Macrodilution Method for Determination of Minimum Inhibitory Concentration of Conventional Antibiotics Against Clinically Isolated Pathogenic Bacteria

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'A thesis report, submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy'

DEDICATION

This Research Work is dedicated to Almighty Allah And my beloved parents and my elder sister.

Declaration by the Research Candidate

I, **Nadia Afrin** hereby declare that the dissertation entitled **"Macrodilution Method for Determination of Minimum Inhibitory Concentration of Conventional Antibiotics Against Clinically Isolated Pathogenic Bacteria"** submitted by me to the Department of Pharmacy, East West University and in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy is a record of original research work carried out by me during 2016, under the supervision and guidance of **Dr. Shamsun Nahar Khan, Chairperson, Department of Pharmacy, East West University** and the thesis has not formed on the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Certificate by the Supervisor

This is to certify that the dissertation entitled **"Macrodilution Method for Determination of Minimum Inhibitory Concentration of Conventional Antibiotics Against Clinically Isolated Pathogenic Bacteria"** submitted to the department of pharmacy, East West University in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy was carried out by **Nadia Afrin (ID: 2011-3-70-011)** under your guidance and supervision and that no part of the research has been submitted for any other degree. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

Dr. Shamsun Nahar Khan

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Certificate by the Chairperson

This is to certify that the thesis entitled **"Macrodilution Method for Determination of Minimum Inhibitory Concentration of Conventional Antibiotics Against Clinically Isolated Pathogenic Bacteria"** submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy is a record of original and genuine research work carried out by **Nadia Afrin** during 2016 of his research in the Department of Pharmacy, East West University.

Dr. Shamsun Nahar Khan

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East West University, Dhaka.

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Abstract

The macrodilution method has employed to observe Minimum Inhibitory Concentration (MIC) of antibiotic standard powder against different clinically isolated pathogenic bacteria. The study was performed to evaluate antimicrobial resistance pattern of antibiotic standard powder named Levofloxacin, Ciprofloxacin, Cephradine, Cefuroxime, Ceftriaxone, Cefixime, Vancomycin, Azithromycin against four clinical isolate of different strains of *E.coli, Klebsiella, Acinetobacter, Staphylococcus aureus*. Serial dilution of antibiotic standard powders has done in different concentration (from 0.015625-128 mcg/mL). Then 10 µl of bacterial suspension added in test tubes. After that, the test tubes were incubated for 18-20 hours at 37.5ºC.The result was observed by measuring MIC (Minimum Inhibitory Concentration) of antibiotic standard powders. Here, 11 strains of *E.coli* showed resistant activity against antibiotic standard powder but some strains gives sensitivity MIC values that are Levofloxacin (0.5 mcg/mL, 0.0625 mcg/mL, 0.015625 mcg/mL) for sample 1, sample 7, sample 8, Ciprofloxacin (0.03125 mcg/mL) for sample 10, Cephradine (1 mcg/mL) for sample 7, Azithromycin (0.5 mcg/mL), For two *Staphylococcus aureus* spp., the sensitivity MIC values are Cephradine (0.125 mcg/mL) for sample 13, Ceftriaxone (4 mcg/mL) for sample 13, Azithromycin (0.5 mcg/mL) for sample 13. In addition, the sensitivity MIC values for five *Klebsiella* spp. : Levofloxacin (0.0625 mcg/mL, 0.125 mcg/mL) for sample 15 and sample 17, Ciprofloxacin (0.5 mcg/mL, 1 mcg/mL) for sample 15 and sample 17, Cefuroxime (1 mcg/mL) for sample 15, Ceftriaxone (0.5 mcg/mL, 0.0625 mcg/mL) for sample 15 and sample 17, Cefixime (1 mcg/mL) for sample 15, Azithromycin (0.25 mcg/mL) for both sample 15 and sample 17. On the other hand, *Klebsiella* spp. Sample 16 showed resistance against all antibiotics. These values compelled to the standard value of CLSI, EUCAST and DIN to know if they are sensitive , intermediate or resistance.

Key words : Antibiotics, Clinical isolates, Macrodilution test, Sensitivity , MIC, Bacteria.

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INTRODUCTION

1.1.Overview

Bacterial resistance to antimicrobial drug is one of the most serious threat to global health. Antimicrobial resistance (AMR) threatens the effective treatment of an ever-increasing range of infections caused by bacteria, parasites, fungi, virus. A post-antibiotic era in which common infection can kill- far from being an apocalyptic fantasy, is instead a very real possibility for the 21 st century.

The causes of antibiotic resistance are complex and include human behavior at many levels of society; the consequences affect everybody in the world. Many efforts have been made to describe the different facts of antibiotic resistance and the intravention needed to meet the challenge. The effectiveness of the antibiotics in treating common infection are decreasing quickly. The use of antibiotic is increasing with rising incomes, high rates of hospitalization and high prevalence of hospital infections. (Laxminarayan et al, 2013*).*

Even before penicillin was introduced, resistance strains of bacteria had been detected. The rapid evolution of bacterial resistance is clear in case of β-lactamases class of antibiotics. Nearly 1000 resistance related β-lactamases that inactivate these antibiotics have been identified, a ten times increase since before 1990. Resistance has spread world wide. Hospital data from developing countries suggest that resistance to the WHO recommended regimen of ampicillin and gentamicin in pathogens causing neonatal infections (in the first 28 days of life) is common : 71 % of isolates of *Klebsiella* spp and 50 % 0f *E.coli* are resistance to gentamicin. (Laxminarayan et al, 2013).

In our country, antibiotic resistance is also threat and same factors like other developing countries are responsible for antibiotic resistance. Rational use of antibiotics can improve the situation.

1.2.Definition of Microbiology

The word microbiology has come from the greek word. According to that greek word it is the study of microscopic organisms. These oraganisms include bacteria, fungi, algae, protozoa & viruses. This part of science discuss about their form, structure, reproduction, metabolism & classification.

These microscopic organisms can be unicellular, multicellular or acellular. Eukaryotic microorganisms are membrane bound cell organelles. They are the fungi & protists. On the

other hand all of the prokaryotic organisms are microorganisms. They are conveniently considered as lacking membrane-bound organelles.Some of the microorganisms are beneficial & others are detrimental. (Pelczar et al, 1996).

1.3.Bacteria

Bacteria are the simplest organisms living on earth. They are considered as the first organisms to evolve on earth. They are few micrometers in length. They were the first life forms to appear on earth. They are present in most habitats on the planet. They also live in animals & plants.

Bacteria can be used for the industrial production of amino acids. *Corynebacterium glutamicum* is one of the most important bacterial species with an annual production of more than two million tons of amino acids, mainly L-glutamate and L-lysine. (Vasanthakumari, 2007)

In biotechnology they are greatly used. Bacteria also help in the manufacture of antibiotics & other chemicals.In developed countries, antibiotic resistance is becoming common. The main reason of it is the use of antibiotics to treat bacterial infections & also in farming. (Vasanthakumari, 2007)

1.4.Morphology of bacteria

Bacteria shows a wide variety in case of its size & shape. It is called morphology of bacteria. They are very small. They are approximately 0.5 to 1.0 m in diameter. Some of them are visible to the unaided eye. (Prescott et al, 1993; Ananthanarayan et al, 2005).

Most species of bacteria are of two shapes. One of them is spherical shape, called cocci & rod-shape, called bacilli. Elongation of bacteria is associated with swimming. Some bacteria are of slightly carved rod shaped or comma-shaped like vibrio. Other bacteria are of spiral-shaped like spirilla or are of tightly coil shaped like spirochaetes. A very small number of species are of tetrahedral or cuboidal shapes. (Prescott et al, 1993; Ananthanarayan et al, 2005)

1.5.Structure of bacteria

Bacterial structure contains various components. Some of these are external to the structure which is known as extracellular structure. Others are internal to the call wall which is known as intracellular structure.

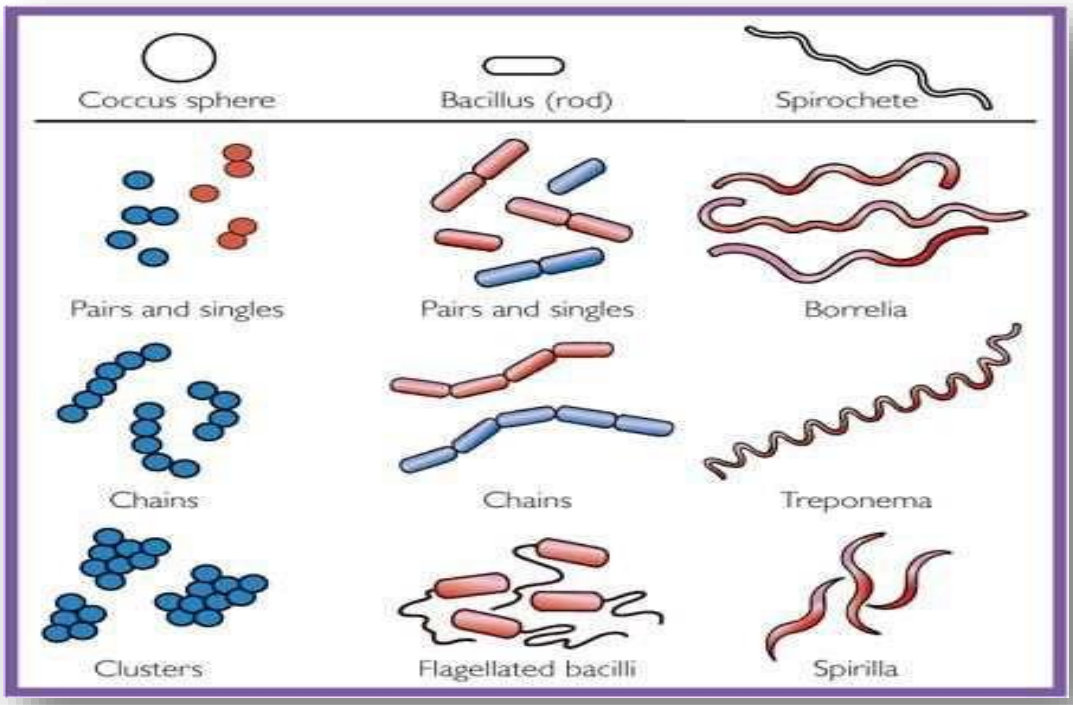

Figure 1.1: Shapes of Bacteria

1.5.1.Intracellular structure

The bacterial cell is made of a lipid membrane. It is also known as a cell membrane or plasma membrane. This membrane encloses the contents of the cell. It acts as a barrier. It holds nutrients, proteins & other essential components of the cytoplasm within the cell. It contains a few large intracellular structures. Nucleus, mitochondria, chloroplasts & the other organelles are absence in their structure. (Naveen, 2010)

Micro-compartments such as carboxysomes give a further level of organization. These are the compartments within bacteria. They are surrounded by polyhedral protein shells instead of by lipid membranes. These polyhedral organelles localize &compartmentalize bacterial metabolism. They follow the whole characteristics of prokaryotic.it is important for them to protect themselves by their cell wall from the antibiotics. They follows many rules to adjust with the environment. Their structure is made in that way to protect themselves. They are specially identified by gram staining test. Some of them retain the crystal violet color. They are called gram-positive bacteria. Some of them do not retain crystal violet color. They are called gram- negative bacteria. They contains different characteristics. As a result they show different

result in case of different antibiotics. They are capable to pass nutrients & other materials through their cell wall.

Many important biochemical reactions use the concentration gradients. These biochemical reactions are energy generation. This reactions use the concentfration gradients across membranes. Electron transports occur across the cell membrane between the cytoplasm & the periplasmic space. The plasma membrane is highly folded in many photosynthetic bacteria. This membrane fills most of the cell with layers of light-gathering membrane. These light-gathering complexes can form lipid-enclosed structures. This is called chlorosomes. It is formed in green sulfur bacteria. Other proteins import nutrients across the cell membrane. They also expel or remove undesired molecules from the cytoplasm. (Pelczar et al, 1996)

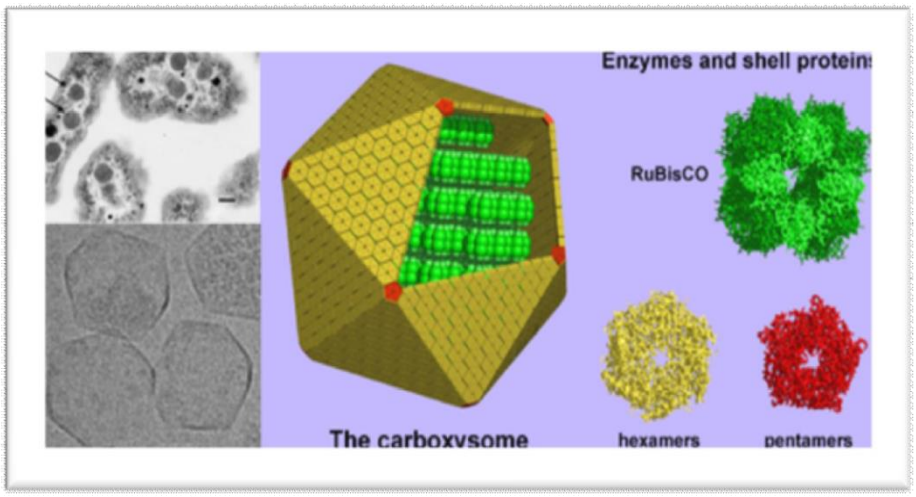

Figure 1.2: Microcompartments of the bacteria

Carboxysomes are organelles that are enclosed by protein. In the top left it is an image of carboxysomes of *Halothiobacillus neapolitanus* by electron microscope. Below this image it is an image of purified carboxysomes & on the right side of it this it is a model of their structure & its Scale bars are 100 nm. In most cases bacteria do not have a membrane-bound nucleus. Their genetic material is typically a single circular chromosome. It is located in the cytoplasm in an irregularly shaped body. This irregularly shaped body is called as nucleoid. It also contains associated proteins & RNA of chromosomes. (Naveen, 2010).

Bacteria contain ribosomes in their intracellular structure. They are often grouped in chains which are called polyribosomes. They remain in their body for the production of proteins. The

structure of the ribosome of bacteria is totally different from that of eukaryotes . Ribosome of bacteria contains a sedimentation rate of 70S. Their subunits have rates of 30S & 50S. Some of the antibiotics bind specifically to 70S ribosomes. This binding inhibits bacterial protein synthesis. These antibiotics kill bacteria without affecting the larger 80S ribosomes. They do not cause any harm to the host. (Naveen, 2010).

Some of the bacteria have intracellular nutrient storage granules. They produce it for later use. They are glycogen, polyphosphate, sulfur or polyhydroxyalkanoates. Some species of bacteria like photosynthetic cyanobacteria can produce internal gas vesicles. They use it to regulate their buoyancy. This allow them to move up or down into water layers with different intensity of light & different levels of nutrient. Membranes of intracellular are called chromatophores. They are also found in the membranes of phototropic bacteria. They are used primarily for photosynthesis & contain bacterichlorophyll pigments & carotenoids (Vasanthakumari, 2007).

At the time before it was considered that bacteria might contain membrane folds termed mesosomes. Now it has shown to are artifacts produced by the chemicals. This is used to prepare the cells for electron microscopy. Inclusions are considered to be nonliving components of the cell. They do not possess metabolic activity. They are not bounded by membranes. Glycogen, lipid droplets, crystals & pigments are the common inclusions (Ananthanarayan et al, 2005).

1.5.2.Extracellular structures

Most of the bacteria contain cell wall on the outside of their cytoplasmic membrane. Cell envelope is consisted of the plasma membrane & cell wall. Peptidoglycan is the common bacterial cell wall material. It is made from polysaccharide chains. This is cross-linked by peptides containing D-amino acids. Cell walls of bacteria are totally different from plants & fungi. Their cell walls are made of cellulose & chitin (Ananthanarayan et al, 2005).

There are two different types of cell wall in bacteria. They are called Gram-positive & Gram-negative. This name of bacteria has originated from the reactions of cells to the Gram stain. This test of Gram stain has been employed for the classification of bacterial species.

1.5.2.1.Gram-negative bacteria

Gram-negative bacteria are the bacteria that show different views comparing to gram positive bacteria in Gram staining protocol. They do not retain crystal violet dye in the Gram staining

protocol. In the test of gram stain a counterstain which is commonly known as safranin is added after the crystal violet which colors all gram-negative bacteria with a red or pink color. In this test the counterstain is used to visualize the colorless gram-negative bacteria. It's much thinner peptidoglycan layer does not retain crystal violet. The test is very useful in classifying two specific types of bacteria. This test is based on the structural differences of their bacterial cell walls. In case of gram-positive bacteria it retains the crystal violet dye after washing in a decolorizing solution. Between these two types of bacteria, gram-negative bacteria are more resistant against antibiotics than gram-positive bacteria. The main reason is that relatively impermeable lipid membrane of gram-negative bacteria (Pelczar et al, 1996).

Lipopolysaccharide layer is a particular component of gram-negative cell envelope. It is also known as LPS or endotoxin layer. This LPS is often associated with the pathogenic capability of gram negative-bacteria. In human body an innate immune response is triggered by LPS. This innate immune response is characterized by cytokine production & immune system activation. The common result of cytokine production is inflammation. It can also produce toxicity in host. Pathogenicity means the ability to cause disease. It is not synonymous with the innate immune response to LPS. Mainly the LPS trigger the innate immune response alone. (Naveen, 2010)

1.5.2.1.1.Characteristics of Gram-negative bacteria

- Cytoplasmic membrane
- Thin peptidoglycan layer which is much thicker in gram-positive bacteria
- In the outer leaflet of its outer membrane contains lipopolysaccharide & in the inner leaflet it contains phospholipids. LPS consists of lipid A, core polysaccharide, & O antigen.
- In the outer membrane porins exist. It acts like pores for particular molecules.
- Between the secondary cell membrane called the periplasmic space & the layers of peptidoglycan there is a space.
- The S-layer rather than the peptidoglycan is directly attached to the outer membrane.
- If present, instead of two flagella have four supporting rings
- They have no teichoic acids or lipoteichoic acids
- In the polysaccharide backbone lipoproteins are attached
- Most of the gram-negative bacteria have Braun's lipoprotein. It serves as a link between the peptidoglycan chain & the outer membrane by a covalent bond.

1.5.2.1.2.Examples of Gram-negative Bacteria

Gram-negative bacteria are also known as proteobacteria. They are *Escherichia coli* or *E. coli, Salmonella, Shigella,* & *Pseudomonas, Moraxella, Stenotrophomonas, Bdellovibrio*, acetic acid bacteria, *Legionella* & numerous others. There are some other notable groups of gram-negative bacteria include the cyanobacteria, spirochaetes, green sulfur & green non-sulfur bacteria.

The gram-negative cocci include the three organisms that causes a sexually transmitted desease *(Neisseria gonorrhoeae),* a meningitis *(Neisseria meningitidis),* & respiratory symptoms *(Moraxella catarrhalis).* Gram-negative bacilli include different types of species. Some of the species cause problems of respiratory systems. They are *Hemophilus influenzae, Klebsiella pneumoniae, Legionella pneumophila, Pseudomonas aeruginosa.* (Ananthanarayan et al, 2005)

1.5.2.2.Gram-positive bacteria

Gram-positive bacteria are stained dark blue or violet in the Gram stain test. They are capable to retain the crystal violet stain. It is because of their thick peptidoglycan layer. That is superficial to the cell membrane. PG layer, cell wall & the plasma membrane are three specific structures. As for example, plant cells contain rigid cell walls in addition to an outer plasma membrane. On the other side, animal cells contain only plasma membranes. For structural support & rigidity cell walls are very important. That is also needed for the survival of plant cells because they are not motile organisms. Their survival also depends on strong & rigid structures. Animal cells & gram- positive cells have some similarities. Both of them are amorphous & can easily change shape (Pelczar, et al, 1996).

1.5.2.2.1.The characteristics of Gram-positive bacteria

- They contain cytoplasmic lipid membrane.
- They have thick peptidoglycan layer. In this layer teichoic acids & lipoids are present. This forms lipoteichoic acids. It serves as chelating agents & also for certain types of adherence.
- Capsule polysaccharides which is only in some species.
- Flagella which is only present in some species. If it is present, it contains two rings for support, because Gram-positive bacteria have only one layer of membrane.
- Pentaglycine chains helps in cross-linking of the individual peptidoglycan molecules. A DD-tranpeptidase enzyme also helps in this cross-linking.

1.5.2.2.2.Examples of Gram-positive bacteria

Gram-positive bacteria are Staphylococcus, Streptococcus, Bacillus, Clostridium, Corynebacterium, Mycobacterium etc (Byarugaba, 2010).

1.6.Difference of structure between Gram-negative & Gram-positive bacteria

Although Gram-positive bacteria & Gram-negative bacteria have a membrane called an S-layer, in case of Gram-negative bacteria it is attached directly to the outer membrane. In case of Gram-positive bacteria, S-layer is attached to the peptidoglycan layer. The basic difference between these two types of bacteria is the presence of teichoic acids in the cell wall. Some of the teichoic acids, lipoteichoic acids contain a lipid component (Prescott et al, 1993).

1.7.Mechanism of Bacterial Pathogenecity

Two broad qualities of pathogenic bacteria underlie the means by which they cause disease:

1. Invasiveness
2. Toxigenesis

1. **Invasiveness:** Invasiveness is the ability to invade tissues. It encompasses mechanisms for colonization (adherence and initial multiplication), production of extracellular substances which facilitate invasion (invasins) and ability to bypass or overcome host defense mechanisms.

2. **Toxigenesis :** Toxigenesis is the ability to produce toxins. Bacteria may produce two types of toxins called exotoxins and endotoxins. Exotoxins are released from bacterial cells and may act at tissue sites removed from the site of bacterial growth. Endotoxins are cell-associated substance. (In a classic sense, the term endotoxin refers to the lipopolysaccharide component of the outer membrane of Gram-negative bacteria). However, endotoxins may be released from growing bacterial cells and cells that are lysed as a result of effective host defense (e.g. lysozyme) or the activities of certain antibiotics (e.g. penicillins and cephalosporins). Hence, bacterial toxins, both soluble and cell-associated, may be transported by blood and lymph and cause cytotoxic effects at tissue sites remote from the original point of invasion or growth. Some bacterial toxins may also act at the site of colonization and play a role in invasion. (ELSEVIER, 2003).

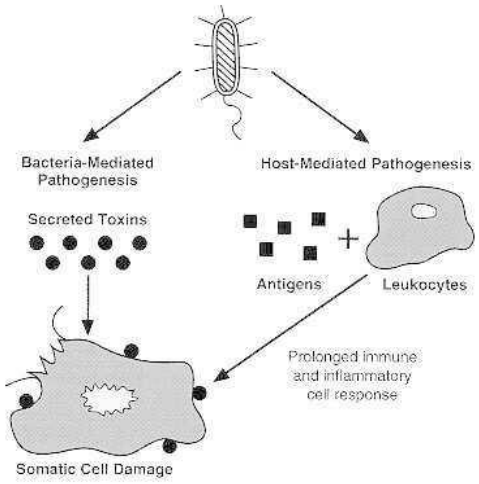

Figure 1.3 : General mechanism of bacterial pathogenecity

1.8.Some Pathogenic Bacteria

1.8.1.*Staphylococcus aureus* sp.

Staphylococcus aureus is a facultative anaerobic, gram-positive coccal bacterium also known as "golden staph" . In medical literature, the bacterium is often referred to as *Staph aureus.*

Scientific Classification

Domain : Bacteria

Kingdom : Eubacteria

Phylum : Firmicutes

Class : Coccus

Order : Bacillales

Family : Staphylococcaceae

Genus : *Staphylococcus*

Species : *S. aureus*

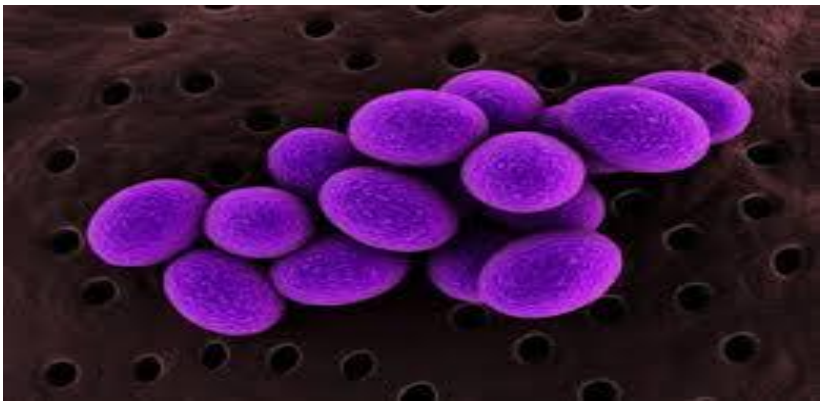

Figure 1.4.1: Image of *Staphylococcus aureus sp*

Transmission of *Staphylococcus aureus*

S. aureus may occur commonly in the environment. *S. aureus* is transmitted through air droplets or aerosol. When an infected person coughs or sneezes, he or she releases numerous small droplets of saliva that remain suspended in air. These contain the bacteria and can infect others. Another common method of transmission is through direct contact with objects that are contaminated by the bacteria or by bites from infected persons or animals. (Konard et al, 2009).

***Staphylococcus aureus* Infection**

Staphylococcus aureus infections range from mild to life threatening. The bacteria tend to infect the skin. However, the bacteria can travel through the bloodstream causing bacteremia) and infect almost any site in the body, particularly heart valves (endocarditis) and bones (osteomyelitis). The bacteria also tend to accumulate on medical devices in the body, such as artificial heart valves or joints, heart pacemakers, and catheters inserted through the skin into blood vessels. (Konard et al 2009)

Treatment

- Ciprofloxacin, rifampin, vancomycin, daptomycin are the choices of antibiotics for the treatment of Endocarditis.
- For bone and joint infections, oxacillin, cefazolin, nafcillin, gentamycin may be used.
- Brain and meninges infection (meningitis) - for MSSA oxacillin, cefazolin, nafcillin, gentamycin etc. may be used.

- Lung infections or pneumonia – for MRSA cases Linezolid, Vancomycin, Clindamycin etc. may be used.

1.8.2.*Klebsiella sp*.

Klebsiella is a genus of non-motile, gram- negative, facultative anerobe, rod-shapped bacteria. *Klebsiella* organism can lead to a wide range of disease states,pneumonia, urinary tract infection, soft tissue infection. (Podschun & Ullmann, 1998).

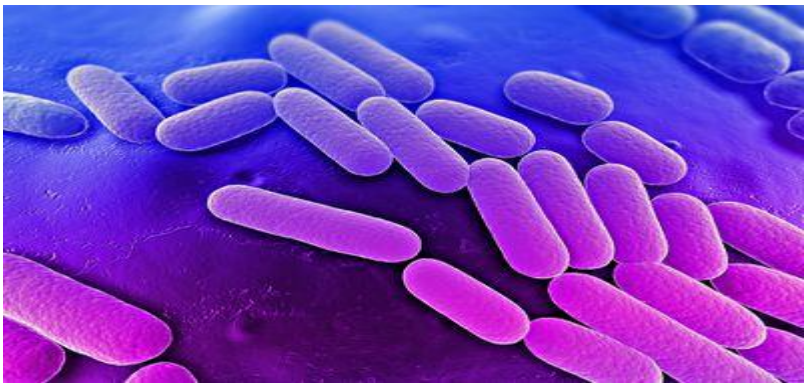

Figure 1.4.2 : Image of *Klebsiella sp.*

Scientific Classification

Kingdom : Bacteria

Phylum : Proteobacteria

Class : Gammaproteobacteria

Order : Enterobacteriales

Family : Enterobacteriaceae

Genus : *Klebsiella*

Species *: Klebsiella oxytoca,*

Klebsiella terrigena

Transmission of *Klebsiella sp.*

Klebsiella spp. can be transmitted through skin contact with environmentally contaminated surfaces or objects; examples include Loofah sponges, medical equipment, and blood products. Fecal transmission has also been suggested for some cases of bacteremia caused by *Klebsiella* spp. *K. rhinoscleromatis* can be transmitted from person-to-person via airborne secretions. (Podschun & Ullmann, 1998).

***Klebsiella* Infection**

- *K. pneumoniae* and *K. oxytoca* cause community-acquired meningitis and brain abscesses. Clinical symptoms include: headaches, fever, altered conciousness, seizures, and septic shock.
- *K. granulomatis* causes donovanosis or granuloma, a chronic ulcerative disease that primarily affects the genitalia. Symptoms include development of small papule or ulcer at the site of inoculation that later develop into large red ulcers that extend along the moist folds of the genitalia. (Podschun & Ullmann, 1998).

Treatment

- Rhinoscleroma is treated with combination antimicrobial therapy for 6-8 weeks. Therapeutic choices include aminoglycosides, tetracycline, sulfonamides, rifampin, and quinolones.
- Ozena may be treated with a 3-month course of ciprofloxacin. Intravenous aminoglycosides and trimethoprim/sulfamethoxazole are also useful in the treatment of these conditions. Susceptibility testing is usually required.
- *For Klebsiella meningitis,* third-generation cephalosporins are the drugs of choice because of superior central nervous system penetration. Reports indicate success with cefotaxime, and meropenem is a useful alternative. (Matthew et al, 2005).

1.8.3.*E.coli sp*

E.coli is a gram- negative, facultative anaerobic, rod-shapped bacterium of the genus of *Escherichia* that is commonly found in the lower intestine of human that cause bacterial meningitis, pneumonia and urinary tract infections.(Nerino et al, 2013).

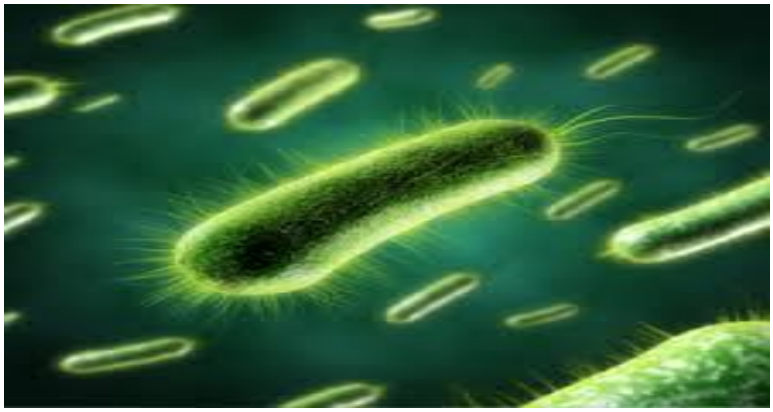

Figure 1.4.3: *E.coli*

Scientific Classification

Domain : Bacteria

Phylum : Proteobacteria

Class : Gammaproteobacteria

Family : Enterobacteriaceae

Genus : *Escherichia*

Species *: E. coli*

Transmission of *E.coli sp.*

E.coli can be transmitted thrugh consumption of contaminated water, raw milk as well as uncooked fruits and vagetables. It can be passed through person to person through improper hygiene. *E.coli* can spread when a person fails to wash his or her hands after having a bowel movement.(Nerino et al, 2013).

***E.coli* Infection**

Escherichia coli is one of the most frequent causes of many common bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and traveler's diarrhea, and other clinical infections such as neonatal meningitis and pneumonia. (Eisenstein et al, 2000).

Treatment of *E.coli* Infection

- *E coli* pneumonia requires respiratory support, adequate oxygenation, and antibiotics, such as third-generation cephalosporins or fluoroquinolones.
- *E coli* cholecystitis requires antibiotics such as third-generation cephalosporins that cover *E coli* and *Klebsiella* organisms.
- *E coli* enteric infections require fluid replacement with solutions containing appropriate electrolytes. Antimicrobials known to be useful in cases of traveler's diarrhea include doxycycline, fluoroquinolones. (Medscape, 2016).

1.8.4.*Acinetobacter* sp.

Acinetobacter is a genus of gram-negative bacteria belonging to the wider class of Gammaproteobacteria. These species are non-motile, oxidase-negative and occur in pairs under magnification (Joon et al, 2014).

Figure 1.4.4 : *Acinetobacter sp.*

Scientific Classification

Kingdom : Bacteria

Phylum : Proteobacteria

Class : Gammaproteobacteria

Order : Pseudomonadales

Family : Moraxellaceae

Genus : *Acinetobacter*

Species *: Acinetobacter apis*

Transmission of *Acinetobacter* sp.

Acinetobacter poses very little risk to healthy people. However, people who have weakened immune systems, chronic lung disease, or diabetes may be more susceptible to infections with *Acinetobacter*. Hospitalized patients, especially very ill patients on a ventilator, those with a prolonged hospital stay, those who have open wounds, or any person with invasive devices like urinary catheters are also at greater risk for *Acinetobacter* infection. *Acinetobacter* can be spread to susceptible persons by person-to-person contact or contact with contaminated surfaces. (Joon et al, 2014)

***Acinetobacter* Infection**

- Blood infection may occur if the germ enters through a catheter placed in the vein. It can also happen when an infection from another place in body spreads to blood.
- Pneumonia is an infection of the lungs. *Acinetobacter baumannii* can get into lungs through the mouth or nose.
- A urinary tract infection (UTI) is an infection of the kidneys, ureters, or bladder. This may happen when the germ enters in to the body. It may also enter through a catheter that is used to drain the urine. (Joon et al, 2014)

Treatment of *Acinetobacter* Infection

- Group II carbapenems (imipenem and meropenem) are the agents of choice for the treatment of severe infections caused by Acinetobacter spp. isolates susceptible to this antimicrobial group, but infection with carbapenem-resistant strains is increasingly encountered.
- Combination antimicrobial therapy is often used to treat infections caused by such multidrug-resistant strains. (Pubmed, 2008)

1.9.Antibiotic

The word antibiotic has come from two greek word, anti & bios. Here *anti* means against & *bios* means life. Antibiotics are the drugs that inhibit the growth of bacteria. These drugs also destroy the bacteria that cause infection. These drugs do not work against any type of viral diseases. As for example, the common cold or influenza is the viral diseases. Microbial infections are the infections that caused by microorganisms. (Ananthanarayan, et al, 2005)

1.10.How Antibiotics Work

Antibiotics are of two types. They may be bacteriostatic or bactericidal. Bacteriostatic means that can easily prevent bacteria from multiplying & bactericidal means that kill bacteria. In most infections, these two types of antibiotics show effectiveness equally. A bactericidal antibiotic is usually more effective, when the immune system is impaired. Bactericidal antibiotic is also effective if the individual has a severe infection. Sometimes bactericidal drugs can be bacteriostatic against some microorganisms. Bacteriostatic drugs can also be bactericidal against some microorganisms. (Katzung, 2010)

1.11.Classification of Antibiotics

There are varieties of antibiotics produced by naturally, semi-synthetically or synthetically. They shows difference in primary target & variation of effectiveness on the species. From them most commonly used antibiotics are discussed here (Vasanthakumari, 2007).

Fluoroquinolones

They are DNA synthesis inhibitor. Example of fluroquinolones are Nalidixic acid, ciprofloxacin, levofloxacin & gemifloxacin. They are Synthetic. their primary target is topoisomerase II & topoisomerase IV (Rang, 2012)

Trimethoprim–sulfamethoxazole

They are DNA synthesis inhibitor such as Co-trimoxazole. It is combination of trimethoprim & sulfamethoxazole. They are synthetic. Their primary target is tetra hydrofolic acid synthesis inhibitors (Rang, 2012)

Rifamycins

They are RNA synthesis Inhibitor. Rifampin & rifapentine are the drug of this class. They are both Natural & semi-synthetic. Their primary target is DNA- dependent RNA polymerase (Rang, 2012).

β-lactams

They are Cell wall synthesis inhibitors. Penicillins like penicillin, ampicillin, oxacillin & cephalosporins like cefazolin, cefoxitin, ceftriaxone, cefepime & carbapenems like imipenem are the drug of this class. They are both natural & semi-synthetic. Their primary target is Penicillin-binding proteins. (Vasanthakumari, 2007).

Glycopeptides & glycolipopeptides

They are Cell wall synthesis inhibitors. Vancomycin is the drug of this class. They are both natural & semi-synthetic.their primary target is Peptidoglycan Units. (Vasanthakumari, 2007).

Lipopeptides

They are Cell wall synthesis inhibitors. Daptomycin & polymixin B are drugs of this class. They are both natural & semi-synthetic. Their primary target is cell membrane (Pomares, 2011).

Aminoglycosides

They are protein synthesis inhibitors. Gentamicin, tobramycin, streptomycin & kanamycin are drug of this class. They are both natural & semi-synthetic. Their primary target is 30S ribosome.

Tetracyclines

They are protein synthesis inhibitors. Tetracycline & doxycycline is the drug of this class. They are both natural & semi-synthetic. Their primary target is 30S ribosome. (Pomares, 2011).

Macrolides

They are protein synthesis inhibitors. Erythromycin & azithromycin is the drug of this class. They are both natural & semi-synthetic. Their primary target is 50S ribosome. (Pomares, 2011).

Phenicols

They are protein synthesis inhibitors. Chloramphenicol is the drug of this class. They are both natural & semi-synthetic. Their primary target is 50S ribosome. (Pomares, 2011).

1.12.Mode of Action Antibiotic

Different antibiotics have different modes of action. It depends on the nature of their structure & degree of affinity to certain target sites within bacterial cells. When the cells of humans & animals do not have cell walls, it becomes so tough for the survival of bacterial species. Drugs that target cell walls can easily kill or inhibit bacterial oraganisms. As for example these drugs are penicllins, cephalosporins, bacitracin & vancomycin. (Vasanthakumari, 2007).

Cell membranes are the important barriers. They play a great role to segregate & regulate the intra & extracellular flow of substances. Any disruption or damage to this structure can be happened.Then it could result in leakage of important solutes essential for the cell's survival. The main reason is that this this structure is found in both eukaryotic and prokaryotic cells. The actions of this class of antibiotics are often poorly selective. They can often be toxic for systemic use in the mammalian host. Example of these types of drugs is polymixin B and colistin. (Vasanthakumari, 2007)

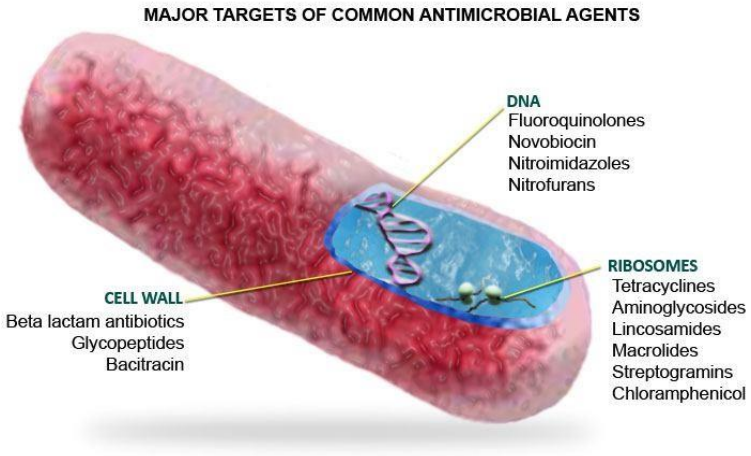

Figure 1.5: Different modes of action of antibiotic

Several types of antibacterial agents target bacterial protein synthesis. It can be happened by binding to either the 30S or 50S subunits of the intracellular ribosomes. Enzymes & cellular structures are made of protein. Protein synthesis is an essential process especially for the multiplication & also for the survival of all bacterial cells. This activity then helps in the disruption of the normal cellular metabolism of the bacteria. It then consequently leads to the death or inhibition of growth of the organism & multiplication. Aminoglycosides, macrolides,

lincosamides, streptogramins, chloramphenicol, tetracyclines show this type of action. (Vasanthakumari, 2007)

DNA and RNA play main role in the replication of all living forms. Some antibiotics perform by binding to components involved in the process of DNA or RNA synthesis. It causes interference of the normal cellular processes. It will ultimately compromise bacterial multiplication & survival. Quinolones, metronidazole, & rifampin are these types of antibiotics. Other antibiotics performed on selected cellular processes. These are essential for the survival of the bacterial pathogens. As for example, both sulfonamides & trimethoprim disrupt the folic acid pathway. It is a necessary step for bacteria to produce precursors important for DNA synthesis. Sulfonamides target & bind to dihydropteroate synthase. Trimethophrim inhibit dihydrofolate reductase. Folic acid is a vitamin synthesized by bacteria, but not humans. Both of these enzymes are essential for the production of folic acid. (Vasanthakumari, 2007)

1.13.Therapeutic Use of Antibiotics

The use of antibiotics as therapeutically means to treat clinically ill animals. The importance of good management of medicine must not be underestimated. Antimicrobial therapy has superior power. It can easily address many diseases condition that is difficult to address by any other therapy. In case of animals the use of antibiotics as therapeutically is complicated. This therapeutic use of antibiotics is less complicated in case of human medicine. It gives the variations between species. (Molly, 2013).

The main target of doing antimicrobial susceptibility test is to determine the various available options for therapy that will be suitable. We have to consider not only about the bacterial susceptibility at the time of selecting an antibiotic. There are some factors that have to be considered during the selection of an antibiotic from a range of option. They are the drug's attributes like pharmacodynamics, pharmacokinetics, toxicity & tissue distribution. The second one is the host characteristics like age, species, & immune status. The accountability to the public & cost effectiveness of antibiotic should also be considered. Each of these issues is important to make a proper decision during the selection of an antibiotic. (Molly, 2013).

1.14. Antibiotic Resistance

People take an antibiotic for a long period of time. As for example, in case of rheumatic fever, the targeted bacteria may develop their own defense against the drug. An enzyme is a complex

protein. It is capable of inducing chemical changes. It remains unchanged. This can easily destroy the drug. This enzyme may produced by the bacteria. Cell wall can become resistant to being broken by the action of the antibiotic. After being resistant to the antibiotic, an individual become fast against the drug. It means that antibiotic is no longer able to fight the infection. At that time another type of antibiotic must be administered (Vasanthakumari, 2007).

Several mechanisms have evolved in bacteria which confer them with antibiotic resistance. These mechanisms can chemically modify the antibiotic, render it inactive through physical removal from the cell, or modify target site so that it is not recognized by the antibiotic. (Vasanthakumari, 2007)

This figure shows different types of mechanisms involve in antibiotic resistance. The most common mode is enzymatic inactivation of the antibiotic. An existing cellular enzyme is modified to react with the antibiotic in such a way that it no longer affects the microorganism. (Vasanthakumari, 2007).

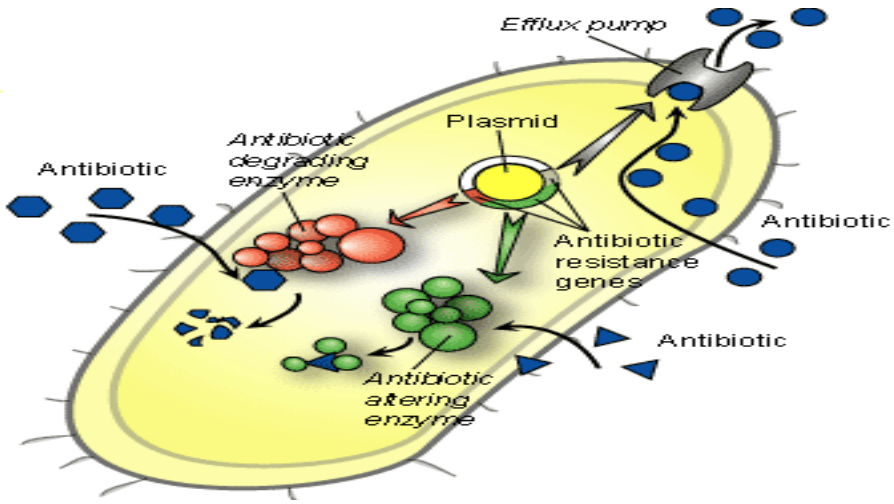

Figure 1.6: Mechanisms of antibiotic resistance

1.15.Some Important Antibiotics

1.15.1.Ciprofloxacin

Ciprofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class. It is a second-generation fluoroquinolone antibacterial. It also inhibits the activity of topoisomerase IV, leads to separation of DNA molecules and subsequent interference with cellular replication. It kills bacteria by inhibiting the activity of DNA gyrase that cause DNA to rewind

after being copied, which stops synthesis of DNA and of protein. Ciprofloxacin is highly active in vitro against a wide range of gram-negative and gram- positive organisms. Ciprofloxacin is indicated for the treatment of acute sinusitis , lower respiratory tract infections, Acute uncomplicated cystitis in females.(Rang, 2012)

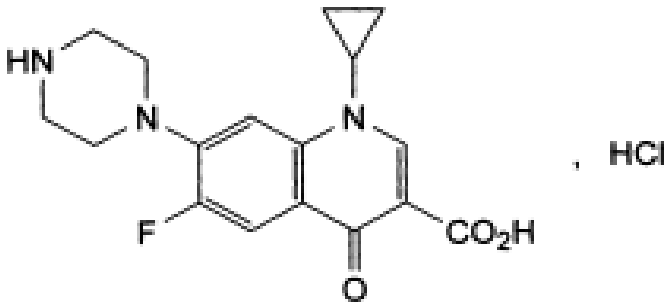

Figure 1.7.1: Ciprofloxacin Hydrochloride

1.15.2.Levofloxacin

Levofloxacin is a synthetically derived third generation fluoroquinolone. Chemically, levofloxacin, a chiral fluorinated carboxyquinolone, is the pure (-)-(S)-enantiomer of the racemic drug substance ofloxacin. Levofloxacin has *in vitro* activity against Gram-negative and Gram-positive bacteria. The mechanism of action of levofloxacin and other fluoroquinolone antimicrobials involves inhibition of bacterial topoisomerase IV and DNA gyrase enzymes required for DNA replication, transcription, repair and recombination. The cells of mammals have the enzyme topoisomerase II instead of DNA gyrase or topoisomerase IV which possesses very little affinity for levofloxacin resulting in minimal damage to the host tissue. Levofloxacin is indicated for the treatment of acute bacterial exacerbation of chronic bronchitis, community-acquired pneumonia, Plaque, chronic bacterial prostatitis. (Rang, 2012)

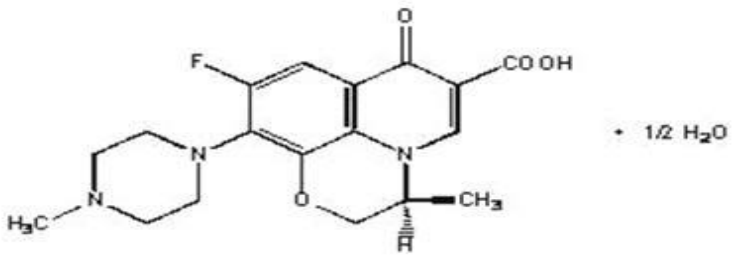

Figure 1.7.2: Structure of Levofloxacin

1.15.3.Azithromycin

Azithromycin is a macrolide antibiotic.It is derived from erythromycin and very active against gram-negative bacteria. Azithromycin is a bacteriostatic drug acts by binding reversibly to 50 S ribosomal subunits of sensitive microorganism. The drug interferes with transpeptidation and translocation thus there is inhibition of protein synthesis and hence inhibition of cell growth.

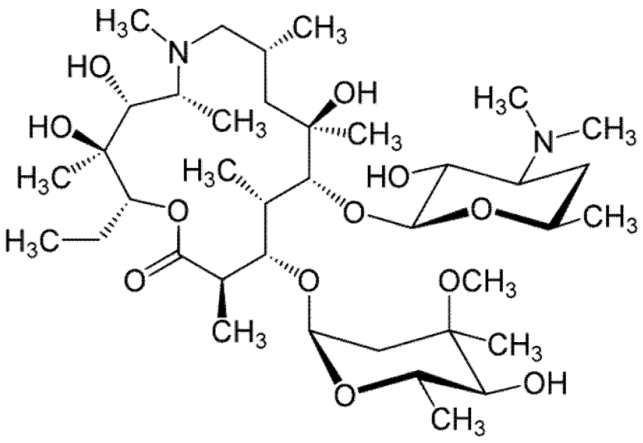

Figure 1.7.3: Structure of Azithromycin

Azithromycin is effectively used as a second line treatment for strep throat. It is used to treat gastrointestinal infections such as traveler's diarrhea, in respiratory tract infections such as pneumonia, cellulitis, Bartonella infection, chancroid cholera, donovanosis, leptospirosis, lyme disease, malaria. (Molly, 2013)

1.15.4.Cephradine

Cephradine is a first generation cephalosporin. It is effective against most gram-positive organisms such as Staphylococcus and Streptococcus. It is not reliably active against Staphylococcus aureus, highly penicillin-resistant Streptococcus pneumoniae, and Enterococcus species. It is generally active against community-acquired Escherichia coli and Klebsiella species, but is frequently resistant to hospital-acquired E coli and Klebsiella species. It Inhibits bacterial cell wall synthesis by binding to one or more of the penicillin-binding proteins (PBPs) which in turn inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell walls, thus inhibiting cell wall biosynthesis. Bacteria eventually lyse due to ongoing activity of cell wall autolytic enzymes while cell wall assembly is arrested. (Rang, 2012)

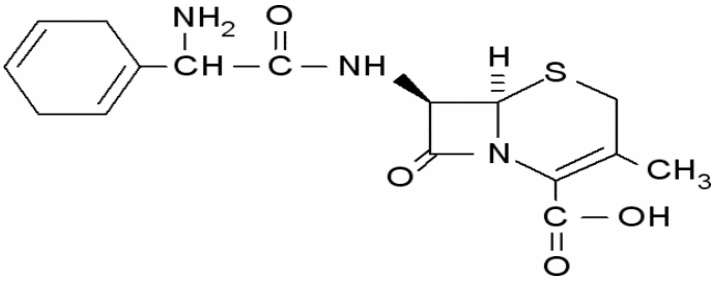

Figure 1.7.4: Structure of Cephradine

The indication of cephradine is similar to other cephalosporin drug. It is used to treat respiratory tract infection caused by group A beta-hemolytic streptococci and *S. pneumoniae* and skin infection caused by staphylococci (penicillin-susceptible and penicillin-resistant) and beta-hemolytic streptococci. (Rang, 2012)

1.15.5.Ceftriaxone

Ceftriaxone is a sterile, semisynthetic, third-generation cephalosporin antibiotic . It is a used in the treatment of bacterial infections caused by susceptible, usually gram-positive, organisms. Ceftriaxone has *in vitro* activity against gram-positive and gram-negative aerobic and anaerobic bacteria. It is stable against hydrolysis by a variety of beta-lactamases, including penicillinases, and cephalosporinases and extended spectrum beta-lactamases. Ceftriaxone binds to penicillin-binding proteins and inactivates penicillin-binding proteins (PBP) located on the inner membrane of the bacterial cell wall. Inactivation of PBPs interferes with the cross-linkage of peptidoglycan chains necessary for bacterial cell wall strength and rigidity. This results in the weakening of the bacterial cell wall and causes cell lysis. The drug is used to treat bone joint infection, intra- abdominal infection and bacterial septicemia. (Molly, 2013).

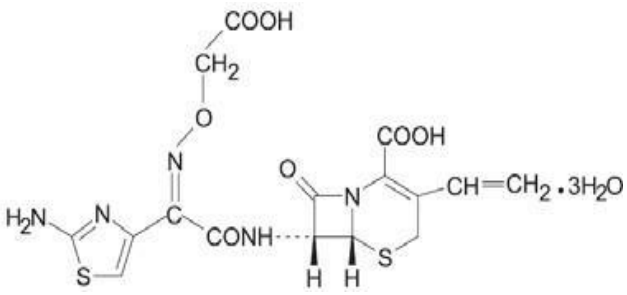

Figure 1.7.5: Structure of Ceftriaxone

1.15.6.Cefuroxime

Cefuroxime is a semisynthetic cephalosporin antibiotic, chemically similar to penicillin. Cefuroxime is effective against a wide variety of bacteria.Cefuroxime is indicated for the treatment of mild-to-moderate pharyngitis/tonsillitis; to treat for sinus infections. (Hadi et al, 2011).

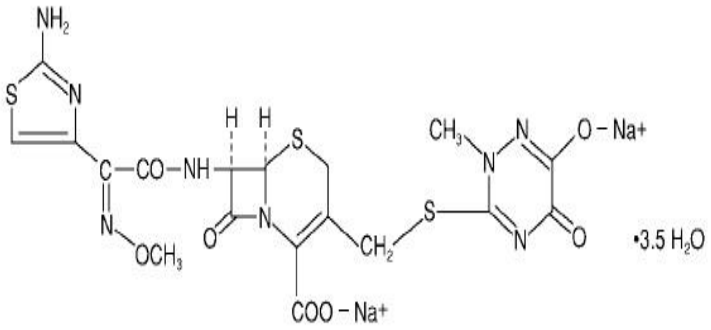

Figure 1.7.6: Structure of Cefuroxime

1.15.7.Cefixime

Cefixime is a third-generation cephalosporin antibiotic. Cefixime is highly stable in the presence of beta-lactamase enzymes. Cefixime is highly active against gram negative cocci, gram negative bacilli and anaerobes than gram positive cocci and bacilli. It is not active against pseudomonas. Like other cephalosporins, cefixime possesses a mechanism of action similar to penicillins. (Harold et al, 2011)

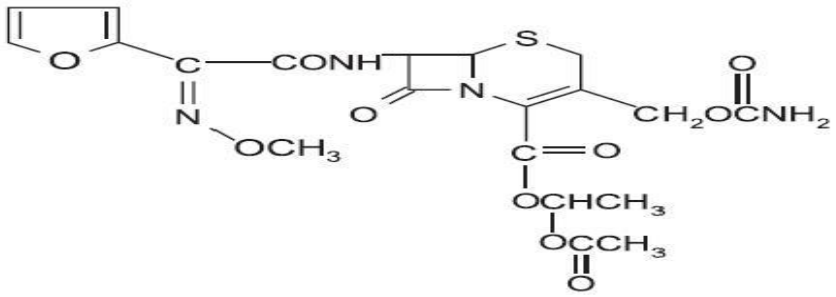

Figure 1.7.7: Structure of Cefixime

Cefixime is used to treat Pharyngitis and Tonsillitis caused by *Streptococcus pyogenes,* Chronic Bronchitis caused by *Streptococcus pneumoniae* and *Haemophilus influenza.* (Harold et al,2011)

1.15.8.Vancomycin

Vancomycin is a tricyclic glycopeptide antibiotic derived from *Amycolatopsis orientalis* (formerly *Nocardia orientalis*). Vancomycin is not active *in vitro* against gram-negative bacilli, mycobacteria, or fungi. It is active against aerobic gram positive bacteria (*Enterococcus faecalis ,Staphylococcus aureus* etc *).* Vancomycin prevents incorporation of N-acetylmuramic acid (NAM)- and N-acetylglucosamine (NAG)-peptide subunits from being incorporated into the peptidoglycan matrix; which forms the major structural component of Gram-positive cell walls. The binding of vancomycin to the D-Ala-D-Ala prevents the incorporation of the NAM/NAG-peptide subunits into the peptidoglycan matrix. Vancomycin is effective in the treatment of staphylococcal endocarditis, and in other infections due to staphylococci, including septicemia, bone infections, lower respiratory tract infections, skin and skin structure infections. (Stan, 2009)

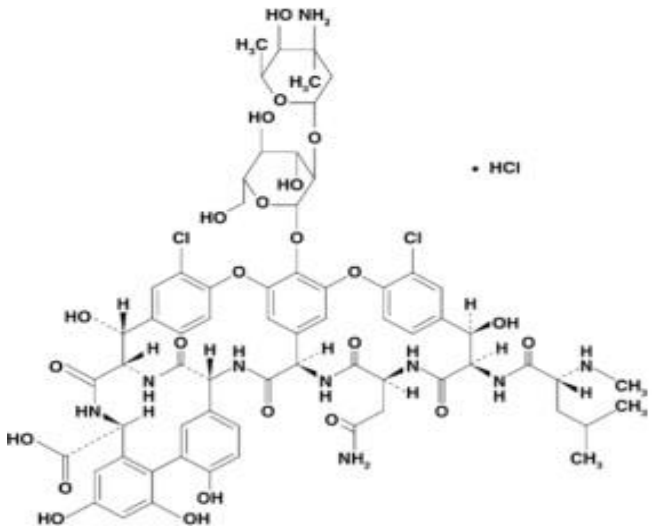

Figure 1.7.8: Structure of Vancomycin

1.16.Methods of antimicrobial sensitivity test

There are different types of antibiotic sensitivity testing methods. They are the dilution methods, disk diffusion methods, E-test etc. (Schwalbe, et al, 2007)

1.16.1.Dilution Method

In dilution method broth & agar media both is used. In case of broth dilution the isolation of a series of concentrations of antimicrobial agents is done. It is performed in a broth environment. In the microdilution test, 0.05 to 0.1 ml total broth volume is used. It can be easily performed in a microtiter format. In macrodilution test, 1.0 ml broth volume is used in standard test tube. For both of these dilution methods, in microdilution & in macrodilution the lowest concentration is used. In this lowest concentration the isolate is completely inhibited. This lowest concentration is known as minimal inhibitory concentration or MIC. This inhibition is confirmed by the absence of visible bacterial growth. In this way MIC is the minimum concentration of the antibiotic. It will ultimately inhibit the particular isolate. The test is only considered as valid in two conditions. These two conditions are if the positive control shows bacterial growth & negative control shows no bacterial growth. In case of agar dilution same procedure of broth dilution is followed. In agar dilution method, it follows to establish the lowest concentration of the serially diluted antibiotic concentration. (Schwalbe, et al, 2007)

1.16.2.Disk Diffusion Method

Disk diffusion method is most probably widely used method. In this process a growth medium is used which is known as Mueller-Hinton agar. At first this agar medium is first seeded throughout the plate. In this plate bacterial isolation is given after diluting this isolation at a standard concentration. Commercially prepared disks are used. Each of the disks is impregnated with a standard concentration of a particular antibiotic. All of these disks are then evenly dispensed & lightly pressed onto the agar surface. The antibiotic test is immediately begun to diffuse outward from the disks. At that time it starts to create a gradient of antibiotic concentration in the agar. As a result the highest concentration is found to close to the disk. The concentration decreases further away from the disk. An overnight incubation is done. Then the bacterial growth around each disk is observed. A clear area of no growth is observed around that particular disk. It means the test isolate is susceptible to a particular antibiotic. (Schwalbe, et al, 2007; Pelczar, et al, 1996)

Zone of inhibition is known as the zone around an antibiotic disk with no growth. This confirms that the minimum antibiotic concentration is sufficient to prevent the growth of the test isolate. After that the zone is then measured in mm. Then this measurement is compared to a standard Interpretation chart.This chart is used to categorize the isolate as susceptible &

intermediately susceptible or resistant. By this qualitative testing method, MIC measurement cannot be determined. This test simply classifies the isolate as susceptible, intermediate or resistant. (Pelczar, et al, 1996)

Chapter Two

LITERATURE REVIEW AND OBJECTIVE OF THE STUDY

A study has been carried out to compare to compare antibacterial activity of standard and different brands of cefixime, against standard sample and clinical isolates of *E. coli* and *S.aureus* collected from the different hospitals of Karachi, Pakistan. Standard sample and clinical isolates of *E. coli* and *S. aureus* were separately cultured in Muller Hinton Broth. After the bacterial incubation, 5 mL solution of each standard cefixime and its different brands were added in the test tubes containing bacterial culture. Cefixime samples were added in a concentration of 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 mcg/mL to separate test tubes. The culture were again incubated and the culture samples were analyzed by UV-Spectrophotometer, minimum inhibitory concentration of all samples were determined. The analysis and interpretation of the results were determined by a single factor ANOVA. An MIC of 0.75 mcg/mL and 8 mcg/mL of standard cefixime was found for *E. coli* and *S.aureus* respectively. Standard cefixime and its six selected brand, exhibited a higher MIC range for the clinical isolates of *S.aureus* than clinical isolates of *E. coli*. Higher MIC value for standard cefixime and its different brands were observed and indicated that both the organisms have developed resistance to cefixime in comparson to standard microorganism acquired from ATCC. (Hafiz, Omair & Muhammad, 2012).

Another study was conducted in clinical microbiology laboratory of the university of Milan, Italy to compare antibacterial activity of levofloxacin and ciprofloxacin aganst urinary tract pathogens, by evaluating MICs and MBCs in accordance with NCCLS susceptibility test, time-kill curves and interference with bacterial adhesion to uroepithelial cells. A total of 200 clinical isolates was tested including the species *E.coli, Klebsiella pneumonia, Proteus vulgaris, Proteus marbills, Providencia rettgeri, Pseudomonas aeroginosa, Enterococcus faecalis, Staphylococcus aureus* and *staphylococcus epidermidis*. All *E.coli* isolates were susceptible to levofloxacin, only one was resistance to ciprofloxacin, *K. pneumonia* strains resistance to ciprofloxacin were also resistance to levofloxacin. *S. epidermidis* strains were susceptible to levofloxacin and ciprofloxacin with the exception of two isolates. Inhibition of adhesion ranged from 36 to 43 % when bacteria were incubated in presence of 0.25× MIC of levofloxacin and ciprofloxacin and from 10 to 27 % at 0.125× MIC. These findings suggest that levofloxacin is an alternative effective to ciprofloxacin in treatment of urinary tract infections. (L. Drago et al, 2001).

Another study has been carried out in an attempt to evaluate antimicrobial pattern with special reference to susceptibility of *Salmonella typhi* to ciprofloxacin isolated from blood culture. The study is also designed to find out the MIC of ciprofloxacin by E-test. Blood samples were taken for culture sensitivity, widal test and ICT from 100 clinically suspected cases of Typhoid fever in 1st week of illness who attented at out patient department of Rajshahi Medical College Hospital (RMCH). The study was done in microbiology department of Rajshahi Medical College and Shishu Hospital. Diagnosis of patients was based on history of fever, blood culture, Widal test and ICT. The antimicrobial susceptibility pattern of isolates from blood culture was recorded. Further more, the minimum inhibitory concentration of ciprofloxacin was determined by E-test for the isolates resistance to ciprofloxacin. Out of 100 suspected cases of typhoid fever, blood culture positive for *S. typhi* were 16%. Antimicrobial susceptibility pattern of 16 isolates of S. typhi showed that no isolates was resistance to ceftriaxone and ceftazidime, only 03 (18.75%) were resistance to ciprofloxacin and azithromycin whereas 10 (62.5%) were MDR showing resistance to Ampicillin, Co-trimoxazole and chloramphenicol which are first line antityphoidal drugs. On the other hand, all (100%) isolates were resistant to Nalidixic acid. The study revealed that ceftriaxone and ceftazidime are the most effective drugs in the treatment of typhoid fever. Moreover, E-test has been bound to be helpful to determine coerrect dose of ciprofloxacin specially in case of drug resistance and pediatric population. (Hasan et al, 2011).

Another study has done to evaluate the sensitivity to honey of gram-positive cocci of clinical significance in wounds. Methicillin resistance Staphylococcus strains and vancomycin resistance enterococci strains were isolated from infected wounds and some enterococci strains isolated from hospital environment surfaces. Using a agar incorporation technique to determine minimum inhibitory concentration, their sensitivity to two natural honeys of median level of antibacterial activity was established and compared with an artificial honey solution. For all of the strains tested, the MIC values against manuka and pasture honey were below 10%(V/V), but the concentration of artificial honey at least three times higher were required to achieve equivalent inhibition in vitro. Comparison of MIC values of antibiotic sensitive strains with their respective antibiotic resistance strains demonstrated no marked difference in their susceptibilities to honey. (Cooper, Molan & Harding, 2002).

A comparative study of broth macrodilution and microdilution methods for the susceptibility testing of fluconazole, itraconazole, flucytosine and amphotericin B with 273 yeasts in the university of Texas Health Science Center, Brazil. The clinical isolates *were Candida tropicalis, Candida krusei, Candida parapsilosis, Candida lusitanae, Trichosporon beigilii*. Both methods were performed according to National Committee for Clinical Laboratory Standards (NCCLS) recommendations. For fluconazole, itraconazole, flucytosine, the end point was the tube that showed 80% growth inhibition compared with growth control for the macrodilution method and and the well with slightly hazy turbidity compared with microdilution methods. For the amphotericin B, the end point was the tube and well in which there is absence of growth. For the reference macrodilution method, MICs were determined after 48 hours of incubation for Candida spp. And *T. beigelii* and after 72 hours for *C. neoformans*. For microdilution method, either the first day MIC (24 hours) and the second day MICs (48 and 72 h respectively) were evaluated. The agreement within one dobling dilution of macrodilution reference for all drugs was higher with the second day MICs than the first day MICs microdilution test for most of the tested strains. General agreement was 92% for fluconazole, 85.7% for itraconazole and 98.3% for flucytosine and 96.4% for amphotericin B. For *C. neoformans* and *T. beigelii*, the agreement of first day reading was higher than second day reading for fluconazole. The study indicates that microdilution technique performed following the NCCLS guideline with second day reading is a valid alternative method for testing fluconazole, itraconazole, flucytosine and amphotericin B against these isolates (Francesco et al, 1994).

In another study, gram-negative bacterial isolates obtained from Oklahoma Animal Diaease Diagnostic Laboratory during 1983-1987 were tested for the antimicrobial susceptibility. Minimum Inhibitory concentrations were determined for the following antimicrobials using microdilution method : ampicillin, cephalotin, Penicillin G, Chloramphenicol, erythromycin, gentamycin, kanamycin, oxytetracycline, spectinimycin, sulfachlorpyrazidine, sulfadimethoxine and tylosine. Results for isolates from cattle, dogs, horses and pigs were presented. In only a few instances were differences in MICs appearant among bacterial isolates from different tissues. Aminocyclitol MICs for equine uterine isolates of *Klebsiella Pneumoniae* of different for MICs isolates from other tissues and ampicillin, kanamycin and

spectinomycin MICs for bovine fecal isolates of *E.coli* differed from MICs for isolates obtained from other tissues. In several instances, bimodal distribution of susceptibilities for ampicillin and kanamycin. There was also a bimodal distribution pattern for erythromycin against *Pasteurella homolytica* of bovine origin. (Burrows, Morton & Fales, 1993).

A multicenter study was done to investigate the accuracy and reproducibility of study of a method for determining MICs of antimicrobials following cycloserine, amikacin, ciprofloxacin, clofazimine, ethambutol, Ethionomide, rifampin and streptomycin against *mycobacterium avium* complex in 7H12 broth with the BACTEC system. In phase I (2.0, 4.0, 8.0 mcg/mL), with eight druga and 10 strains the intralaboratory reproducibility was 95.7 to 100% allowing 1 dilution difference upon repeat testing. The results of phase II (1.0, 2.0, 4.0, 8.0 mcg/mL) testing with 41 additional strains were consistant with those obtained in phase I, with good intralaboratory reproducibility. The radiometric method validated by sampling and plating of same broth cultures and determining, by a number of C.F.U per mL, the lowest drug concentration that inhibited more than 99% of initial bacterial population. Three test concentration of each drug and the tentative interpretation of results are proposed. Radiometric MIC determination has the potential to become the method of choice for clinical microbiology laboratory and evaluation of new agents for the treatment of *mycobacterium avium* infections, both pulmonary and disseminated. (Salman et al, 1993).

Bacteriophages represent a rich and unique resource of anti-infectives to counter the growing world-wide problem of antibiotic resistance.This study compared the host range of lytic bacteriophages and temperate phagesbelonging to various genera, namely *Staphylococcus, E. coli* and *Salmonella,* with a range of clinical isolates using two methods: the classical agar overlay method and a newly developed MIC method. MIC was only observed with isolates that were susceptible to phage infection, which correlated with the agar overlay assay, whereas no MIC was detected with isolates that were resistant to phage infection. The simple MIC method was useful in determining phage adsorption and host range, and detecting possible prophage contamination in phage preparations. Interestingly, this method was also applicable to strain differentiation through phage susceptibility testing using a 96-well, high throughput format that proved to be easy, cost-effective, fast and reliable. (Aradhana et al, 2013).

A study has performed to evaluate in-vitro antibacterial activity of Karanj (Pongamia pinnata) and Neem (Azadirachta indica) seed oil against fourteen strains of pathogenic bacteria. Using the tube dilution technique, it was observed that 57.14 and 21.42% of the pathogens were inhibited at 500 microl/ml; 14.28 and 71.42% at 125 microl/ml; and 28.57 and 7.14% at 250 microl/ml of Karanj and Neem oils, respectively. The activity with both the oils was bactericidal and independent of temperature and energy. Most of the pathogens were killed more rapidly at 4 degrees C than 37 degrees C. The activity was mainly due to the inhibition of cell-membrane synthesis in the bacteria. (Baswa M, 2001).

The main objective of Minimum Inhibitory Concentration (MIC) testing are-

- To identify bacterial resistance pattern of a pathogen.
- To measure sensitivity of an isolate to a range of antibiotics.
- Data used to revise standard prescribing policies.
- An appropriate choice of an antibiotic that will increase chances of treatment success. and help in the fight to slow antibiotic resistance.

Chapter Three

MATERIALS & METHOD

3.1.Study design

Different Active Pharmaceutical Ingredient (API) was collected from Incepta Pharmaceutical and Asiatic Laboratory for in vitro antimicrobial susceptibility test.Various strains of *E. coli, Pseudomonas spp.and Salmonella typhi, Klebsiella, Acinetobactor,Staphylococusaureus* and *Enterococci* were collected from Pathology department of Berdem Hospital.Then the clinical isolates of these microorganisms were subcultured and MIC test was performed by measuring the minimum concentration value according to dilution method.

3.2.Period and place of the study

The duration of this study was 1 year and all the tests have performed in the microbiological

laboratory of Pharmacy department of East West University.

Table 3.1. List of Microorganism used for antimicrobial Susceptibility test

Sl	Microorganism type	Scientific name
1	**Gram-Positive bacteria**	*Staphylococcus aureus*
2	**Gram-Negative bacteria**	*Pseudomonas Spp.*
3		*Klebsiella*
4		*E.coli*
5		*Acinobactor*
6		*Salmonella typhi*

Table 3.2. List of Antibiotic standard powder Used in the Test:

Sl	API Ingredient	Name of Company	Potency
1	Levofloxacin USP	Asiatic laboratory Ltd	95.87%
2	Azithromycin	Incepta Pharmaceutical	99.99%
3	Cephradine	Asiatic laboratory Ltd	91.67%
4	Ciprofloxacin	Incepta Pharmaceutical	99.99%
5	Vancomycin HCL	Incepta Pharmaceutical	99.189%
6	Ceftriaxone	Incepta Pharmaceutical	99.99%
7	Cefuroxime Axetil	Incepta Pharmaceutical	79.51%
8	CefiximeMicronased	Incepta Pharmaceutical	99.99%

3.3.1.Name of Apparatus

1. Sterile Test tubes
2. Nutrient Agar media & nutrient broth media
3. Inoculating loop
4. Sterile forceps & cotton
5. Sample of Active Pharmaceutical Ingredient
6. Sample of microorganism
7. Sterile Petri dishes
8. Measuring cylinder
9. Micropipettes (2-20µl) & Sterile Micropipette tips
10. Bunsen burners
11. Incubator (BK 4266
12. Hot air oven (FN-500, Niive)
13. Laminar air-flow unit (ESCO, Singapore)
14. Autoclave(HIRAYAMA, Japan)

3.3.2.Name of Solvent

1. Distilled water
2. Sterile saline solution (Sodium Chloride)
3. Ethanol(95%)

3.4. Sterilization process:

Test tube, petri dishes and other glass wares were sterilized by autoclaving at a temperature of 121ºc and a pressure of 15Ib/sq. inch for 20 minutes. The blank discs were kept in a covered Petri dish and then subjected to dry heat sterilization for 1 hour at 180ºc. After completion of sterilization, both the autoclave glass wares and discs were kept in a laminar hood under UV light for 30 minutes. UV light was switched on before one hour working in laminar hood to avoid any accidental contamination.

3.5.Preparation of inoculum

The tested organisms were grown overnight at 37.5^0C in nutrient broth medium. The broth medium with the organism was dilute in such way that the medium contains about 1.5×10^8 cells/ml. This suspension was used as inoculum. The following procedure describes a method for preparing the desired inoculum by comparison with a 0.5 McFarland standard.

3.5.1.McFarland Standard: McFarland standards are suspensions of either barium sulfate or latex particles that allow visual comparison of bacterial density. These often include a Wickerham card, which is a small card containing parallel black lines. A 0.5 McFarland standard is equivalent to a bacterial containing between 1×10^8 CFU/ml of E.coli.

A.0.5 McFarland standard was prepared in Lab as describe below:

1.Add a 0.5-ml aliquot of a 0.048M $BaCl_2$(1.175% w/v $Bacl_2.2H_2O$) to 99.5mL of 0.18 M H_2SO_4 (1% v/v) with constant stirring to maintain a suspension.

2. Verify the correct density of the turbidity standard by measuring absorbance using a spectrophotometer with a 1-cm light path and matched cuvette. The absorbance at 625nm should be 0.08 to 0.13nm for the 0.5McFarland standards.

3. Transfer the barium sulfate suspension in 4 to 6 ml aliquots into screw-cap tubes of the same size as those used in standardizing the bacterial inoculums.

4. Tightly seal the tubes to prevent loss by evaporation.

5. Store in the dark at room temperature(22° to 25°C).

B.**Use of the McFarland standard in the Macro dilution Procedure**:

1. Prior to use, vigorously agitate the barium sulfate standard on a mechanical vortex mixer and inspect for a uniformly turbid appearance. Replace the standard if large particles appear. If using a standard composed of latex particles, mix by inverting gently, not on a vortex mixer.

2. After overnight broth culture or adds bacterial colonies to the broth in the "preparation of the inoculum" step of the procedure, used to compare the resulting suspension to the McFarland

standard. This is done by holding both the standard and the inoculum tube side by side and no more than 1 inch from the face of the Wickerham card (with adequate light present) and comparing the appearance of the lines through both suspensions. Do not hold the tubes flush against the card. If the bacterial suspension appears lighter than the 0.5 McFarland standards, more organisms should be added to the tube from the culture plate. If the suspension appears denser than the 0.5 McFarland standards, additional NaCl saline should be added to the inoculum tube in order to dilute the suspension to the appropriate density.

3. In order to ensure appropriate density of bacterial suspension also used spectrophotometer with a 1-cm light path and matched cuvette. The absorbance at 625 nm should be 0.08nm to 0.13nm for bacterial suspension as like to the 0.5 McFarland standards.

Figure 3.1: Mc Farland Standard

3.6.Preparation of Solution:

Use a calibrated analytical balance to weight antimicrobial agents. Allowance for the potency of the powder can be made by use of the following formula:

Weight of powder (mg) =

$$\frac{\text{Volume of solution (mL)} \times \text{Concentration (mg/L)}}{\text{Potency of powder (mg/g)}}$$

3.7.Procedure:

1.Seventeen autoclaved test tube were taken, of which fourteen were marked as 1,2,3,4,5,6,7,8,9,10,11,12,13,14 and the rest three were assigned as C_M (medium), C_S (medium+ sample) and C_I (medium+ inoculums).

2. 1 ml of sterile nutrient broth medium was taken on each test tube.

3. 1 ml of the sample solution or drug was added in first test tube and shaked well in such a way so that no bubble can not be fomed.

4.1 ml content from the first test tube was transferred to the second test tube, was mixed uniformly and again 1 ml of this mixture transferred to the third test tube. This process of serial dilution was continued up to the fourteenth test tube.

5. Then 10µl of the diluted inoculums of organism (1.5×10^6cells/ml) was added to each of the fourteen test tubes and mixed well.

6.1 ml of the sample solution was added to the control test tube, C_S and mixed well and 1 ml of this mixed content was discarded. This was done to check the clarity of the medium in presence of diluted solution of the compound.

7.10µl of the inoculums (1.5×10^6cells/ml) was added to the control test tube C_I for observing the growth of the organism used in the medium. The control test tube C_M containing medium only was used to confirm the sterility of the medium.

8. At last all the tubes were incubated at 37ºC for 12-18 hours.

The same procedure was also applied to determine the Minimum Inhibitory Concentration (MIC) against organisms.

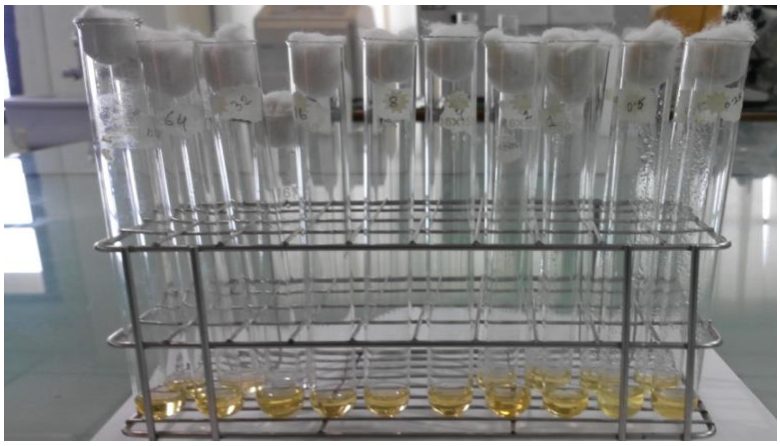

Figure 3.2: Macrodilution method

3.8.List of machines

Here are some names of machines that are used in the antimicrobial susceptibility test.

3.8.1.Analytical balance

An analytical balance is used in our research work. It is designed to measure small mass in the sub-milligram range. The measuring pan of this balance is remained inside a transparent enclosure with doors. So, it is impossible for the entry of dust into this machine. Another advantage of using this balance is that no air currents in the room can affect the balance's operation. (Vasanthakumari, 2007).

Fig 3.3.1: Analytical balance

3.8.2.Autoclave

An autoclave is a device that is used to sterilize. This machine is widely used in microbiology & also in other sector of science. They vary in size. Their function depends on the size of the load of the contents. In this machine high pressure is provided with saturated steam at 121 °C. This machine is able to neutralize potentially infectious agents by utilizing pressurized steam & superheated water. (Vasanthakumari, 2007).

Figure 3.3.2: Autoclave

3.8.3.Hot air oven

Hot air ovens are electrical devices that are greatly used in sterilization. This ovens use dry heat. This heat helps to sterilize articles. 50 to 300 °C are maintained in this machine. (Vasanthakumari, 2007).

Figure 3.3.3: Hot air oven

3.8.4.Laminar flow cabinet

A laminar flow cabinet is also known as laminar flow closet or tissue culture hood. This device is carefully enclosed bench. This device is designed to prevent contamination of semiconductor wafers, biological samples, or any particle sensitive device. In this device air is drawn through a HEPA. Here the air is blown in a very smoothly & laminar flow towards the user. The device is usually made of stainless steel. (Vasanthakumari, 2007)

Figure 3.3.4: Laminar flow cabinet

3.8.5.Incubator

This device is used to grow & maintain microbiological cultures. It maintains the optimal temperature, humidity & other conditions. In other conditions carbon dioxide & oxygen content of the atmosphere are included. In this device the temperature is maintained at 28 to 30°C for bacteria. (Vasanthakumari, 2007)

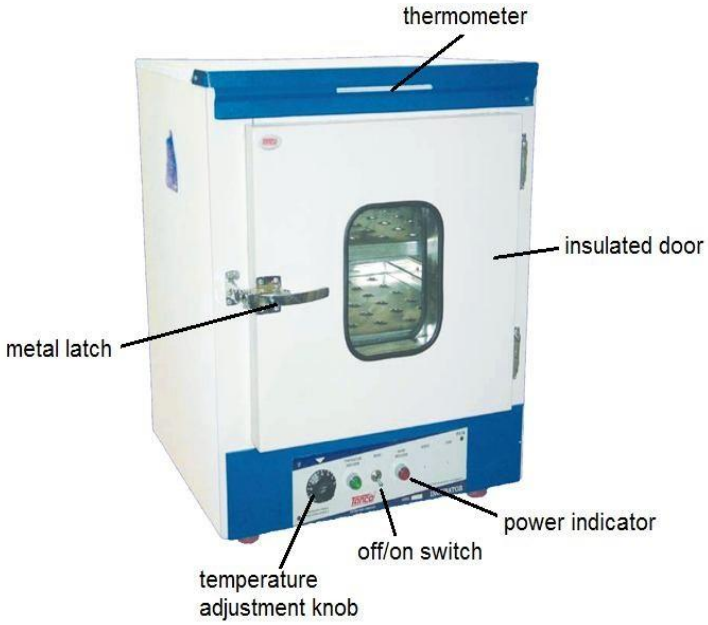

Figure 3.3.5: Incubator

Chapter Four

DISCUSSION AND RESULT

Result and Discussion

The study only carried out only on the clinical isolates to observe the sensitivity pattern on clinically isolated bacteria used conventional antibiotic standard powder. In this study, we have tried to discuss on broad spectrum antibiotics including fluroquininoles, Cephalosporins, Macrolide. Vancomycin, a glycopeptides also included in this sensitivity studies.

Many research have been done on antibiotics for the development of antibiotic resistant as many antibiotics are becoming resistance on human body. Cephalosporin group of drugs have drawn a lot of attention and as a result they are possessing a new generation of drugs. We mainly tried to cover all four generations of cephalosporins. We selected cephradin from first generation, Cefuroxime from second generation, and Ceftriaxone and cefixim from third generation. We also selected ciprofloxacin from second generation quinonoles, Levofloxacin from third generation quinonoles, and also azithromycin from macrolide group of drugs.

***Escherichia coli* Resistance pattern:**

Discussion of different strains of *Escherichia coli* resistance pattern is given below. MIC (Minimum Inhibitory Concentration) of antibiotics measured in mcg/ml or µg/ml.

Sample 1:

The *Escherichia coli* sample collected from the urine of a female patient (45 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotic standard powder has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) Quinonole group antibiotic, levofloxacin (0.5 mcg/mL) showed sensitivity against this clinical isolate; with a MIC range ≤2 mcg/mL, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI). On the other hand, Minimum Inhibitory Concentration(MIC) of ciprofloxacin was found at 4 mcg/mL which shows resistance activity according to standard (≥ 4 mcg/mL).

Cephalosporin group antibiotic, Cephradine (32 mcg/mL) showed resistance according to standard MIC value which is greater or equal (≥) to 4 mcg/mL. In addition, the Minimum Inhibitory Concentration(MIC) of cefixime was found at 2 mcg/mL which can be considered as

intermediate following reported data (2 mcg/mL). On the other hand, ceftriaxone and cefuroxime did not show any activity.

In case of vancomycin, the minimum inhibitory concentration was 32 mcg/mL but it is not clinically effective according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Macrolide group antibiotic, Minimum Inhibitory Concentration of azithromycin was 16 mcg/mL but it is clinically insignificance according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Table 4.1:Antibacterial study of *Escherichia coli* against conventional antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	0.5	≤ 2	4	≥ 8
Ciprofloxacin	4	≤ 1	2	≥ 4
Cephradine	32	≤ 2	--	≥ 4
Ceftriaxone	--	≤ 1	--	≥ 2
Cefixime	2	≤ 1	2	≥ 4
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	32	--	--	--
Azithromycin	16	--	--	--

Sample 2

The *Escherichia coli* sample collected from the urine of a female patient (31 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin (64 mcg/mL) showed resistance against this clinical isolate; with a MIC range ≥ 8 mcg/mL. On the other hand, ciprofloxacin did not show any activity against this *E.coli*.

Cephalosporin group antibiotic, cephradine, cefixime, ceftriaxone and cefuroxime did not show any activity.

In case of vancomycin, the minimum inhibitory concentration was 16 mcg/mL but it is not clinically effective according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Macrolide group antibiotic, Minimum Inhibitory Concentration of azithromycin was 16 mcg/mL but it is clinically insignificance according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Table 4.2:Antibacterial study of *Escherichia coli* against conventional antibiotics standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	64	≤ 2	4	≥ 8
Ciprofloxacin	--	≤ 1	2	≥ 4
Cephradine	--	≤ 2	--	≥ 4
Ceftriaxone	--	≤ 1	--	≥ 2
Cefixime	--	≤ 1	2	≥ 4
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	16	--	--	--
Azithromycin	16	--	--	--

Sample 3

The *Escherichia coli* sample collected from the urine of a male patient (57 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European

Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin (32 mcg/mL) showed resistance against this clinical isolate; with a MIC range ≥ 8 mcg/mL. On the other hand, ciprofloxacin did not show any activity against this *E.coli*.

Cephalosporin group antibiotic, Ceftriaxone (64 mcg/mL) showed resistance according to standard MIC value which is greater or equal (≥) to 2 mcg/mL.On the other hand, cefixime, cephradine and cefuroxime did not show any activity.

In case of vancomycin, the minimum inhibitory concentration was 64 mcg/mL but it is not clinically effective according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Macrolide group antibiotic, Minimum Inhibitory Concentration of azithromycin was 16 mcg/mL but it is clinically insignificance according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Table 4.3:Antibacterial study of *Escherichia coli* against conventional antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	32	≤ 2	4	≥ 8
Ciprofloxacin	--	≤ 1	2	≥ 4
Cephradine	--	≤ 2	--	≥ 4
Ceftriaxone	64	≤ 1	--	≥ 2
Cefixime	--	≤ 1	2	≥ 4
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	64	--	--	--
Azithromycin	16	--	--	--

Sample 4

The *Escherichia coli* sample collected from the pus of a male patient (75 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI),The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin (64 mcg/mL) showed resistance against this clinical isolate; with a MIC range ≥ 8 mcg/mL. On the other hand, ciprofloxacin did not show any activity against this *E.coli*.

Cephalosporin group antibiotic, cephradine, cefixime, ceftriaxone and cefuroxime did not show any activity.

In case of vancomycin, the minimum inhibitory concentration was 16 mcg/mL but it is not clinically effective according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Macrolide group antibiotic, Minimum Inhibitory Concentration of azithromycin was 16 mcg/mL but it is clinically insignificance according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Table 4.4:Antibacterial study of *Escherichia coli* against conventional antibiotic powder

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	64	≤ 2	4	≥ 8
Ciprofloxacin	--	≤ 1	2	≥ 4
Cephradine	--	≤ 2	--	≥ 4
Ceftriaxone	--	≤ 1	--	≥ 2
Cefixime	--	≤ 1	2	≥ 4
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	16	--	--	--
Azithromycin	16	--	--	--

Sample 5

The *Escherichia coli* sample collected from the pus of a male patient (39 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin and ciprofloxacin did not show any activity against this *E.coli*.

Cephalosporin group antibiotic, cephradine, cefixime, ceftriaxone and cefuroxime did not show any activity.

In case of vancomycin, the minimum inhibitory concentration was 8 mcg/mL but it is not clinically effective according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Macrolide group antibiotic, azithromycin did not show activity.

Table 4.5:Antibacterial study of *Escherichia coli* against conventional antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	--	≤ 2	4	≥ 8
Ciprofloxacin	--	≤ 1	2	≥ 4
Cephradine	--	≤ 2	--	≥ 4
Ceftriaxone	--	≤ 1	--	≥ 2
Cefixime	--	≤ 1	2	≥ 4
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	8	--	--	--
Azithromycin	--	--	--	--

Sample 6

The *Escherichia coli* sample collected from the urine of a female patient (27 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin did not show any activity against this *E.coli* but the Minimum Inhibitory Concentration(MIC) of ciprofloxacin was found at 2 mcg/mL which can be considered as intermediate following reported data (2 mcg/mL).

Cephalosporin group antibiotic, Ceftriaxone (32 mcg/mL) showed resistance according to standard MIC value which is greater or equal (≥) to 2 mcg/mL.On the other hand, cefixime, cephradine and cefuroxime did not show any activity.

In case of vancomycin, the minimum inhibitory concentration was 8 mcg/mL but it is not clinically effective according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Macrolide group antibiotic, Minimum Inhibitory Concentration of azithromycin was 16 mcg/mL but it is clinically insignificance according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Table 4.6:Antibacterial study of *Escherichia coli* against antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	--	≤ 2	4	≥ 8
Ciprofloxacin	2	≤ 1	2	≥ 4
Cephradine	--	≤ 2	--	≥ 4
Ceftriaxone	32	≤ 1	--	≥ 2
Cefixime	--	≤ 1	2	≥ 4
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	8	--	--	--
Azithromycin	16	--	--	--

Sample 7

The *Escherichia coli* sample collected from the urine of a female patient (57 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin (0.0625 mcg/mL) showed vey high sensitivity against this clinical isolate; with a MIC range ≤2 mcg/mL, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI). On the other hand, ciprofloxacin did not provide any activity.

Cephalosporin group antibiotic, cephradine (1 mcg/mL) showed sensitivity against this clinical isolate; with a MIC range ≤2 mcg/mL, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI). On the other hand, cefixime, ceftriaxone and cefuroxime did not show any activity.

In case of vancomycin, the minimum inhibitory concentration was 4 mcg/mL but it is not clinically effective according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Macrolide group antibiotic, Minimum Inhibitory Concentration of azithromycin was 16 mcg/mL but it is clinically insignificance according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Table 4.7:Antibacterial study of *Escherichia coli* against conventional antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	0.0625	≤ 2	4	≥ 8
Ciprofloxacin	--	≤ 1	2	≥ 4
Cephradine	1	≤ 2	--	≥ 4
Ceftriaxone	--	≤ 1	--	≥ 2
Cefixime	--	≤ 1	2	≥ 4
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	4	--	--	--
Azithromycin	16	--	--	--

Sample 8

The *Escherichia coli* sample collected from the urine of a female patient (65 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin (0.015625 mcg/mL) showed vey high sensitivity against this clinical isolate; with a MIC range ≤2 mcg/mL, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI). On the other hand, ciprofloxacin did not provide any activity.

Cephalosporin group antibiotic, Cephradine (32 mcg/mL) showed resistance according to standard MIC value which is greater or equal (≥) to 4 mcg/mL. In addition, ceftriaxone, cefixime and cefuroxime did not show any activity.

In case of vancomycin, the minimum inhibitory concentration was 16 mcg/mL but it is not clinically effective according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Macrolide group antibiotic, Minimum Inhibitory Concentration of azithromycin was 16 mcg/mL but it is clinically insignificance according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Table 4.8:Antibacterial study of *Escherichia coli* against conventional antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	0.015625	≤ 2	4	≥ 8
Ciprofloxacin	--	≤ 1	2	≥ 4
Cephradine	32	≤ 2	--	≥ 4
Ceftriaxone	--	≤ 1	--	≥ 2
Cefixime	--	≤ 1	2	≥ 4
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	16	--	--	--
Azithromycin	16	--	--	--

Sample 9

The *Escherichia coli* sample collected from the urine of a female patient (55 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. The zone of inhibition has been measured. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin did not show any activity against this *E.coli* but the Minimum Inhibitory Concentration(MIC) of ciprofloxacin was found at 16 mcg/mL which can be considered as resistance following standard data (≥ 4 mcg/mL).

Cephalosporin group antibiotic, cephradine, cefixime, ceftriaxone and cefuroxime did not show any activity.

In case of vancomycin and azithromycin, they did not show any activity.

Table 4.9:Antibacterial study of *Escherichia coli* against conventional antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	--	≤ 2	4	≥ 8
Ciprofloxacin	16	≤ 1	2	≥ 4
Cephradine	--	≤ 2	--	≥ 4
Ceftriaxone	--	≤ 1	--	≥ 2
Cefixime	--	≤ 1	2	≥ 4
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	--	--	--	--
Azithromycin	--	--	--	--

Sample 10

The *Escherichia coli* sample collected from the pus of a female patient (35 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin did not show any activity against this *E.coli* but the Minimum Inhibitory Concentration(MIC) of ciprofloxacin was found at 0.03125 mcg/mL which showed very high sensitivity be following standard data (≤ 1 mcg/mL).

Cephalosporin group antibiotic, Cephradine (64 mcg/mL) showed resistance according to standard MIC value which is greater or equal (≥) to 4 mcg/mL. In addition, ceftriaxone, cefixime and cefuroxime did not show any activity.

In case of vancomycin, the minimum inhibitory concentration was 32 mcg/mL but it is not clinically effective according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Macrolide group antibiotic, Minimum Inhibitory Concentration of azithromycin was 0.5 mcg/mL but it is clinically insignificance according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Table 4.10:Antibacterial study of *Escherichia coli* against conventional antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	--	≤ 2	4	≥ 8
Ciprofloxacin	0.03125	≤ 1	2	≥ 4
Cephradine	64	≤ 2	--	≥ 4
Ceftriaxone	--	≤ 1	--	≥ 2
Cefixime	--	≤ 1	2	≥ 4
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	32	--	--	--
Azithromycin	0.5	--	--	--

Sample 11

The *Escherichia coli* sample collected from the urine of a female patient (33 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin (8 mcg/mL) showed resistance against this clinical isolate; with a MIC range ≥ 8 mcg/mL. On the other hand, ciprofloxacin did not show any activity against this *E.coli*.

Cephalosporin group antibiotic, cephradine, cefixime, ceftriaxone and cefuroxime did not show any activity.

In case of vancomycin, the drug did not show any activity.

Macrolide group antibiotic, Minimum Inhibitory Concentration of azithromycin was 32 mcg/mL but it is clinically insignificance according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Table 4.11:Antibacterial study of *Escherichia coli* against conventional antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	8	≤ 2	4	≥ 8
Ciprofloxacin	--	≤ 1	2	≥ 4
Cephradine	--	≤ 2	--	≥ 4
Ceftriaxone	--	≤ 1	--	≥ 2
Cefixime	--	≤ 1	2	≥ 4
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	--	--	--	--
Azithromycin	32	--	--	--

Resistance pattern of *Acinetobacter* :

Discussion of strain of *Acinetobacter* resistance pattern is given below. MIC (Minimum Inhibitory Concentration) of antibiotics measured in mcg/ml or µg/ml.

Sample 12

The *Acinetobacter* sample collected from the pus of a female patient (56 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin (64 mcg/mL) showed resistance against this clinical isolate; with a MIC range ≥ 8 mcg/mL. On the other hand, ciprofloxacin did not show any activity against this *Acenobacter*.

Cephalosporin group antibiotic, cephradine, cefixime, ceftriaxone and cefuroxime did not show any activity.

In case of vancomycin and azithromycin, they did not show any activity.

Table 4.12:Antibacterial study of *Acinenobacter* against conventional antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	64	≤ 2	4	≥ 8
Ciprofloxacin	--	≤ 1	2	≥ 4
Cephradine	--	--	--	--
Ceftriaxone	--	≤ 8	16-32	≥ 64
Cefixime	--	--	--	--
Cefuroxime	--	--	--	--
Vancomycin	--	--	--	--
Azithromycin	--	--	--	--

***Staphylococcus aureus* Resistance pattern:**

Discussion of *Staphylococcus aureus* resistance pattern is given below. MIC (Minimum Inhibitory Concentration) of antibiotics measured in mcg/ml or µg/ml.

Sample 13

The *Staphylococcus aureus* sample collected from the pus of a male patient (37 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin and ciprofloxacin did not show any activity against this *staphylococcus aureus.*

Cephalosporin group antibiotic, Cephradine (0.125 mcg/mL) showed sensitivity according to standard MIC value which is less or equal (≤) to 2 mcg/mL. Ceftriaxone (4 mcg/mL) showed sensitivity according to standard MIC value which is less or equal (≤) to 8 mcg/mL. On the other hand, cefixime and cefuroxime did not show any activity.

In case of vancomycin, the minimum inhibitory concentration was 8 mcg/mL which can be considered as intermediate according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Macrolide group antibiotic, Minimum Inhibitory Concentration of azithromycin was 0.5 mcg/mL which showed sensitivity with standard MIC range (≤ 2 mcg/mL) according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Table 4.13:Antibacterial study of *Staphylococcus aureus* against conventional antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	--	≤ 1	2	≥ 4
Ciprofloxacin	--	≤ 1	2	≥ 4
Cephradine	0.125	≤ 2	--	≥ 4
Ceftriaxone	4	≤ 8	16-32	≥ 64
Cefixime	--	≤ 1	--	≥ 2
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	8	≤ 2	4-8	≥ 16
Azithromycin	0.5	≤ 2	4	≥ 8

Sample 14

The *Staphylococcus aureus* sample collected from the pus of a male patient (70 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The

European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin (16 mcg/mL) showed resistance against this clinical isolate; with a MIC range ≥ 4 mcg/mL. On the other hand, ciprofloxacin did not show any activity against this *Staphylococcus aureus*.

Cephalosporin group antibiotic, cephradine, cefixime, ceftriaxone and cefuroxime did not show any activity.

In case of vancomycin, the drug did not show any activity.

Macrolide group antibiotic, Minimum Inhibitory Concentration of azithromycin was 16 mcg/mL which showed resistance with standard MIC range (≥ 8 mcg/mL) according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Table 4.14:Antibacterial study of *Staphylococcus aureus* against conventional antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	16	≤ 1	2	≥ 4
Ciprofloxacin	--	≤ 1	2	≥ 4
Cephradine	--	≤ 2	--	≥ 4
Ceftriaxone	--	≤ 8	16-32	≥ 64
Cefixime	--	≤ 1	--	≥ 2
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	--	≤ 2	4-8	≥ 16
Azithromycin	16	≤ 2	4	≥ 8

Resistance pattern of *Klebsiella*:

Discussion of *Klebsiella* resistance pattern is given below. MIC (Minimum Inhibitory Concentration) of antibiotics measured in mcg/ml or µg/ml.

Sample 15

The *Klebsiella* sample collected from the urine of a female patient (44 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin (0.0625 mcg/mL) showed sensitivity against this clinical isolate; with a MIC range ≤2 mcg/mL, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI). On the other hand, Minimum Inhibitory Concentration(MIC) of ciprofloxacin was found at 0.5 mcg/mL which shows sensitivity according to standard (≤ 1 mcg/mL).

Cephalosporin group antibiotic, Cephradine did not show any activity. In addition, ceftriaxone (0.5 mcg/mL), cefixime (1 mcg/mL) and cefuroxime (1 mcg/mL) showed sensitivity according to standard MIC value which are ≤ 1 mcg/mL, ≤ 1 mcg/mL and ≤ 4 mcg/mL respectively.

In case of vancomycin, the drug did not show any activity.

Macrolide group antibiotic, Minimum Inhibitory Concentration of azithromycin was 0.25 mcg/mL but it is clinically insignificance according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Table 4.15:Antibacterial study of *Klebsiella sp.* against conventional antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	**Intermediate**	**Resistance**
Levofloxacin	0.0625	≤ 2	4	≥ 8
Ciprofloxacin	0.5	≤ 1	2	≥ 4
Cephradine	--	≤ 2	--	≥ 4
Ceftriaxone	0.5	≤ 1	--	≥ 2
Cefixime	1	≤ 1	2	≥ 4
Cefuroxime	1	≤ 4	8-16	≥ 32
Vancomycin	--	--	--	--
Azithromycin	0.25	--	--	--

Sample 16

The *Klebsiella* sample collected from the pus of a male patient (46 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin and ciprofloxacin did not show any activity against this *Klebsiella.*

Cephalosporin group antibiotic, cephradine, cefixime, ceftriaxone and cefuroxime did not show any activity.

In case of vancomycin and azithromycin, the drugs did not show any activity.

Table 4.16:Antibacterial study of *Klebsiella* against conventional antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	--	≤ 2	4	≥ 8
Ciprofloxacin	--	≤ 1	2	≥ 4
Cephradine	--	≤ 2	--	≥ 4
Ceftriaxone	--	≤ 1	--	≥ 2
Cefixime	--	≤ 1	2	≥ 4
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	--	--	--	--
Azithromycin	--	--	--	--

Sample 17

The *Klebsiella* sample collected from the urine of a female patient (37 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin (0.125 mcg/mL) showed sensitivity against this clinical isolate; with a MIC range ≤2 mcg/mL, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI). On the other hand, Minimum Inhibitory Concentration(MIC) of ciprofloxacin was found at 1 mcg/mL which shows sensitivity according to standard (≤ 1 mcg/mL).

Cephalosporin group antibiotic, Ceftriaxone (0.0625 mcg/mL) showed resistance according to standard MIC value which is less or equal (≤) to 1 mcg/mL. In addition, cephradine, cefixime and cefuroxime did not show any activity.

In case of vancomycin, the drug did not show any activity.

Macrolide group antibiotic, Minimum Inhibitory Concentration of azithromycin was 0.25 mcg/mL but it is clinically insignificance according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Table 4.17:Antibacterial study of *Klebsiella* against conventional antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	0.125	≤ 2	4	≥ 8
Ciprofloxacin	1	≤ 1	2	≥ 4
Cephradine	--	≤ 2	--	≥ 4
Ceftriaxone	0.0625	≤ 1	--	≥ 2
Cefixime	--	≤ 1	2	≥ 4
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	--	--	--	--
Azithromycin	0.25	--	--	--

Sample 18

The *Klebsiella* sample collected from the urine of a male patient (48 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin and ciprofloxacin did not show any activity against this *Klebsiella.*

Cephalosporin group antibiotic, cephradine, cefixime, ceftriaxone and cefuroxime did not show any activity.

In case of vancomycin, the drugs did not show any activity.

Macrolide group antibiotic, Minimum Inhibitory Concentration of azithromycin was 16 mcg/mL but it is clinically insignificance according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Table 4.18:Antibacterial study of *Klebsiella* against conventional antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	--	≤ 2	4	≥ 8
Ciprofloxacin	--	≤ 1	2	≥ 4
Cephradine	--	≤ 2	--	≥ 4
Ceftriaxone	--	≤ 1	--	≥ 2
Cefixime	--	≤ 1	2	≥ 4
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	--	--	--	--
Azithromycin	16	--	--	--

Sample 19

The *Klebsiella* sample collected from the urine of a female patient (45 years old). The sample was subjected to sensitivity test against various conventional antibiotic standard powder. Minimum Inhibitory Concentration (MIC) of these antibiotics has been determined and the value compared with standard MIC value following Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and DIN (German Institute for Standardization).

Quinonole group antibiotic, levofloxacin (64 mcg/mL) showed resistance against this clinical isolate; with a MIC range ≥ 8 mcg/mL, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI). On the other hand, Minimum Inhibitory Concentration(MIC) of ciprofloxacin was found at 128 mcg/mL which shows resistance activity according to standard (≥ 4 mcg/mL).

Cephalosporin group antibiotic, Cephradine (64 mcg/mL) showed resistance according to standard MIC value which is greater or equal (≥) to 4 mcg/mL.On the other hand, cefixime, ceftriaxone and cefuroxime did not show any activity.

In case of vancomycin, the drugs did not show any activity.

Macrolide group antibiotic, Minimum Inhibitory Concentration of azithromycin was 32 mcg/mL but it is clinically insignificance according to Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Table 4.19:Antibacterial study of *Klebsiella* against conventional antibiotic standard powder-

Name of Antibiotic	Sample MIC Value (mcg/mL)	Standard MIC Range (mcg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	64	≤ 2	4	≥ 8
Ciprofloxacin	128	≤ 1	2	≥ 4
Cephradine	64	≤ 2	--	≥ 4
Ceftriaxone	--	≤ 1	--	≥ 2
Cefixime	--	≤ 1	2	≥ 4
Cefuroxime	--	≤ 4	8-16	≥ 32
Vancomycin	--	--	--	--
Azithromycin	32	--	--	--

Chapter Five

CONCLUSION

Conclusion

Antibiotic resistance is an emergence issue in Bangladesh. I have worked on 19 different samples of different strains of pathogenic bacteria. From my research work, I found that pathogenic bacteria have both sensitivity and resistant effect. Here, 11 strains of *E.coli* showed resistant activity against antibiotic standard powder but some strains gives sensitivity MIC values that are Levofloxacin (0.5 mcg/mL, 0.0625 mcg/mL, 0.015625 mcg/mL) for sample 1, sample 7, sample 8, Ciprofloxacin (0.03125 mcg/mL) for sample 10, Cephradine (1 mcg/mL) for sample 7, Azithromycin (0.5 mcg/mL), For the two *Staphylococcus aureus* spp., the sensitivity MIC values are Cephradine (0.125 mcg/mL) for sample 13, Ceftriaxone (4 mcg/mL) for sample 13, Azithromycin (0.5 mcg/mL) for sample 13. In addition, the sensitivity MIC values for the five *Klebsiella* spp. : Levofloxacin (0.0625 mcg/mL, 0.125 mcg/mL) for sample 15 and sample 17, Ciprofloxacin (0.5 mcg/mL, 1 mcg/mL) for sample 15 and sample 17, Cefuroxime (1 mcg/mL) for sample 15, Ceftriaxone (0.5 mcg/mL, 0.0625 mcg/mL) for sample 15 and sample 17, Cefixime (1 mcg/mL) for sample 15, Azithromycin (0.25 mcg/mL) for both sample 15 and sample 17. On the other hand, *Klebsiella* spp. Sample 16 showed resistance against all antibiotics.

From this study, it can be concluded that fluroquinonoles group drugs (ciprofloxacin and levofloxacin) and cephalosporin group drug (cephradine, cefixime, cefuroxime, ceftriaxone) showed sensitivity and resistant activity against both gram positive and gram negative bacteria. In addition, Vancomycin gave resistant activity against gram positive *Staphylococcus aureus* species bacteria. Macrolide group antibiotic, azithromycin showed susceptibility effect against *Staphylococcus aureu*s species.

New resistance mechanisms emerge and spread globally threating our ability to treat common infectious diseases, resulting in death and disability of individuals who until recently could continue a normal curse of life.

Chapter Six

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