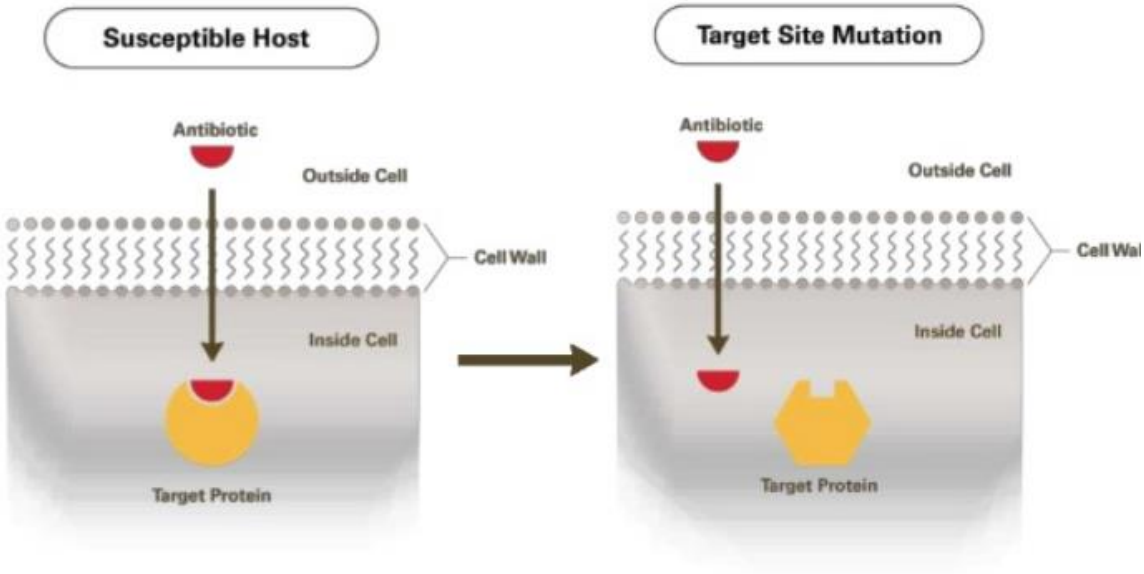

Figure 19: Alteration in target site (Cox & Wright, 2013)

1.9 Background information of *Staphylococcus aureus*

Staphylococcus aureus is a cocci-shaped gram-positive bacterium as shown in Figure 4. It is quite widely found in the environment. Similarly, they are also a part of human normal flora. It is quite often found in the skin as well as in the nasal area (most often in the mucous membrane). Up to 15% of the entire population and half of all adolescents are reported to be colonized by this bacterium. *S. aureus* colonization may differ in different population and work group. For example, medical professionals, patients tend to have a higher degree of colonization than most other people. *S. aureus* living on the skin and mucous membrane don't cause any harm. However, if somehow, they find their way into the blood, they can cause very serious infections (Taylor & Unakal, 2019).

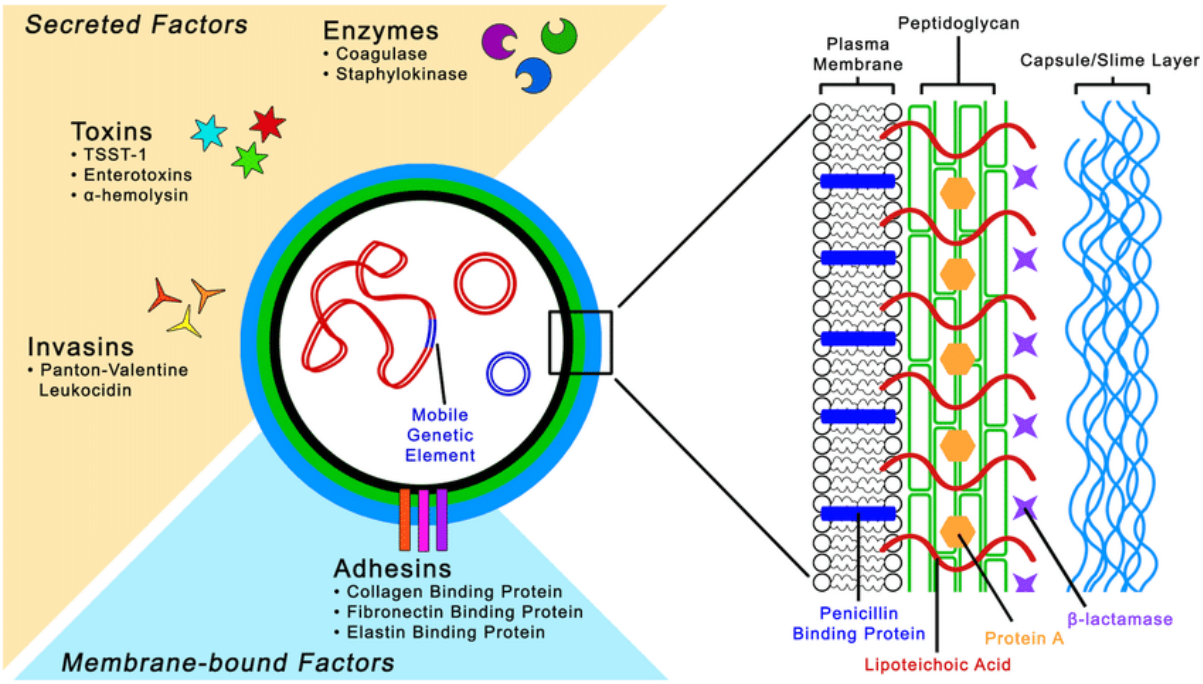

Figure 20: Structure of S. aureus and pathogenetic factor (*Kong EF, Johnson JK, 2016)*

1.10 Resistance of *Staphylococcus aureus*

Staphylococcus aureus has many strains which are resistant to many antibiotics. MRSA are the most commonly found strains. They have a mec gene which is a part of a significant chromosomal cassette mec is responsible for its resistant ability. Penicillin-binding protein 2a is encoded by this mec gene. This PBP-2a is different from other PBPs. This does not have as much affinity to beta-lactam compared to other PBPs. This result in lower binding of PBPs with β-lactam antibiotics which makes it highly resistant. The process can be seen in Figure 21. The bacteria can produce biofilms, different surface proteins to contribute to its resistance (Gordon & Lowy, 2008).

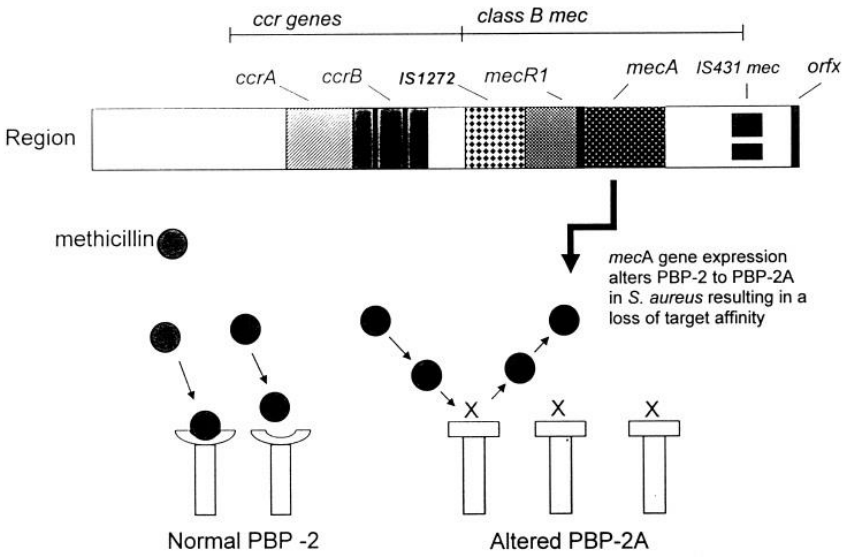

Figure 21: Process of developing methicillin resistance in S. aureus (Gordon & Lowy, 2008).

1.11 Prevalence of *S. aureus* resistance in Bangladesh

In previously conducted study, done on *S. aureus* isolated obtained from cow milk found that cefriaxone, ciprofloxacin, gentamycin caused high numbers of resistance. The sensitivy pattern is shown in Figure 22. Amikacin, imipene, azithromycin did not cause resistance. However, experiments on isolates collected from human patients were not conducted (Jahan et al., 2015).

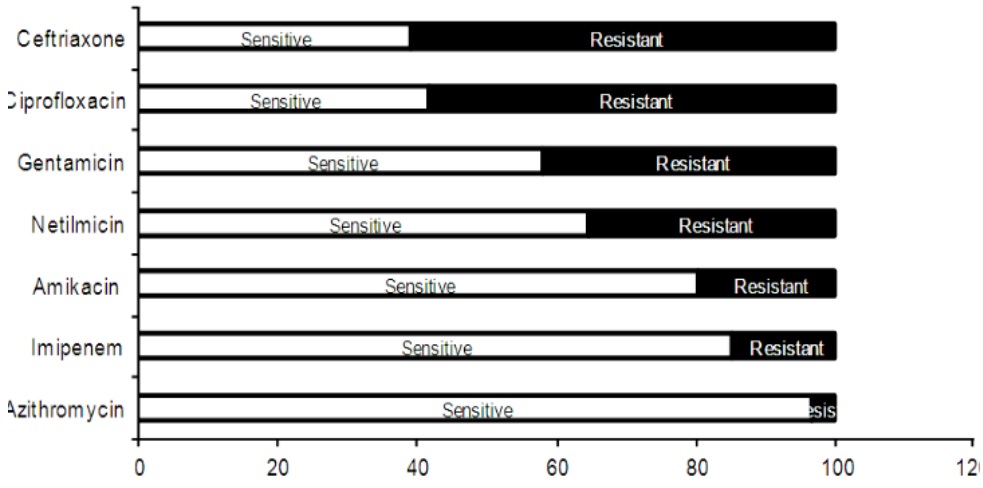

Figure 22: Sensitivity pattern of S. aureus in cow milk (Jahan et al., 2015).

Chapter 2

Aim and methodology

2.1 Aim of the study

The aim of the study is to determine the development of resistance of *Staphylococcus aureus.* Furthermore, the research was conducted to identify the specific antibiotics against which the bacteria is resistant. It was also done to unfold the different causes and factors which can contribute to the emergence of resistance. Lastly, the study was carried out to identify susceptible populations particularly in different age groups and genders.

2.2 Methodology

2.2.1 Research protocol

Staphylococcus aureus isolates were collected from different hospitals and diagnostic centers across Dhaka, Bangladesh. The collection of these samples was done from August to September, 2019. No questionnaires for patients, doctors or diagnostic center officials were used for this study. These were collected from diagnostic labs and hospitals and they were basically test reports which can be termed as secondary data. These data contained age, gender, pattern of sensitivity against the particular microorganism.

Data samples were collected from hospitals and diagnostic labs at random. The data was first divided into the pattern of the specific antibiotics used. The data was further divided according to age. They were basically categorized into 3 age groups. The groups are, patients aged below 25, aged 25 to 45 and aged 45 and above. Additionally, the gender of the patients was also distinguished.

Lab officials in the hospitals and diagnostic centers used disc diffusion method to get insights about particular antibiotics. Therefore, the experiments were highly effective, time efficient

and the cost was kept to a minimum. A standard agar medium with specific concentrations was used to make culture plates. Specific commercially viable antibiotic disks were evenly placed on the plates. Incubating overnight, bacterial formation in every plate was observed. Afterwards, particular areas in the plate where there is no bacterial colony (zone of inhibition) was identified and measured. This determined the sensitivity of that particular drug against the antibiotic.

The different zones of inhibition were plotted on a standard interpretation bar graph and the properties of the isolate was defined for every drug. These properties were specified in the bar graphs. The characteristics was categorized into resistant, sensitive and not applicable. Furthermore, the data was also analyzed by separating the sample according to age and gender. No qualitative measurement tests such as minimum inhibitory concentration (MIC) were not conducted.

Most importantly, all these data collection and work was done with prior permission of the respective authorities. The study protocol was extensively reviewed by my supervisor. Furthermore, the protocol was reviewed and authorized by the authorities in their respective hospitals and diagnostic centers. Patient's privacy was our top priority and it was maintained thoroughly. In addition, names of hospitals and diagnostic centers were also kept anonymous.

2.2.2 Collection of data

Data collection process was conducted with prior permissions of respective authorities. Sensitive information regarding patient, hospital, diagnostic center was handled with care and animosity was maintained. Data sets were compiled and printed for further usage

2.2.3 Analysis of data

The data was computed using SPSS, version 23 (IBM, Inc., Chicago, IL, USA, 2015) and Microsoft office excel, 2016 to generate percentage, valid percentage and cumulative

percentage of each set (resistant, sensitive, not applicable) of data. These software were also used to generate charts for visual representation.

Chapter 3

Results

The analysis was done considering different prospective. Firstly, the population was categorized according to different types of antibiotics used in the antibiogram test. The population was divided into male and female. The population was further divided into different age groups. The 3 age groups were below age 25, aged 25 to 45 and aged 45 and above. Antibiogram tests were conducted to observe different numbers of sensitivity, resistance among the sample population.

3.1 Sensitivity of *S. aureus* in all patients

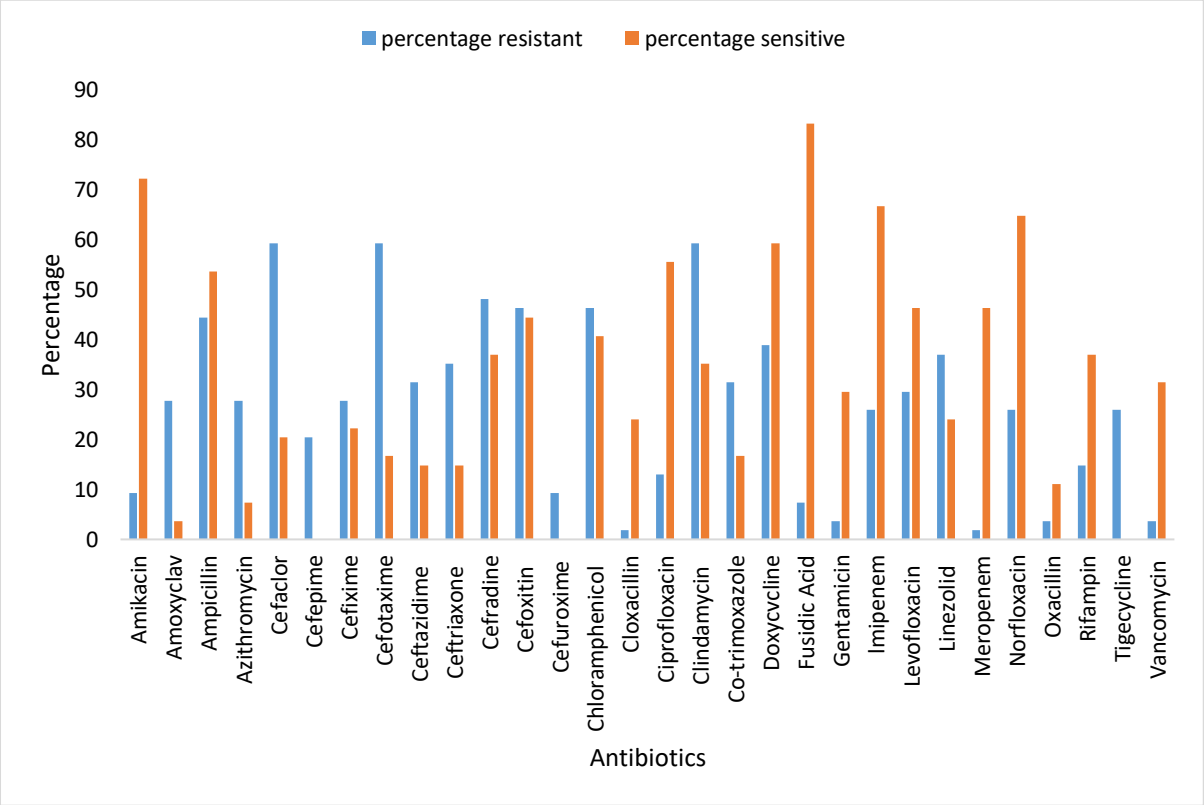

Figure 23: Antibiotics sensitivity in isolates acquired from patients

By computing all the data, we found that isolates are 9.3% resistant and 72.2% sensitive to amikacin, 27.8% resistance and 3.7% sensitive to amoxiclav, 44.4% resistance and 53.7% sensitive ampicillin, 27.8% resistance and 7.4% sensitive to azithromycin, 59.3% resistance

and 20.4% sensitive to cefaclor, 20.4% resistant and none sensitive to cefepime, 27.8% resistant and 22.2% sensitive to cefixime, 59.3% resistant and 16.7% sensitive to cefotaxime, 31.5% resistant and 14.8% sensitive to ceftazidime, 35.2% resistant and 14.8% sensitive to ceftriaxone, 48.1% resistant and 38.0% sensitive to cefradine, 46.3% resistant and 44.4% sensitive to cefoxitin, 9.3% resistant and none sensitive to cefuroxime, 46.3% resistant and 40.7% sensitive to chloramphenicol, 1.9% resistant and 24.1% sensitive to cloxacillin, 13.0% resistant and 55.6% sensitive to ciprofloxacin, 59.3% resistant and 39.2% resistant to clindamycin, 31.5% resistant and 16.7% sensitive to co-trimoxazole, 38.9% resistant and 59.3% sensitive to doxycycline, 7.4% resistant and 83.3% sensitive to fusidic acid, 3.7% resistant and 29.6% sensitive to gentamycin, 25.9% resistant and 66.7% sensitive to imipenem, 29.6% resistant and 46.3% sensitive to levofloxacin, 37% resistant and 24.1% sensitive to linezolid, 1.9% resistant and 46.3% sensitive to meropenem, 25.9% resistant and 64.8% sensitive to norfloxacin, 3.7% resistant and 11.1% sensitive to oxacillin, 14.8% resistant and 38.0% sensitive to rifampin, 25.9% sensitive and none resistant to tigecycline, 3.7% resistant and 31.5% sensitive to vancomycin. The stats can be seen in Figure 23.

3.2 Sensitivity in different groups

The isolates were divided into different categories. Among the patients there were 55.6% male and 44.4% female. Furthermore, they were divided into different age groups.

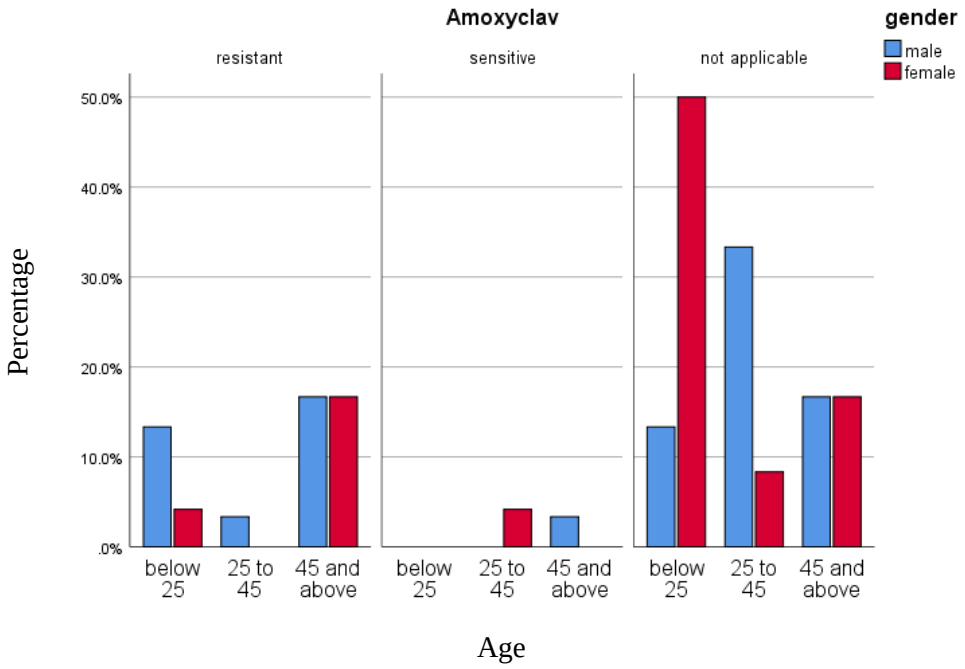

Figure 24: Amoxiclav sensitivity of S. aureus among patients

Figure 24 outlines the sensitivity of amoxiclav among different age groups. Out of 54 isolates of *S. aureus* 15 (27.8) were resistant, 2 (3.7) were sensitive, and 37 (68.5%) were not sensitive nor resistant. It can be seen that resistance was particularly higher in male who are below the age of 25 and between the age of 25 and 45. Sensitivity was also higher in male above 45. However, between the age of 25 and 45 women showed a higher sensitivity. Additionally, women were in higher numbers in below 25 age group who did not develop resistance or sensitivity. On the other hand, male number are significantly higher between the age of 25 and 45.

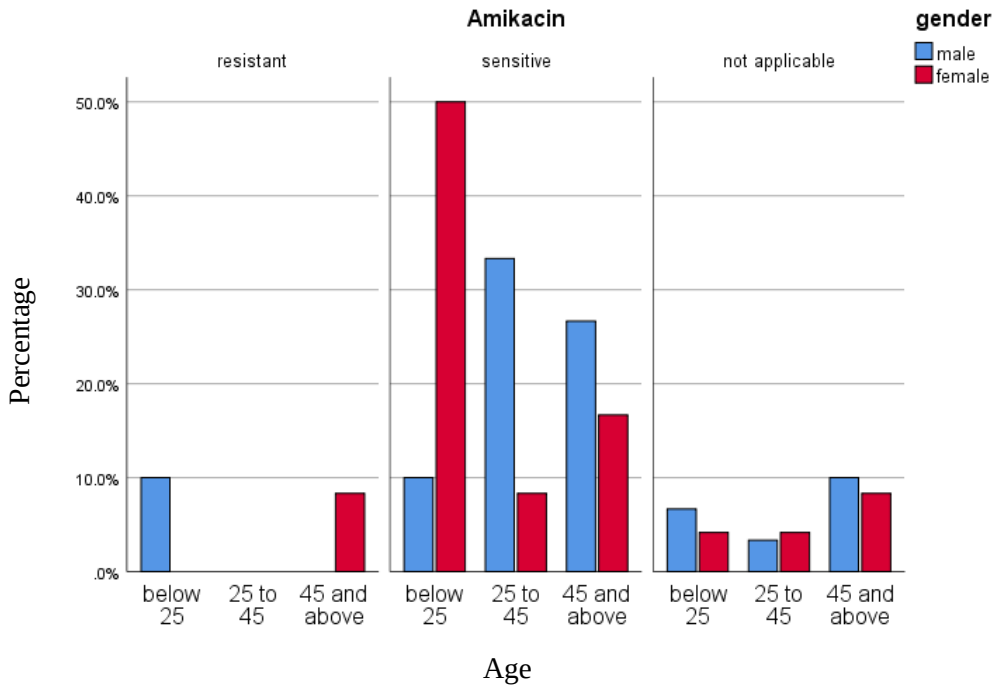

Figure 25: Amikacin sensitivity of S. aureus among patients

From Figure 25 we can see that none of the isolates from patients who are between 25 and 45 are not resistant to amikacin. Interestingly, only men who are below 25 are resistant and only women who are above 45 are resistant. However, both male and female all showed some degree of sensitivity. Lower numbers of patients were normal as did not show resistance or sensitivity. Overall, out of 54 isolates 5 (9.3%) showed resistance, 39 (72.2%) showed sensitivity and 10 (18.5) were normal. Therefore, most of the patients showed sensitivity towards the drug.

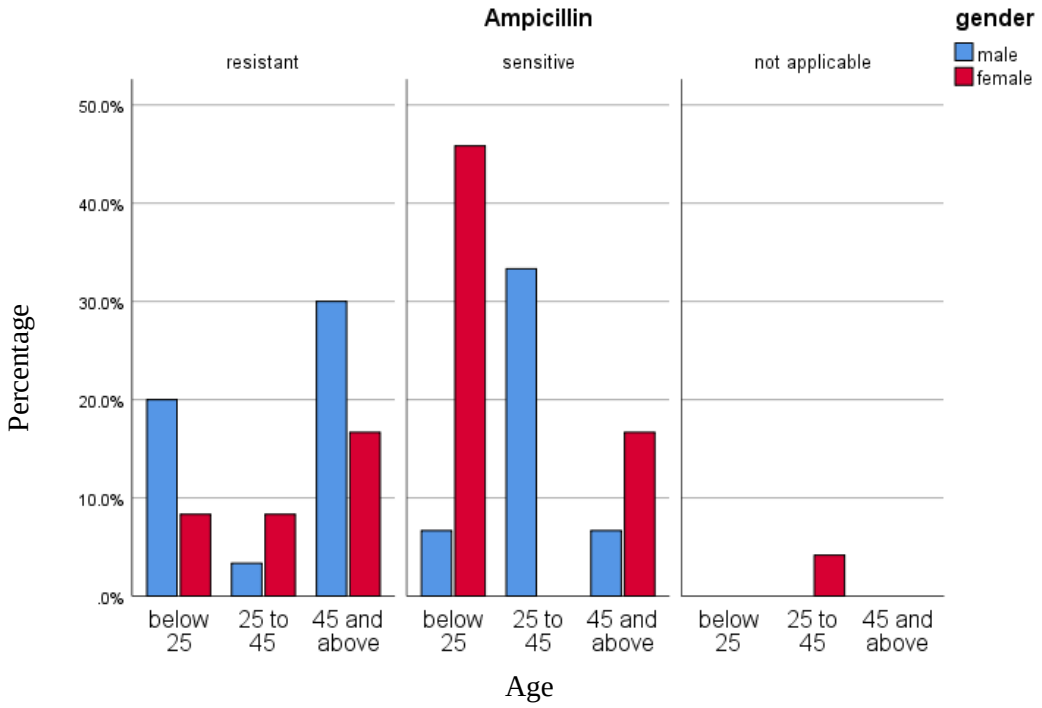

Figure 26: Ampicillin sensitivity of S. aureus among patients

As can be seen from Figure 26 most of the isolates acquired from the patients were sensitive or resistant. Less than 5% (1) of the bacterial isolates showed no resistance or sensitivity. 44.4% (24) of the isolates were resistant and 53.7% (29) were sensitive. Women showed higher sensitivity who are below the age of 25. Although there were no women sensitive cases in 25 to 45 age group. Resistance occurrence was somewhat similar among male and female across all age groups.

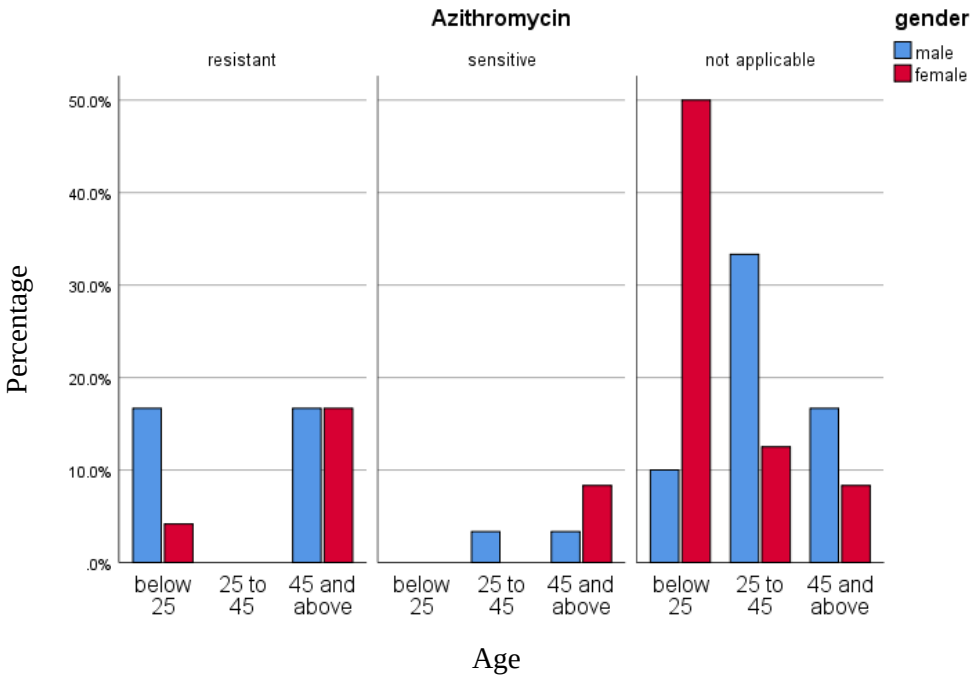

Figure 27: Azithromycin sensitivity of S. aureus among patients

It can be seen from Figure 27 more isolates (64.8%) did not develop resistance nor they are sensitive. Only 4 patients (7.4%) were sensitive to the drug. Furthermore, only men were sensitive to the drug aged 25-45. In case of resistance, no isolates among patients aged 25 to 45 were resistant to the drug. However, equal amounts of isolates were resistant in the above 45 age group. More men developed resistance who are aged below 25. Overall, 15 (44.4%) of the isolates were resistant.

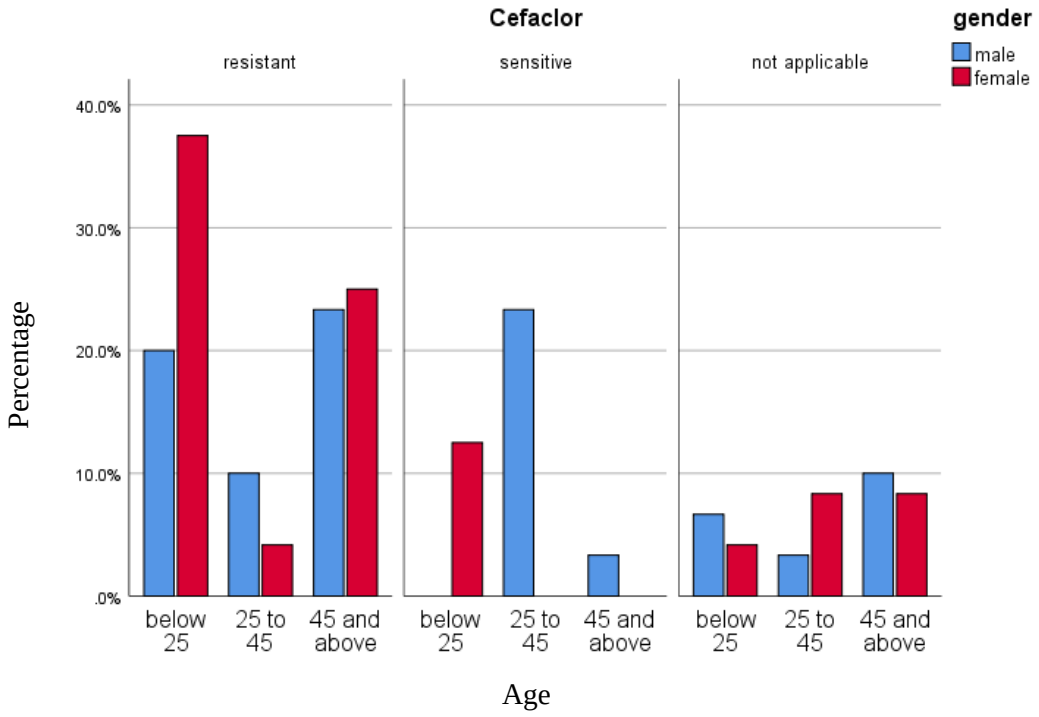

Figure 28: Cefaclor sensitivity of S. aureus among patients

Figure 28 suggests that out of the 54 sample 32 (59.3%) had resistance. Resistance was more prevalent in patients who are aged below 25 and patients who are 45 and above. Interestingly, only women aged below 25 were sensitive and only men who are aged between 25 and 45 showed sensitivity. Similar to sensitivity where 11 samples were considered sensitive, fewer number of isolates (11) among male and female showed no resistance and sensitivity.

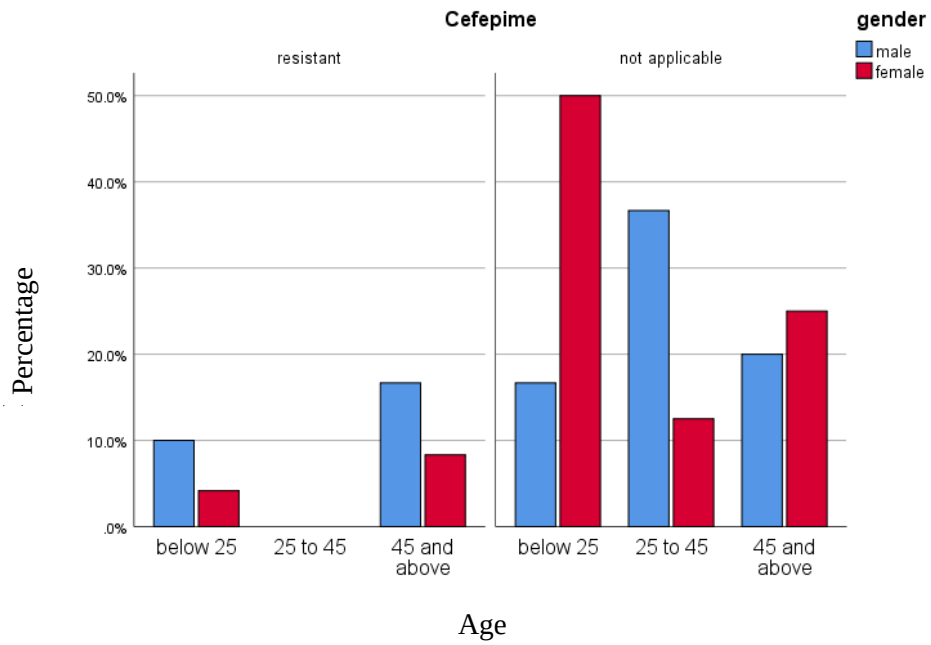

Figure 29: Cefepime sensitivity of S. aureus among patients

From the Figure 29 we can see that, results of lot of samples (79.6%) could not be identified in the antibiogram tests. Only 11 of the isolates (20.4%) were resistance.

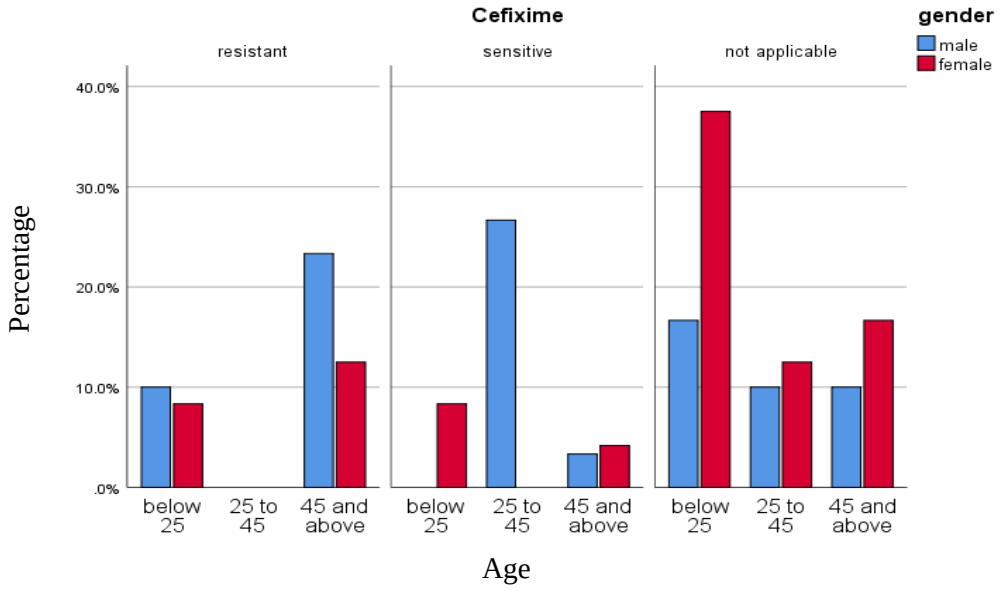

Figure 30: Cefixime sensitivity of S. aureus among patients

More isolates from patients were not resistant nor sensitive to cefixime as can be seen from Figure 30. 27 isolates (50%) were not resistant and sensitive. Only women below age 25 and only men aged 25 to 24 were sensitive. Only 8 isolates (14.8%) of the samples were sensitive. On the other hand, no isolates collected from patients aged 25 to 45 were resistant. Prevalence of resistance below the age of 25 were similar between male and female but it was higher in men aged 45 and above.

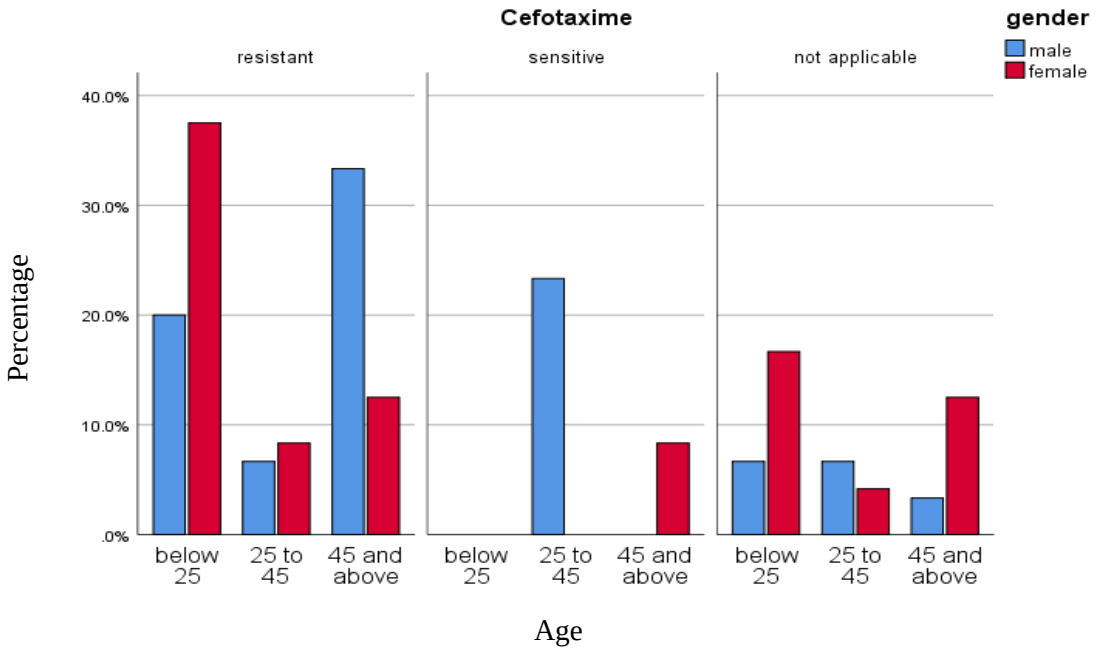

Figure 31: Cefotaxime sensitivity of S. aureus among patients

In this case, high number of isolates showed resistance as shown in Figure 31. Almost 60% of all the patients were resistant to this antibiotic. Numbers were particularly high in females below age 25 and male age above 45. Only 9 patients (16.7%), most of them male age 25 to 45 and women aged above 45 are sensitive to this drug. 13 patients (24.1%) were categorized not applicable.

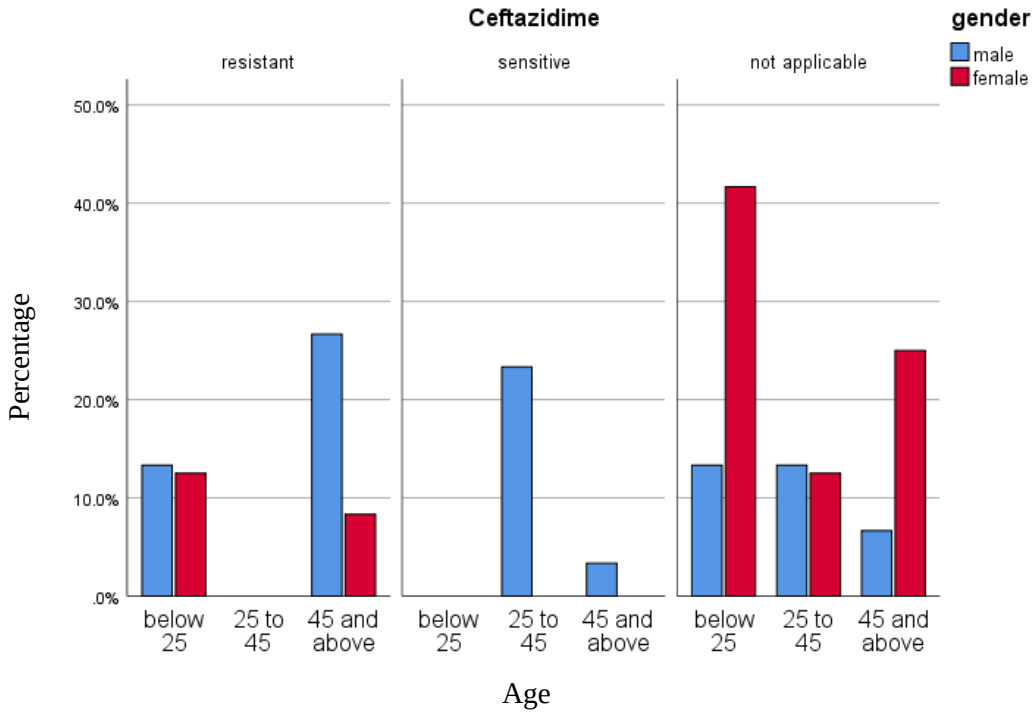

Figure 32: Ceftazidime sensitivity of S. aureus among patients

As Figure 32 suggests, more than half (53.7%) of the isolates were non-resistant, non-sensitive. Only 8 samples were considered sensitive and all of them were men aged above 25. Resistance did occur in any patients aged between 25 and 45. Occurrence of resistance in aged 45 and above were significantly higher in men. No resistance occurred any isolates obtained from patients aged between 25 to 45.

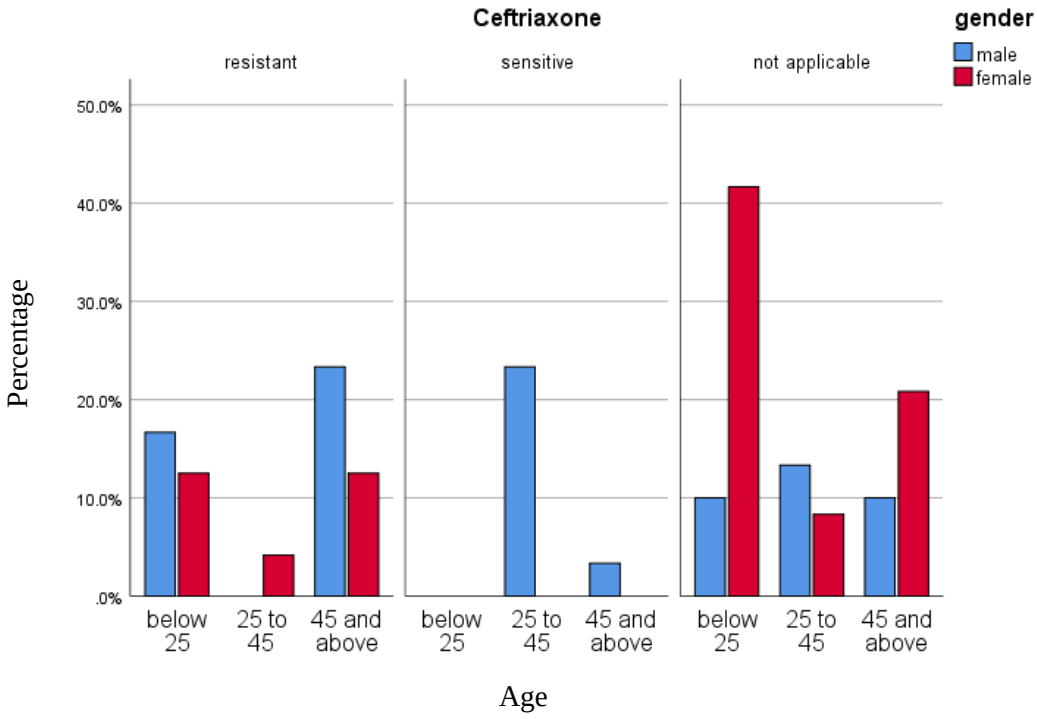

Figure 33 Ceftriaxone sensitivity of S. aureus among patients

As can be seen from Figure 33, half of the isolates were non-resistant, non-sensitive. Only 8 samples were considered sensitive and all of them were men aged above 25. Resistance occurred in 19 (35%) isolates. Furthermore, no man who are aged 25 to 45 did not develop resistance. Occurrence of resistance in the other two groups was quite similar between men and women.

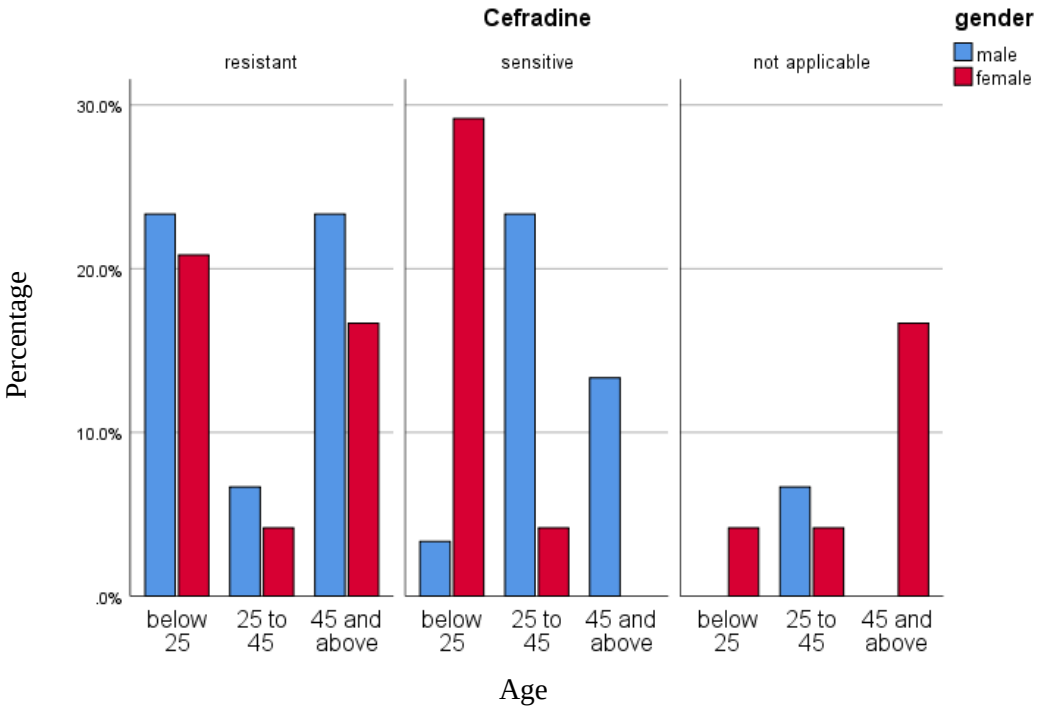

Figure 34: Cefradine sensitivity of S. aureus among patients

From Figure 34 we can see high numbers of sensitivity and resistance. 26 (48.1%) of isolates were resistant, most of them were in below 25 and above 45 age group. Prevalence in men and women were quite similar. In case of sensitivity, more women aged below 25 were sensitive to cefradine. However, in the group 25 to 45 there are fewer women than man and in age 45 and above group there are no women at all. Altogether 20 isolates (37%) were sensitive. Only about 15% of the isolates were not applicable where in below 25 and 45 and above age group there was no isolates acquired from men.

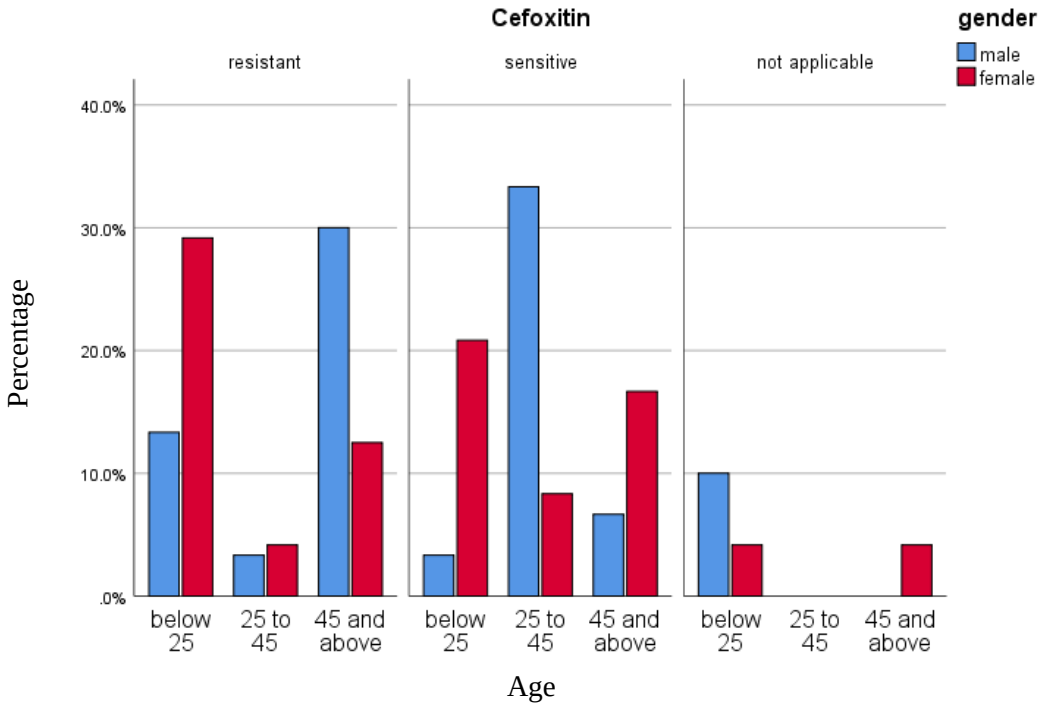

Figure 35: Cefoxitin sensitivity of S. aureus among patients

From Figure 35 we can see high numbers of sensitivity and resistance. 25 (46.3%) of isolates were resistant, most of them were in below 25 and above 45 age group. Prevalence in women was higher in below 25 age group and it was higher in men in 45 and above age group. In case of sensitivity, more women aged below 25 were sensitive to the antibiotic. Moreover, in the age group 45 and above there are fewer men than women. However, in the age group 25 to 45 resistance among men was more prevalent. Altogether 24 isolates (44.4%) were sensitive. Only about 9.3% of the isolates were not applicable.

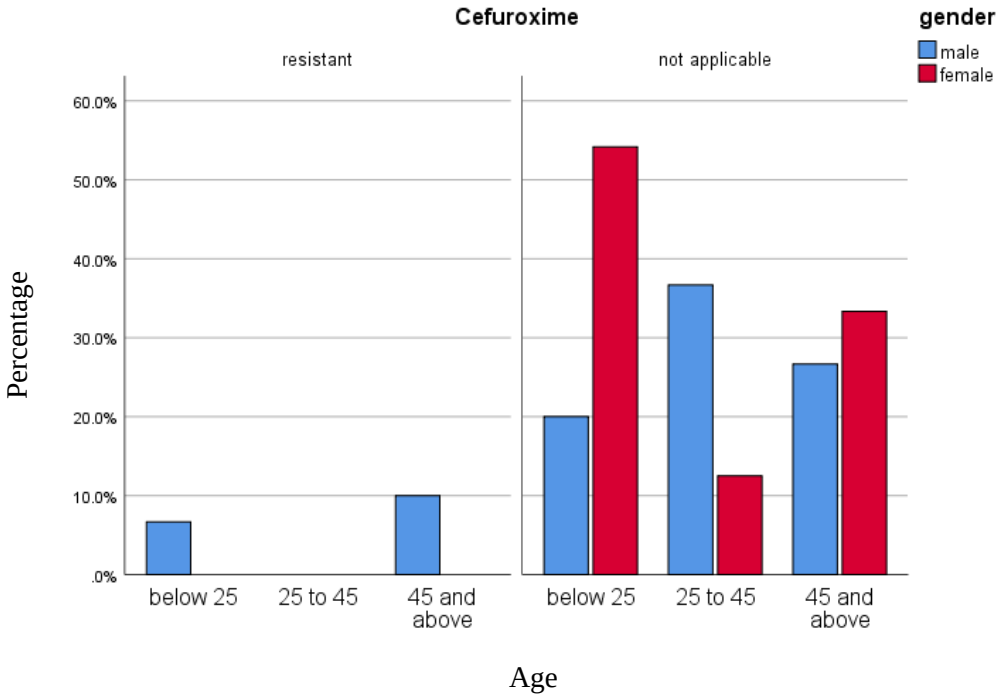

Figure 36: Cefuroxime sensitivity of S. aureus among patients

Much like cefepime, there was also a high amount of not applicable isolates during antibiogram test of cefuroxime. 9.3% of the isolates were sensitive in which all of them came from male patients. No sensitive isolated were found (Figure 36).

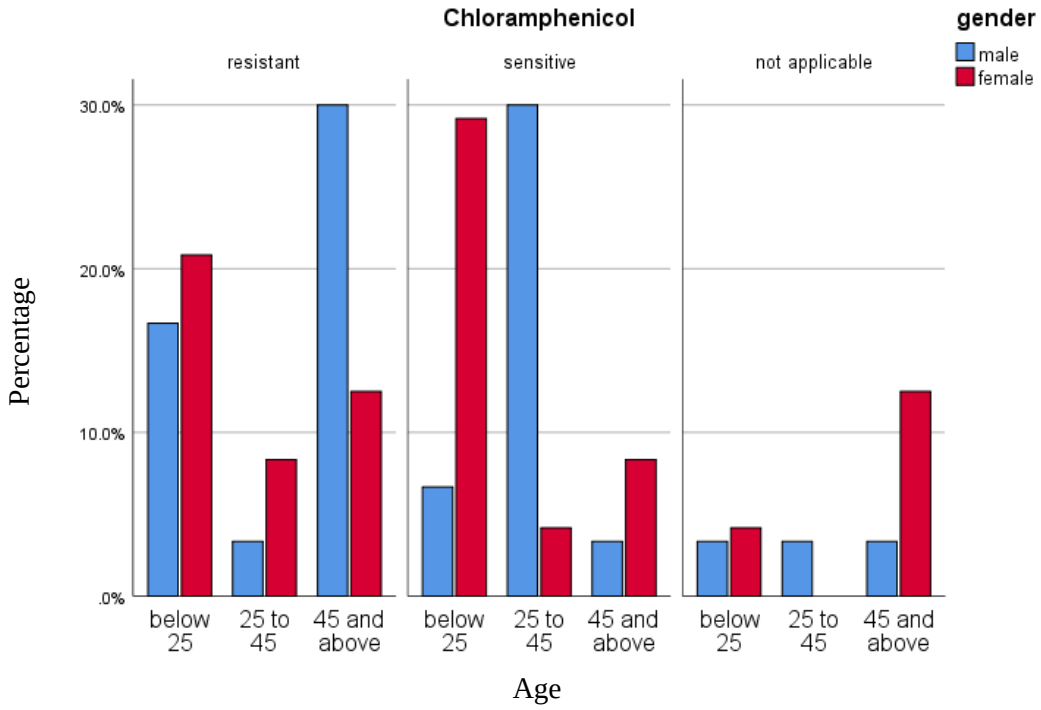

Figure 37: Chloramphenicol sensitivity of S. aureus among patients

From Figure 37 we can see that similar to cefoxitin and cefradine there are high numbers of sensitivity and resistance. 25 (46.7%) of the isolates were resistant and 22 (40.3%) of them were sensitive. Prevalence of resistance was quite higher in male who are 45 and above. Sensitivity was higher in women who are below 25. However, sensitivity in between 25 to 45 age group were significantly higher. Only 7 (13%) of the isolates did not show any sensitivity or resistance.

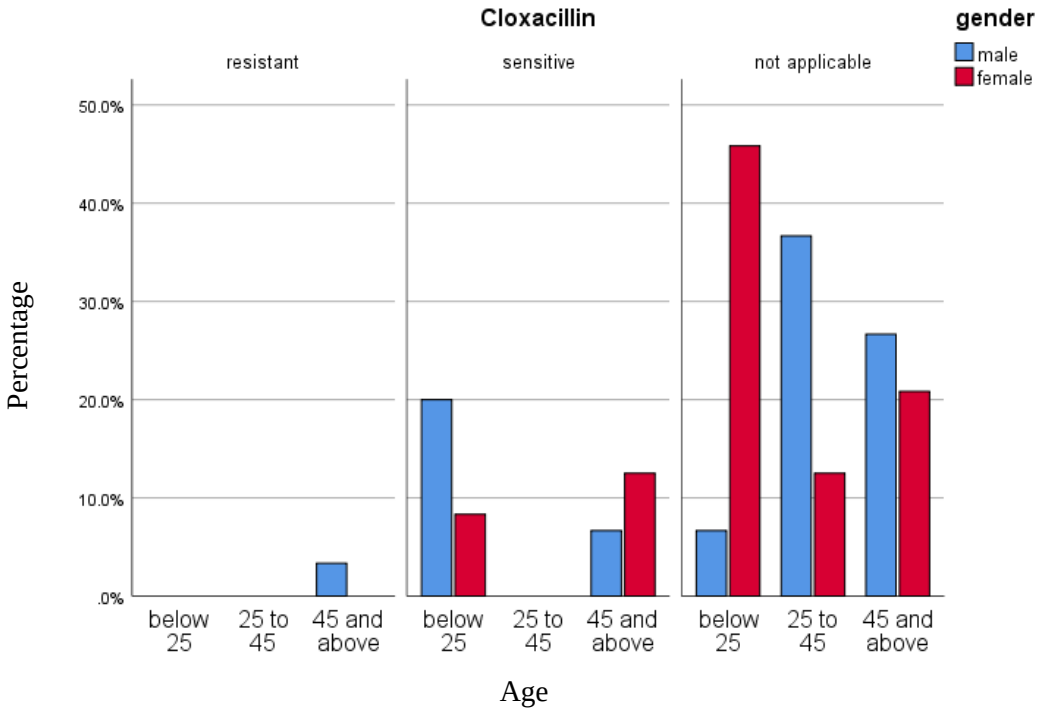

Figure 38: Cloxacillin sensitivity of S. aureus among patients

From Figure 38 we can see that a staggering 40 isolates (74.1%) did not show any resistance of sensitivity. Only 1 isolate obtained from a male patient aged 45 and above showed resistance. 13 isolates (24.1%) were sensitive. No patients aged between 25 to 45 had shown sensitivity.

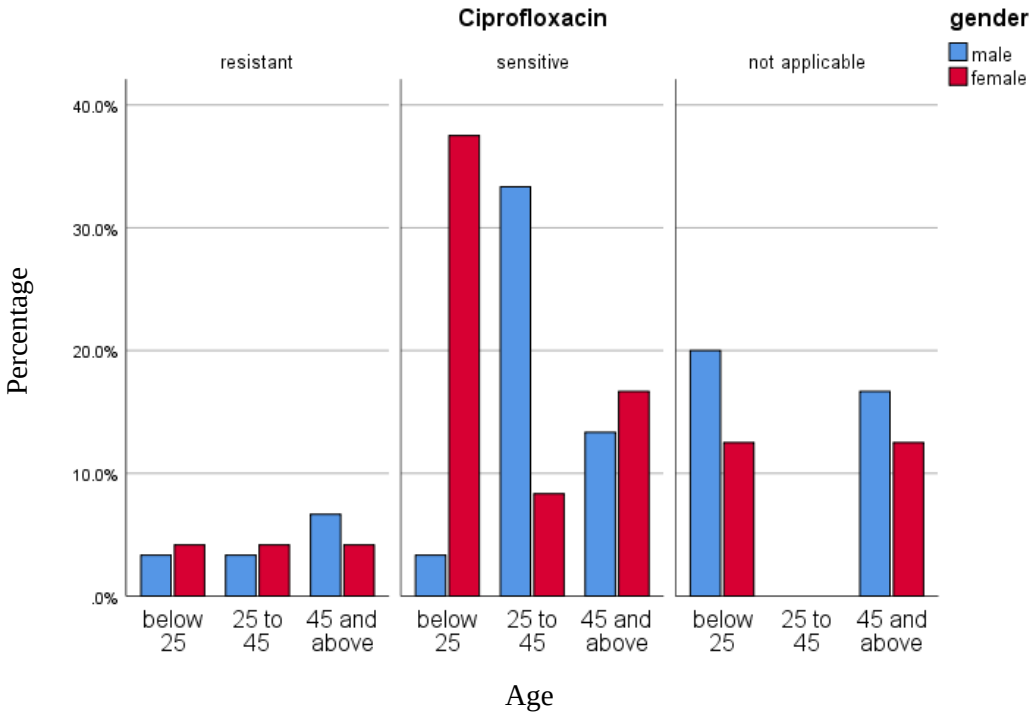

Figure 39: Ciprofloxacin sensitivity of S. aureus among patients

As Figure 39 suggests, out of the 54 isolates only 7(13%) showed resistance toward ciprofloxacin. Resistance was equally prevalent in all age groups among male and female patients. More than half (55.6%) of the isolates were sensitive. Women aged below 25 had a higher occurrence of sensitivity than men. However, in the age group 25 to 45 occurrence of sensitivity was more common in men. 17 (31.5%) of all the isolates did not show any resistance or sensitivity.

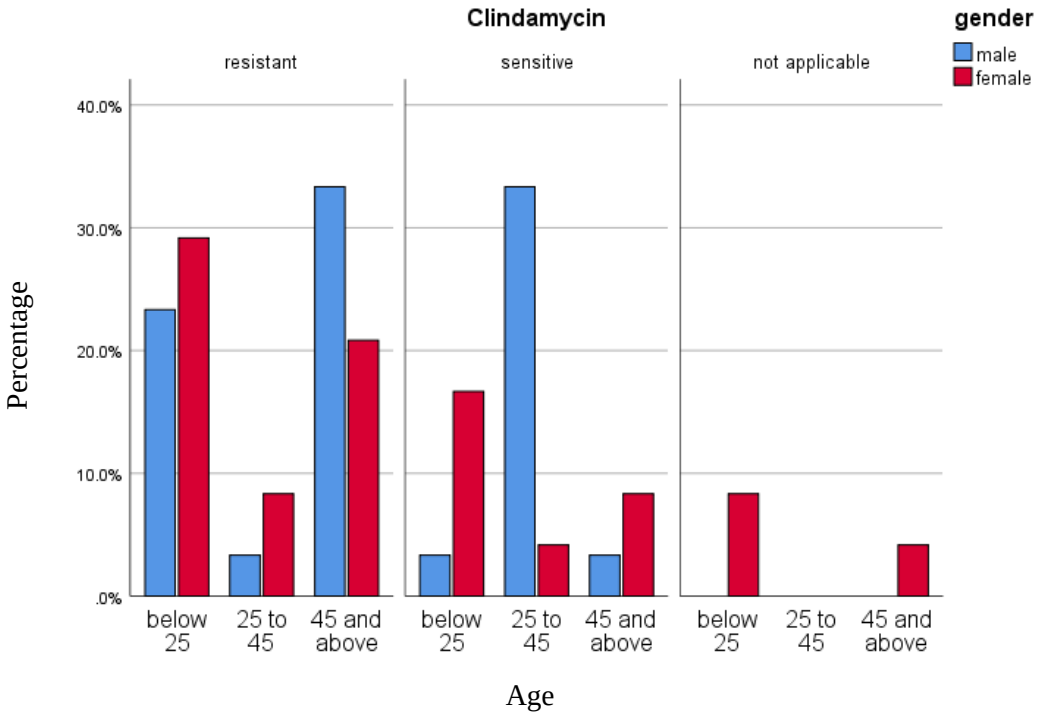

Figure 40: Clindamycin sensitivity of S. aureus among patients

From Figure 40 we can see that similar to cefoxitin and cefradine there are high numbers of sensitivity and resistance. 32 (59.3%) of the isolates were resistant and 19 (35.2%) of them were sensitive. Prevalence of resistance was quite higher in male who are 45 and above. Sensitivity was higher in women who are below 25. However, sensitivity in men between 25 to 45 age group were significantly higher. Only 3 (5.6%) of the isolates did not show any sensitivity or resistance and all of them were obtained from females.

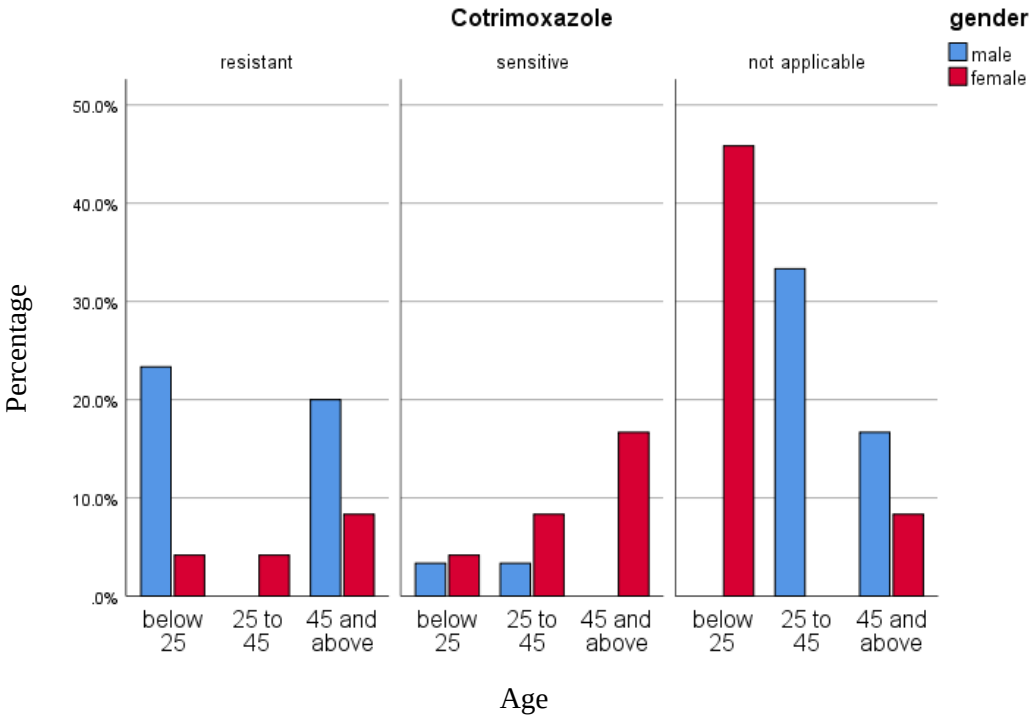

Figure 41: Co-trimoxazole sensitivity of S. aureus among patients

As can be seen from Figure 41, co-trimoxazole sensitivity or resistance did not occur in 28 (51.9%) of all the isolates. There were only men in age group 25 to 45 and there were only women in the age group below 25. 17 isolates (31.5%) were resistance. Prevalence of resistance was higher in men aged below 25 and above 45. However, there were no resistance in men aged 25 to 45. Only 9 (16.7%) of the isolates showed sensitivity towards the drug.

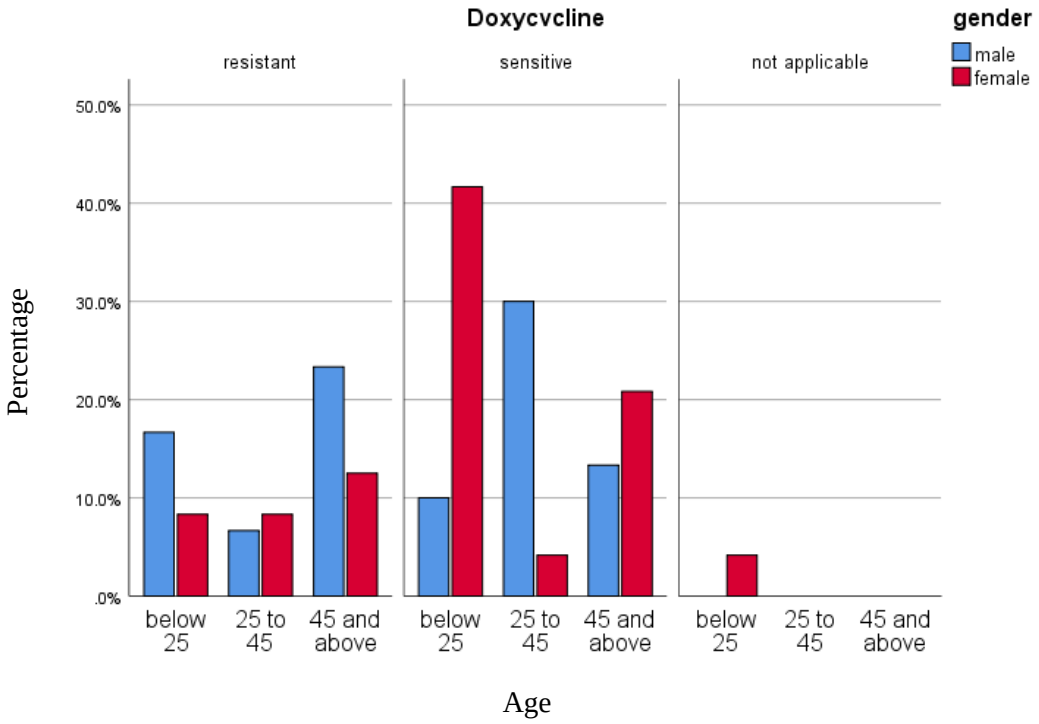

Figure 42: Doxycycline sensitivity of S. aureus among patients

From Figure 42 we can see high numbers of sensitivity and resistance. 21 (38.9%) of isolates were resistant, all of them equally prevalent in all age and sex groups. In case of sensitivity, more women aged below 25 were sensitive to the antibiotic. Moreover, in the age group 45 and above there are fewer men than women. However, in the age group 25 to 45 resistance among men was more prevalent. Altogether 32 isolates (59.3%) were sensitive. Only about 1 of the isolates were not applicable and that was from a woman aged below 25.

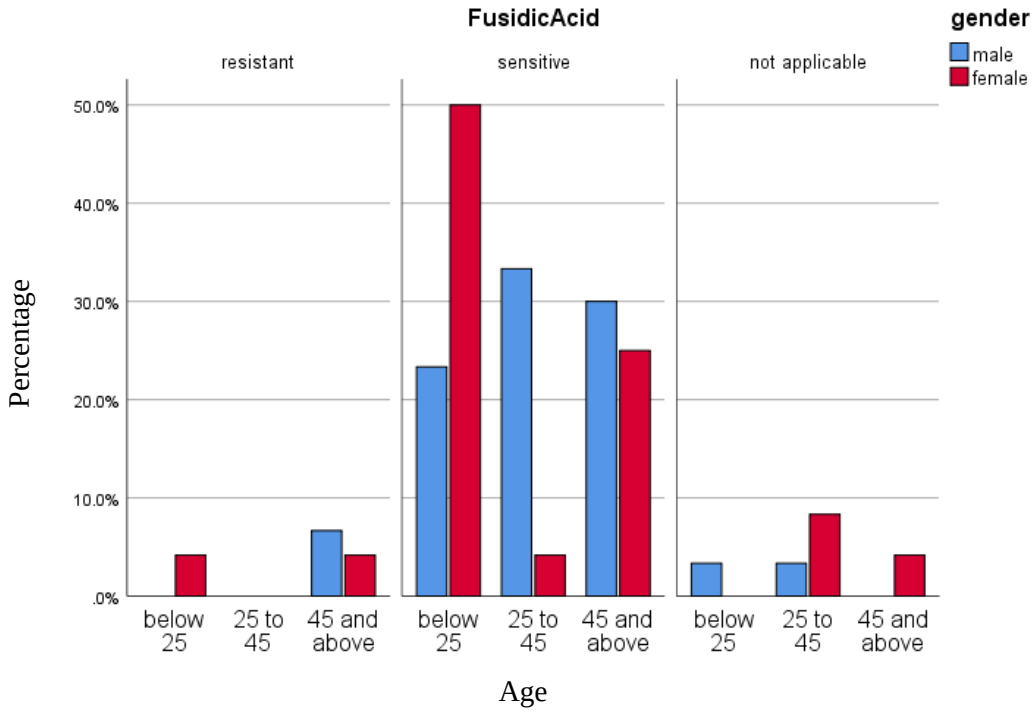

Figure 43: Fusidic acid sensitivity of S. aureus among patients

As Figure 43 suggests, out of the 54 isolates only 4 (7.4%) showed resistance toward the antibiotic. Resistance did not occur in any patient aged between 25 to 45. Furthermore, men aged below 25 did not show resistance. A staggering 45 (83.3%) of the isolates were sensitive. Women aged below 25 had a higher occurrence of sensitivity than men. However, in the age group 25 to 45 occurrence of sensitivity was more common in men. 5 (9.3%) of all the isolates did not show any resistance or sensitivity.

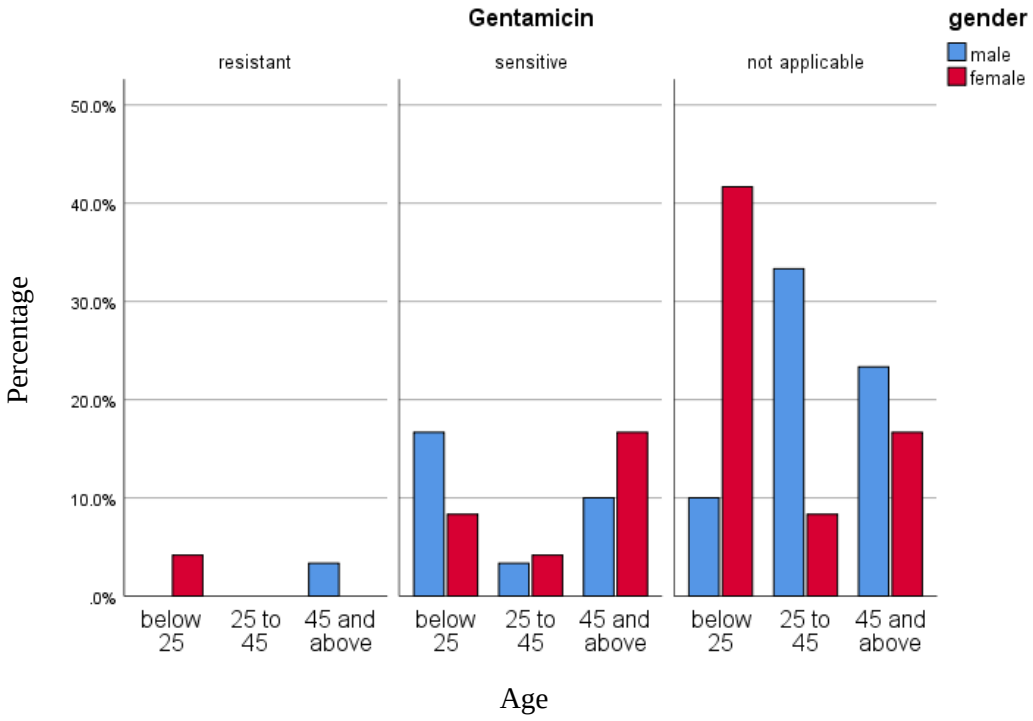

Figure 44: Gentamycin sensitivity of S. aureus among patients

It can be seen from Figure 44, more isolates (66.7%) did not develop resistance nor are they sensitive. Only 2 patients (3.7%) were resistant to the drug. Furthermore, 16 isolates showed sensitivity. Sensitivity was equally prevalent among men and women across different age groups.

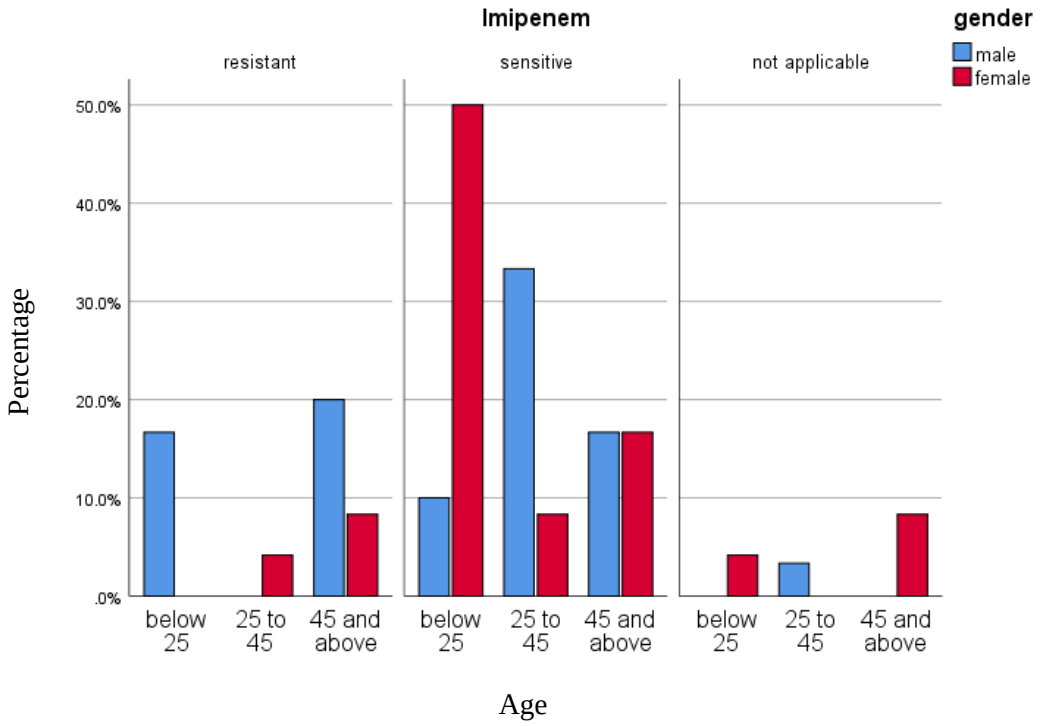

Figure 45: Imipenem sensitivity of S. aureus among patients

It can be seen from Figure 45, out of the 54 isolates 14 (25.9%) showed resistance toward the antibiotic. Resistance did not occur in any men aged between 25 to 45 and in any women aged below 25. A staggering 36 (66.7%) of the isolates were sensitive. Women aged below 25 had a higher occurrence of sensitivity than men. However, in the age group 25 to 45 occurrence of sensitivity was more common in men. 4 (7.4%) of all the isolates did not show any resistance or sensitivity.

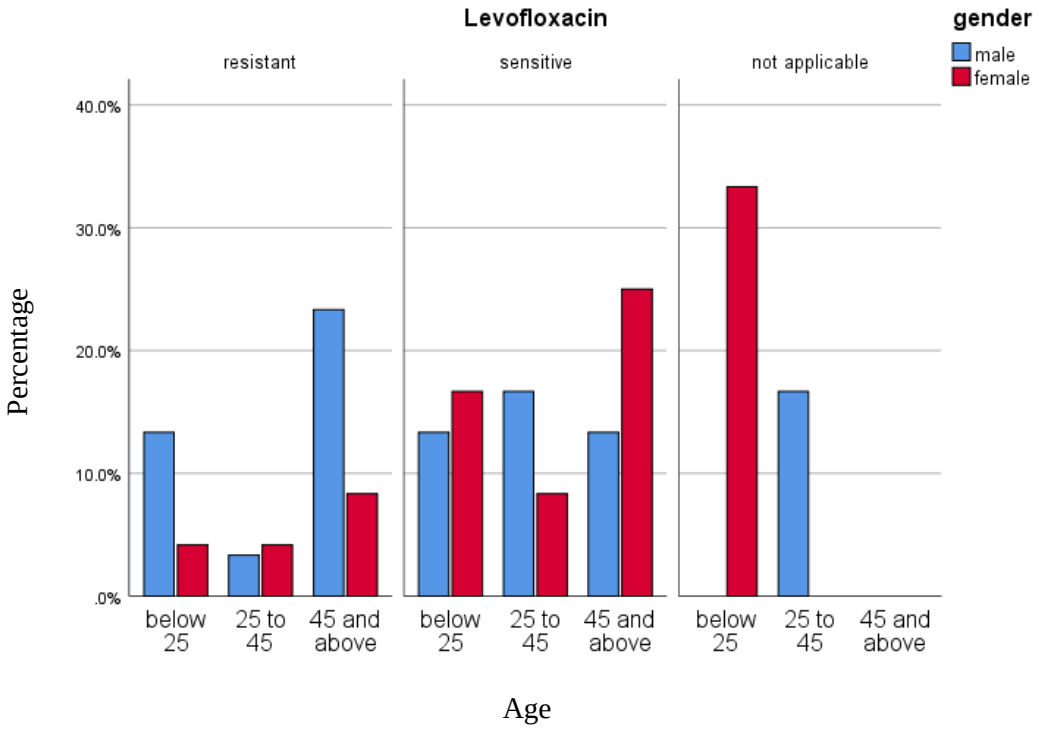

Figure 46: Levofloxacin sensitivity of S. aureus among patients

As Figure 46 suggests, out of the 54 isolates 16 (29.6%) showed resistance toward the antibiotic. Prevalence of resistance was higher in men aged below 25 and above 45. 25 (46.3%) of the isolates were sensitive. Women aged below 25 had a higher occurrence of sensitivity than men. However, in the age group 25 to 45 occurrence of sensitivity was more common in men. 13 (24.1%) of all the isolates did not show any resistance or sensitivity. In this group there were only women aged below 25 and only men aged 25 to 45.

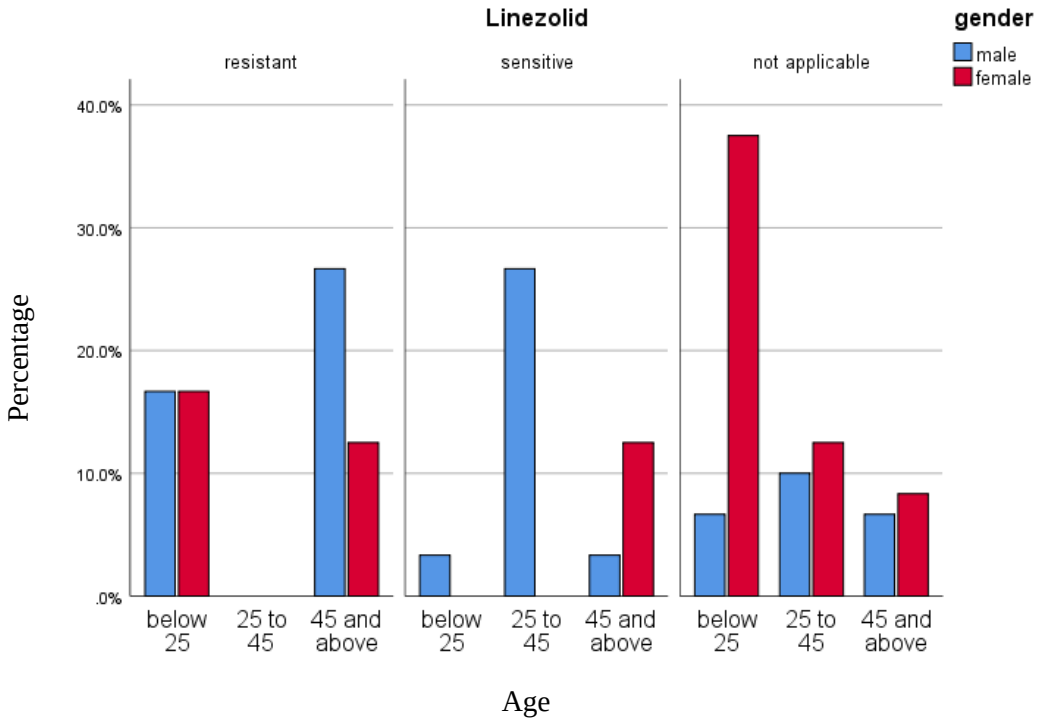

Figure 47: Linezolid sensitivity of S. aureus among patients

As can be seen from Figure 47, 20 (37%) showed resistance toward the antibiotic. Prevalence of resistance was higher in men above 45. There was no patient aged between 25 and 45 who developed resistance. 13 (24.1%) of the isolates were sensitive. There were only men who developed sensitivity aged below 25 and 25 to 45. However, in the age group 25 to 45 occurrence of sensitivity was more common in women. 21 (38.9%) of all the isolates did not show any resistance or sensitivity. Women showed a higher tendency of nonresistance and non-sensitivity across all age groups.

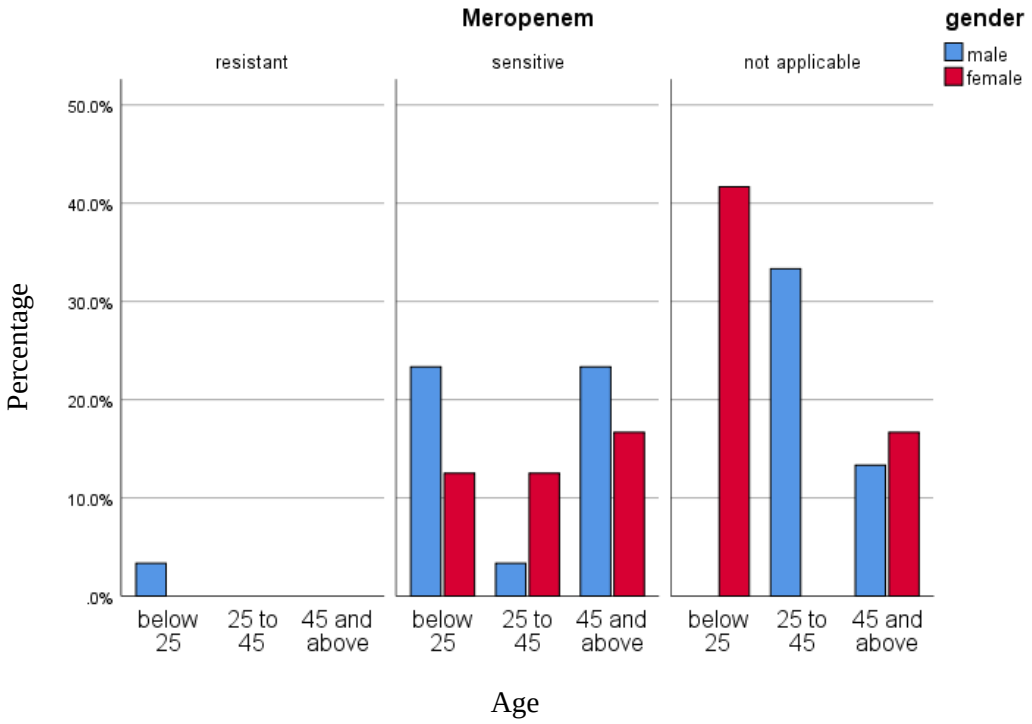

Figure 48: Meropenem sensitivity of S. aureus among patients

It can be seen from Figure 48, 28 isolates (51.9%) did not develop resistance nor are they sensitive. Only 1 male patient aged below 25 was resistant to the drug. Furthermore, 25 isolates showed sensitivity. Sensitivity was higher in men in below 25 and above 45 age group. However, number of women was higher in developing sensitivity in the age group 25 to 45.

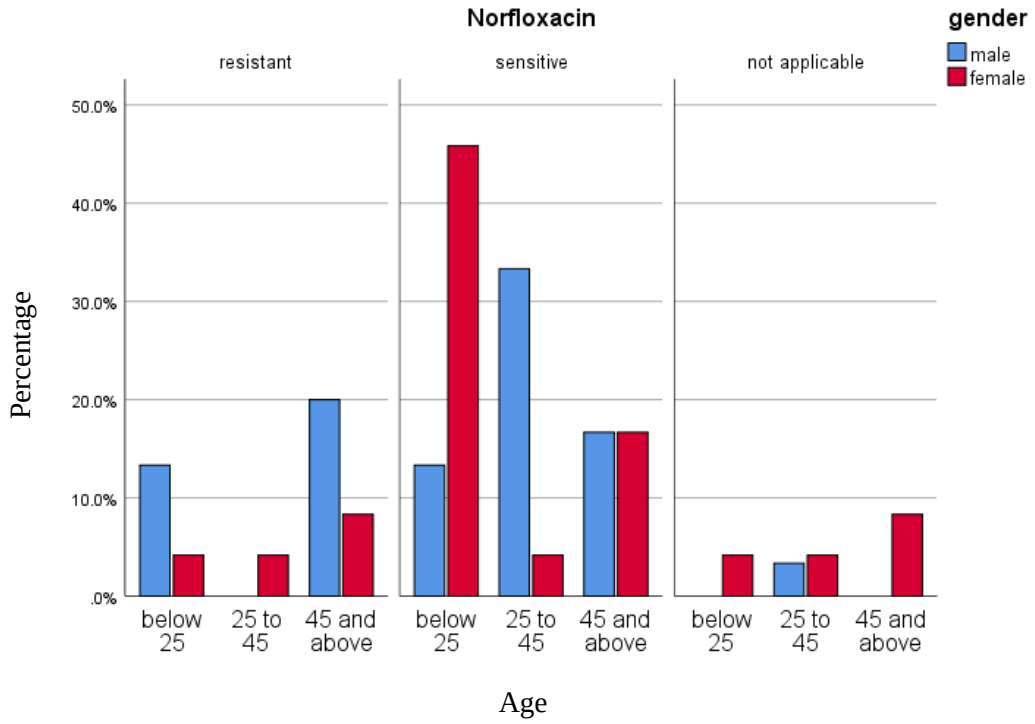

Figure 49: Norfloxacin sensitivity of S. aureus among patients

As Figure 49 suggests, out of the 54 isolates only 5 (9.3%) did not show any resistance or sensitivity towards the antibiotic. 35 (64.8%) of all the isolates were sensitive. Sensitivity was higher in women aged below 25. However, it was higher in men aged between 25 and 45. 14 (25.9%) of the sample showed resistance with higher occurrence in men aged below 25 and above 45. However, there was no man who developed resistance aged between 25 to 45.

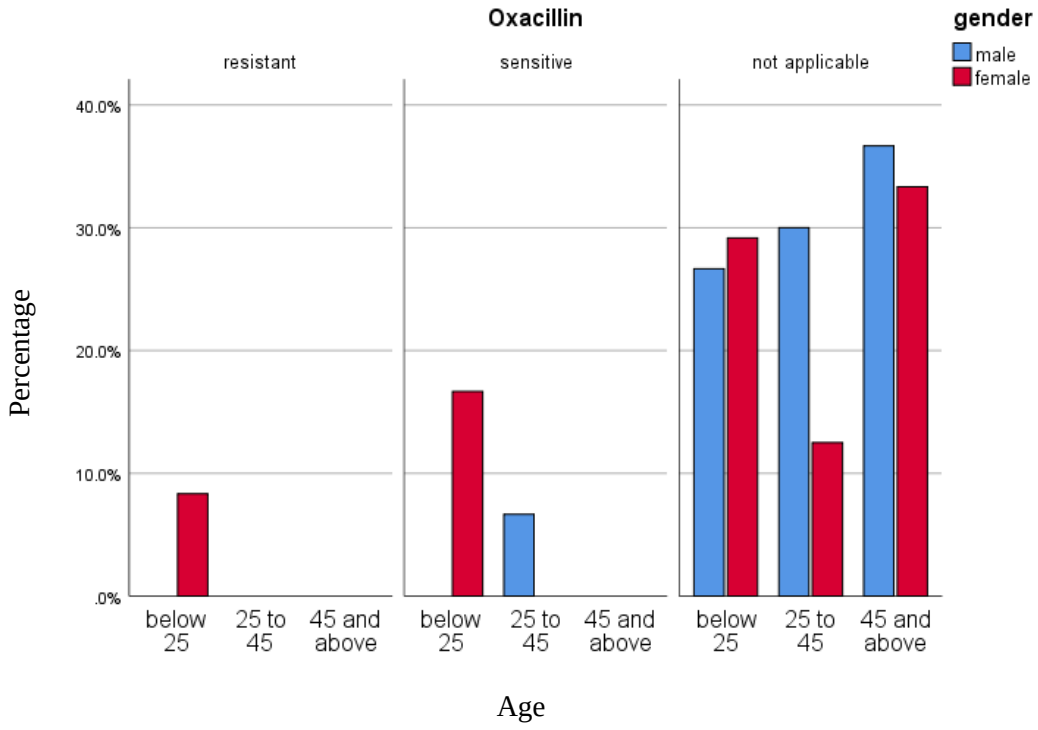

Figure 50: Oxacillin sensitivity of S. aureus among patients

It can be seen from Figure 50 that a staggering 46 isolates (85.2%) were not resistant and sensitive. Only 2 women aged below 25 developed resistance. Only men aged between 25 to 45 and only women aged below 25 developed sensitivity towards the drug. Altogether only 6 isolates (11.1%) showed sensitivity.

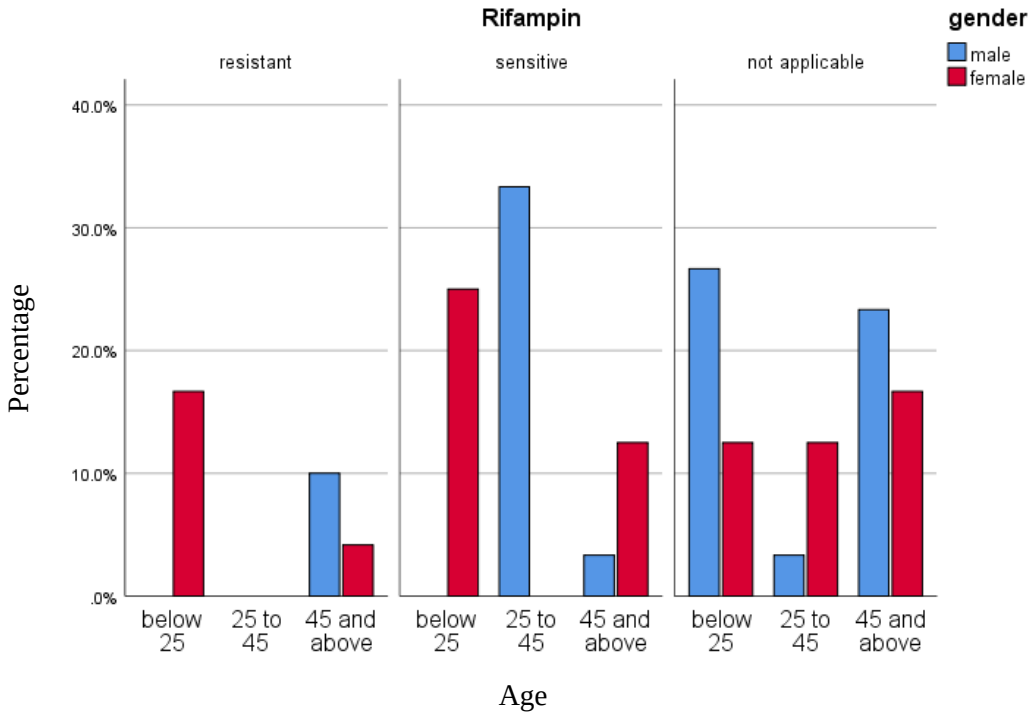

Figure 51: Rifampin sensitivity of S. aureus among patients

As Figure 51 suggests, out of the 54 isolates 26(48.1%) did not show any resistance or sensitivity towards the antibiotic. 20 (37.0%) of all the isolates were sensitive. Sensitivity was shown only in women aged below 25 and in mean aged 25 to 45. 8 (14.8%) of the sample showed resistance with higher occurrence in men aged above 45. However, there was no man who developed resistance aged between 25 to 45 and below 25. There was no patient who developed resistance in 25 to 45 age group.

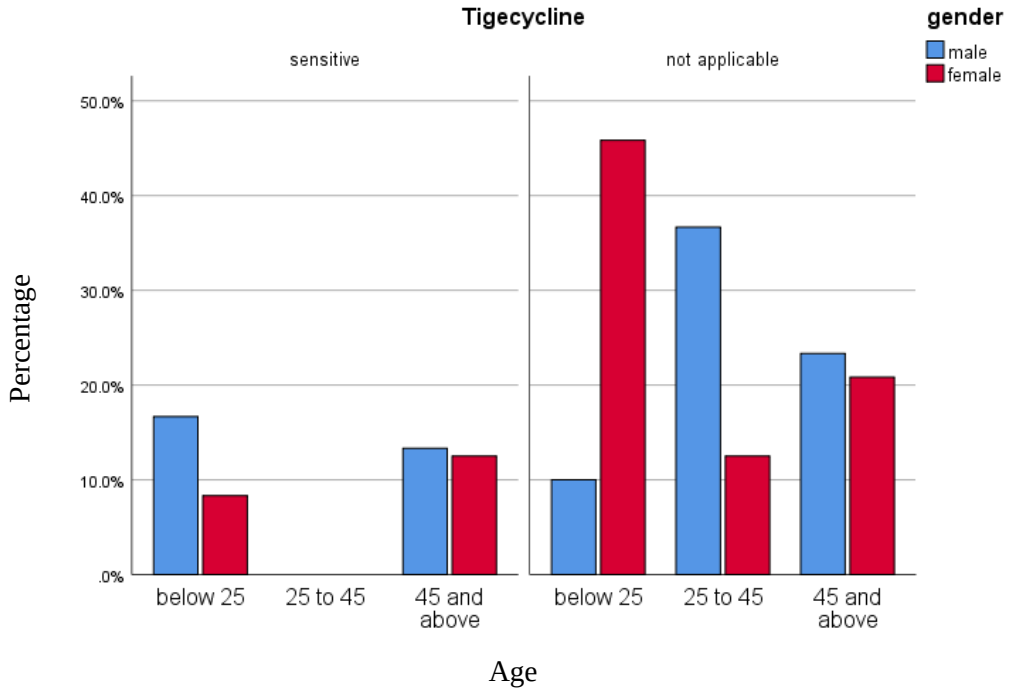

Figure 52: Tigecycline sensitivity of S. aureus among patients

There were no resistant isolates found as the Figure 52 suggests. Properties in most of the isolates could not been identified. 25.9% of the isolates were sensitive.

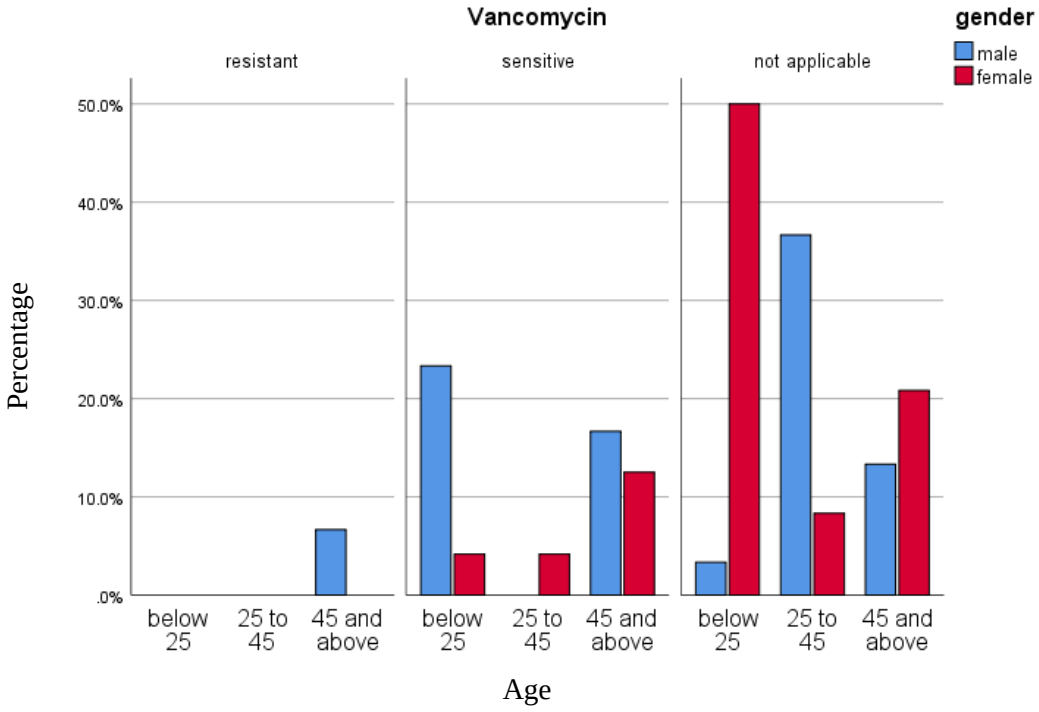

Figure 53: Vancomycin sensitivity of S. aureus among patients

It can be seen from Figure 53, more isolates (64.8%) did not develop resistance nor they are sensitive. Only 2 patients (3.7%) were resistant to the drug, both of them are men aged above 45. Furthermore, 17(31.5%) isolates showed sensitivity. Sensitivity was higher in men aged below 25 and above 45. However, there was no man who developed sensitivity in 25 to 45 age group.

Chapter 4

Discussion

This study had implicated resistance in case of cefaclor, clindamycin, cefotaxime, ampicillin. Ampicillin and cefaclor are β-lactam antibiotic. Furthermore, cefotaxime is also a β-lactam antibiotic belonging to the third generation of cephalosporins. The major inhibitory target for β-lactam antibiotics in *S. aureus* is the bifunctional transglycolylase-transpeptidase PBP2 which carries disaccharide pentapeptide. This is a key feature of peptidoglycan. This enzyme shifts the peptide molecule from membrane-bound fats to the polysaccharide chains. In the meantime, transpeptidase (TP) cross links glycine. However, resistance can enable *S. aureus* to produce β-lactamase. The *S. aureus* β-lactamase responsible for resistance to β-lactam antibiotic is a typical serine β-lactamase (BlaZ) that forms the same type of acyl enzyme intermediate as the TP of PBP2). With BlaZ, the addition of water is rapid resulting in regeneration of the active site serine and release of the ring-opened penicilloic acid, a hydrolytic degradation product with no inhibitory activity. The β-lactamase structural gene is carried by the transposon Tn*552* or Tn*552*-like elements. The transposon is either located on a large plasmid, the prototype of which is pI524, or is integrated into the bacterial chromosome. Expression of the enzyme is inducible, being controlled by the BlaI repressor and the BlaR sensor. The enzyme itself is a lipoprotein that is partly located on the outer face of the cytoplasmic membrane where it is strategically located to protect PBP2s while some is released into the surrounding medium causing destruction of the antibiotics. Resistance to clindamycin can be due to the proteins synthesized by mutated *S.aureus* to cause alteration in the target cells as we described earlier.

Amikacin, fusidic acid, imipenem, norfloxacin had good effectiveness against *S. aureus* when we compare with other antibiotics we studied. This was similar for all age groups and genders. This confirmed the previously established study which showed that norfloxacin

work effectively and does not cause resistance in *S aureus* (Tsiodras et al., 2001). This also confirmed previous finding which had shown good effectiveness imipenem and amikacin against *S. aureus.* of Another previously described study found that *S. aureus* was very sensitive to imipenem, norfloxacin (Lowy, 2003). We also found that the Bactria was not highly resistant to vancomycin. This study contradicts with previously published researches which shows that the infections caused by vancomycin-resistant *S. aureus* (VRSA) are of great concern because they reflect both complete resistance and a different mechanism for dissemination. In contrast to the chromosomally mediated resistance for VISA strains, the VRSA strains acquire resistance by conjugal transfer of the *vanA* operon from an *Enterococcus faecalis*, raising the specter of a far more efficient means for dissemination of the resistance gene among strains of staphylococci. (Lowy, 2003).

Our study had its limitations. Such as cefuroxime, amoxiclav, cefepime, gentamycin and oxacillin sensitivity patterns were not well established from our antibiogram tests.

Chapter 5

Conclusion

Throughout the world antibiotic resistance has converted to one of the most important subjects. Antibiotics have transmuted the efficacy of the medicine by saving millions of lives. The appearance of resistant bacteria is occuring worldwide, jeopardizing the efficiency of the lifesaving antibiotics. Bacterial contagions have again become a risk developing issue after many years of curing patients with antibiotics. This problem resulted from the over-do and misuse of antibiotics along with the lack of ability of the pharmaceutical industries to develop new drug. Pharmaceutical industries are facing these problems due to reduced financial incentive and challenging regulatory requirements. Resistant bacteria hover the extraordinary health aids that have been attained with antibiotics. It also place a substantial health and economic burden on the Bangladesh health care system and population. This study conducted to find out resistance pattern of *S. aureus* in Dhaka, Bangladesh. This study had confirmed previous findings and also observed new findings. It showed that the bacteria were highly resistant against ampicillin, clindamycin, cefaclor, cefotaxime. To overcome this problem, synchronized efforts to implement new policies, renew research efforts, and pursue steps to manage the crisis are greatly needed. Hopefully this research will increase awareness and pave the way for future studies.

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Chapter 6

Future directions

This study has revealed a lot of information regarding the resistance pattern of *S. aureus.* However, there are still scopes for future work. For example, the data was collected from only diagnostic centers in Dhaka. The study could be conducted in different districts or divisions across the country. We only collected the antibiogram tests for single drug treatment. In order to get the full idea combined drug treatment antibiogram data can also be collected.

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