In Vitro Minimum Inhibitory Concentration (MIC) of Antibiotics Standard Powder Against Different Clinically Isolated Pathogenic Bacteria & Resistance Pattern

Submitted By

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

DEDICATION

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

Declaration by the Research Candidate

I, **Sharmin Ara Chowdhury** hereby declare that the dissertation entitled "**In Vitro Minimum Inhibitory Concentration (MIC) of Antibiotics Standard Powder Against Different Clinically Isolated Pathogenic Bacteria & Resistance Pattern**" submitted by me to the Department of Pharmacy, East West University and in the partial fulfilment of the requirement for the award of the degree Masters of Pharmacy (M.Pharm) is a record of original research work carried out by me during 2016, under the supervision and guidance of **Dr. Shamsun Nahar Khan,** Chairperson & Associate Professor, 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.

Date: 09.02.2016

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Certificate by the Supervisor

This is to certify that the dissertation entitled "**In Vitro Minimum Inhibitory Concentration (MIC) of Antibiotics Standard Powder Against Different Clinically Isolated Pathogenic Bacteria & Resistance Pattern** " submitted to the department of pharmacy, East West University in partial fulfilment of the requirements for the degree of Masters of Pharmacy (M.Pharm) was carried out by **Sharmin Ara Chowdhury** (2013-3-79-032) under my 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

Supervisor,

Chairperson & Associate Professor,

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East West University, Dhaka.

Certificate by the Chairperson

This is to certify that the thesis entitled "**In Vitro Minimum Inhibitory Concentration (MIC) of Antibiotics Standard Powder Against Different Clinically Isolated Pathogenic Bacteria & Resistance Pattern**" submitted to the Department of Pharmacy, East West University for the partial fulfilment of the requirement for the award of the degree Masters of Pharmacy (M.Pharm) is a record of original and genuine research work carried out by **Sharmin Ara Chowdhury** during 2016 of his research in the Department of Pharmacy, East West University.

Dr. Shamsun Nahar Khan

Chairperson and Associate Professor,

Department of Pharmacy,

East West University.

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Abstract

The conventional broth macrodilution method to test minimum inhibitory concentration (MIC) of antibiotics standard powder against Different Clinically Isolated Pathogenic Bacteria.This study was employed to evaluate the microbial resistance pattern of antibiotics standard powder were Cepharadine, Cefuroxime, Ceftriaxone, Cefixim, Azithromycin, Ciprofloxacin, Levofloxacin & Vancomycin against 6 types clinically isolates 23 strain of pathogenic bacteria (*Staphylococcus aureus, Salmonella typhi, Klebsiella spp., Pseudomonas spp., Escherichia coli & Acinetobactor spp.).* Where antibiotic standard powder was used in various concentrations (in serial dilution from 0.015625-128µg/ml) and 10µl suspension of test organisms (containing 1.5×10^6 cells per ml) were taken in the test tubes and mixed well. Three control test tubes C_S, C_M and C_I were used to perform control test .Then the test tubes were incubated at 37.5ºc for 18-20 hrs. The result was measured by observing minimum inhibitory concentration (MIC) value. Here, *E.coli* has total 9 strains and gives resistant activity against antibiotics standard powder but majority of the strains sample gives highest sensitivity MIC values that are Levofloxacin (0.015625µg/ml) for sample 9, Ciprofloxacin (0.03125µg/ml) for sample 1, Cepharadin (0.5µg/ml) for sample 2, Ceftriaxone (0.0625µg/ml) for sample 5 & Azithromycin (0.0625µg/ml) for sample 7. *E.coli* sample 4 is resistant against all antibiotics. Here, *Klebsiella spp.* total 4 strains sample some gives highest sensitivity MIC values that are Levofloxacin (0.0625µg/ml) for sample 10, Ciprofloxacin (0.25µg/ml) for sample 10, Cepharadin (2µg/ml) for sample 10 & Azithromycin (0.0625µg/ml) for sample 13. *Staphylococcus aureus* total 2 strains some gives highest sensitivity MIC values that are Levofloxacin (0.0625µg/ml), Cepharadine (0.125µg/ml), Cefuroxime (0.5µg/ml), Ceftriaxone (0.5µg/ml) & Azithromycin (0.0625 µg/ml) for sample 14. *Acinetobector spp*. has 1 strain gives a highest sensitivity MIC values that are Levofloxacin (0.5µg/ml) & Ceftriaxone (4µg/ml) for sample 16. *Pseudomonas spp*. total 3 strains some gives highest sensitivity MIC values that are Levofloxacin (1µg/ml) for sample 19, Ciprofloxacin (0.03125µg/ml) for sample 17 & Azithromycin (1µg/ml) for sample 17. *Pseudomonas spp*. sample 18 is resistant against all antibiotics. *Salmonella typhy* total 4 strains some gives highest sensitivity MIC values that are Levofloxacin (0.0625µg/ml) for sample 21, Ciprofloxacin (0.125µg/ml) for sample 20 & 22, Cepharadin (0.25µg/ml) for sample 20 & 22, Ceftriaxone (1µg/ml) for sample 21 & Azithromycin (0.25µg/ml) for sample 23.

Key word: Minimum inhibitory concentration, Macrodilution, Antibiotic standard powder & pathogenic bacteria.

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Chapter-1

Introduction

Introduction

Most bacteria are harmless, but harmful bacteria, also known as pathogenic bacteria, can cause incredible damage to a person's body, including death. Intracellular bacteria are pathogenic bacteria which always cause disease when they enter the human body, in contrast with conditional bacteria, which can cause infections and disease in certain circumstances. Some notable pathogenic bacteria include *Streptococcus, Staphylococcus, Tuberculosis,* and *Escherichia coli,* among many others. Worldwide, these bacteria account for many illnesses and disease epidemics.

Antibiotic resistance occurs when an antibiotic has lost its ability to effectively control or kill bacterial growth; in other words, the bacteria are "resistant" and continue to multiply in the presence of therapeutic levels of an antibiotic.

Multiple-drug resistance occurs when bacteria are resistant to more than one antibiotic. Because of years of antibiotic overuse, multidrug resistance is now the rule rather than the exception among resistant bacteria. This situation has largely occurred through the sequential use of multiple different antibiotics.

Antibiotics, which are natural substances produced by certain groups of microorganisms, and chemotherapeutic agents, which are chemically synthesized. A hybrid substance is a semisynthetic antibiotic, where in a molecular version produced by the microbe is subsequently modified and to achieve desired properties. Furthermore, some antimicrobial compounds, originally discovered as products of microorganisms, can be synthesized entirely by chemical means. In the medical and pharmaceutical worlds, all these antimicrobial agents used in the treatment of disease are referred to as antibiotics, interpreting the word literally.

Antibacterial are used to treat bacterial infections. The toxicity to humans and other animals from antibacterial is generally considered low. However, prolonged use of certain antibacterial can decrease the number of gut flora, which may have a negative impact on health. The discovery, development and clinical use of antibacterial during the 20th century has substantially reduced mortality from bacterial infections.

Resistance occurs when an antibiotic is no longer effective at killing or limiting the growth of bacteria. It can occur naturally (innate ability or genetic mutation), or can be acquired through previous exposure to an antibiotic or through contact with another organism that is resistant (transfer of resistance).

There are several ways that bacteria can resist the effects of antibiotics. Some bacteria develop the ability to neutralize the antibiotic before it can harm them, others can change the antibiotic attack site so it cannot affect the function of the bacteria, and still others can pump the antibiotic out of the cell or prevent the antibiotic from getting into the cell.

Once bacteria are resistant, the infections they cause may not be cured or controlled by antibiotic treatment, or there may be few effective drug choices. In some cases, these illnesses can lead to disability or even death.

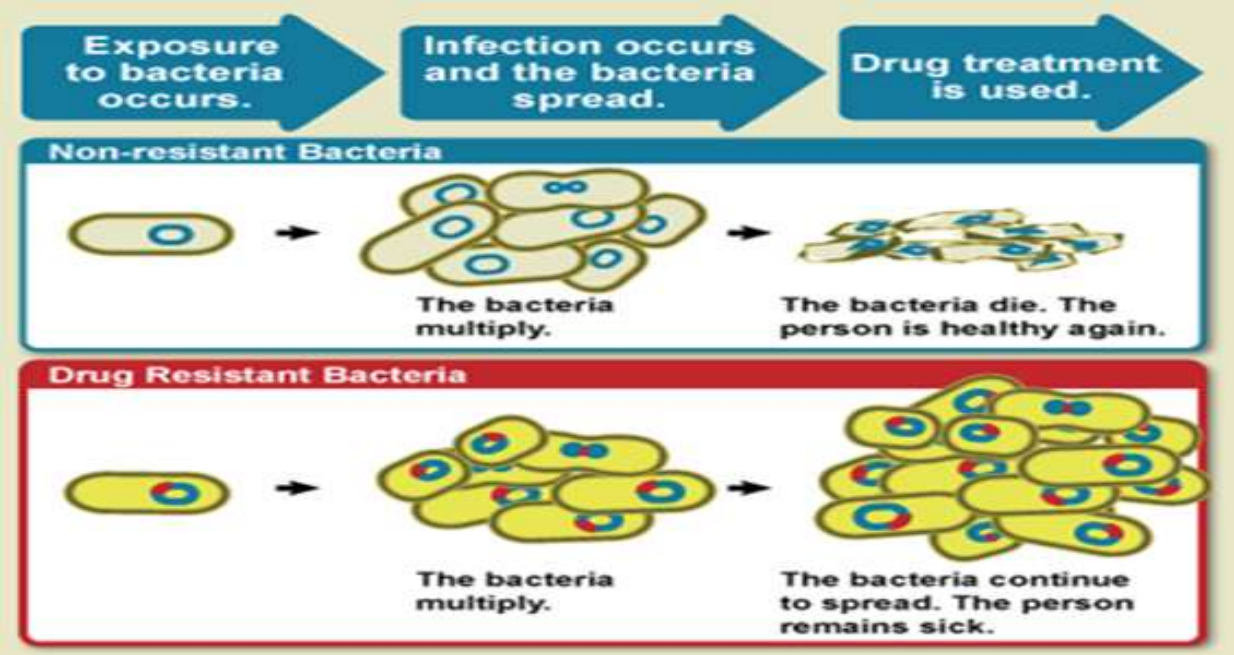

Fig 1.01: on- resistance bacteria and Drug resistance bacteria

1.1: Bacteria: Bacteria are the oldest and most abundant organisms on earth. Bacteria, or prokaryotes, differ from eukaryotes in a wide variety of characteristics, a degree of difference as great as any that separates any groups of organisms.

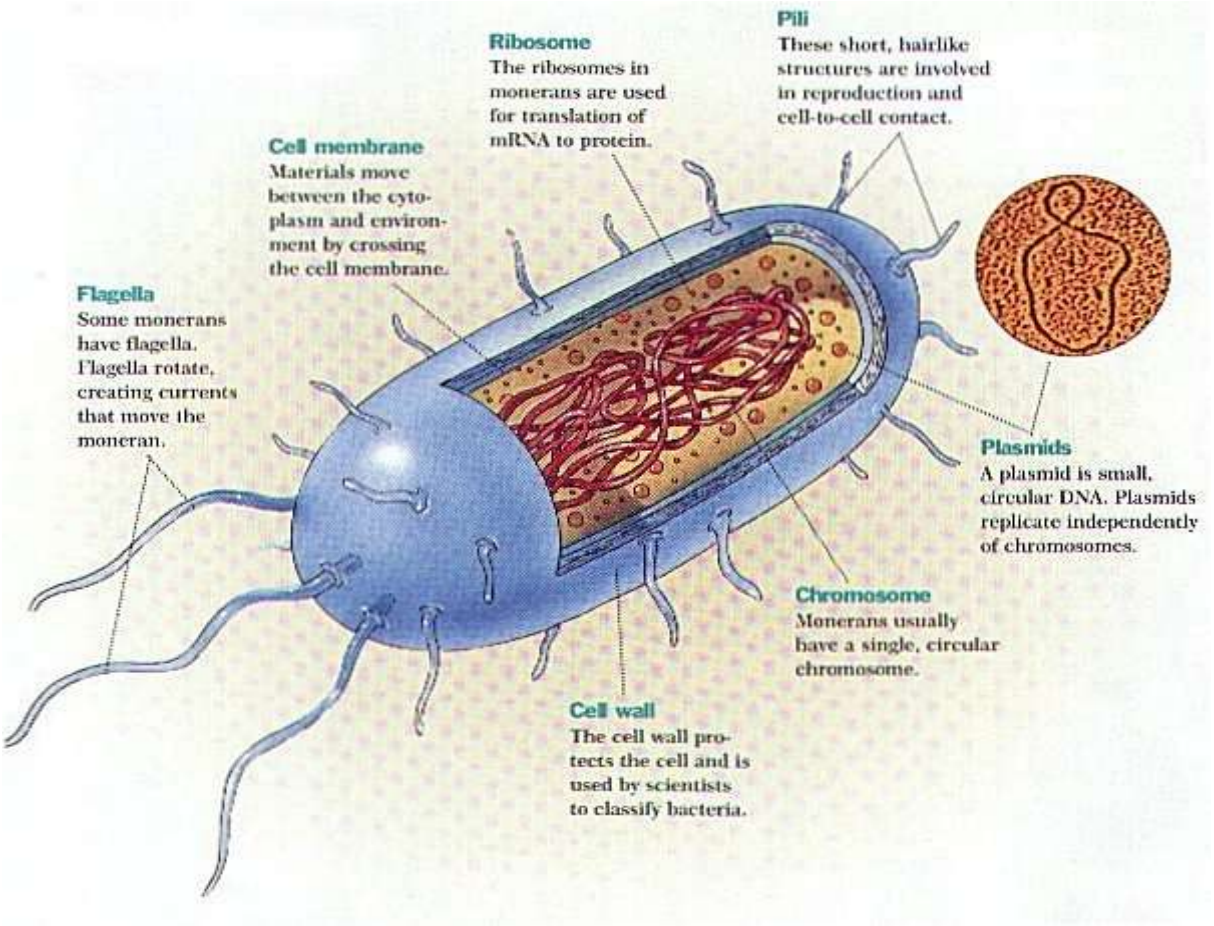

Fig.1.02: Bacteria

1.1.2: Gram staining: Gram staining, also called Gram's Method, is a method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative). The name comes from the Danish bacteriologist Hans Christian Gram, who developed the technique.

1.1.3: Gram-positive: Gram-positive bacteria retain the color of the crystal violet stain in the Gram stain. This is characteristic of bacteria that have a cell wall composed of a thick layer of a particular substance (called peptidoglycan).

The Gram-positive bacteria include *staphylococci, Actinomyces, Bacillus, Streptomyces, Lactobacillus, Clostridium, Corynebacterium, Enterococcus, Mycobacterium,* and *Mycoplasma.*

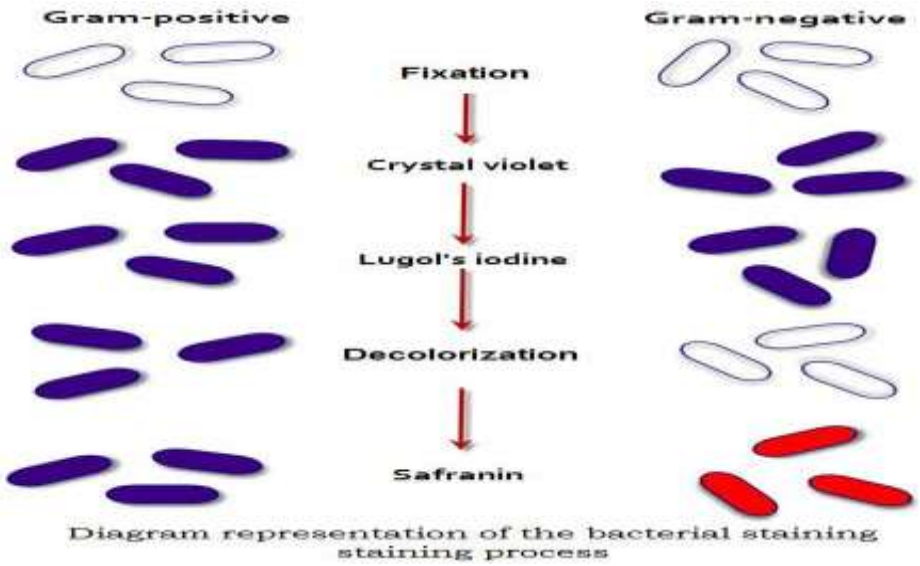

Fig 1.03: Gram staining

1.1.4: Gram-negative: Gram-negative bacteria lose the crystal violet stain (and take the color of the red counterstain) in Gram's Method of staining. This is characteristic of bacteria that have a cell wall composed of a thin layer of a particular substance (called peptidoglycan).

The Gram-negative bacteria include *Thiobacter, Vibrio, Salmonella, Shigella, Rickettsia, Klebsiella, Neisseria, Pseudomonas,* and *E.coli* [Levinson, 2006].

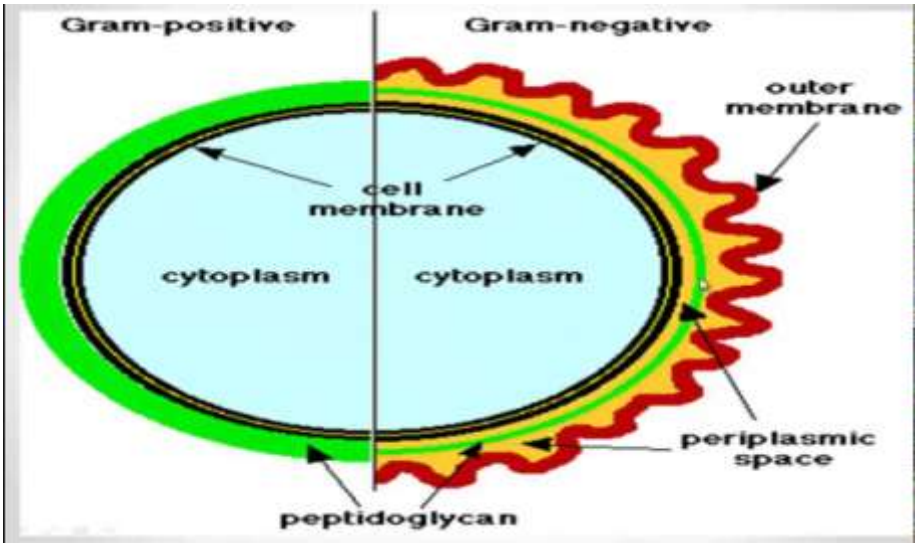

Fig 1.04: Gram positive and Gram negative bacteria

1.1.5: Mechanism of Resistance bacteria: Some bacteria are naturally resistant to certain types of antibiotics. However, bacteria may also become resistant in two ways: 1) by a genetic mutation or 2) by acquiring resistance from another bacterium.

Mutations, rare spontaneous changes of the bacteria's genetic material, are thought to occur in about one in one million to one in ten million cells. Different genetic mutations yield different types of resistance. Some mutations enable the bacteria to produce potent chemicals (enzymes) that inactivate antibiotics, while other mutations eliminate the cell target that the antibiotic attacks. Still others close up the entry ports that allow antibiotics into the cell, and others manufacture pumping mechanisms that export the antibiotic back outside so it never reaches its target.

Bacteria can acquire antibiotic resistance genes from other bacteria in several ways. By undergoing a simple mating process called "conjugation," bacteria can transfer genetic material, including genes encoding resistance to antibiotics (found on plasmids and transposons) from one bacterium to another. Viruses are another mechanism for passing resistance traits between bacteria. The resistance traits from one bacterium are packaged into the head portion of the virus. The virus then injects the resistance traits into any new bacteria it attacks. Bacteria also have the ability to acquire naked, "free" DNA from their environment.

Any bacteria that acquire resistance genes, whether by spontaneous mutation or genetic exchange with other bacteria, have the ability to resist one or more antibiotics. Because bacteria can collect multiple resistance traits over time, they can become resistant to many different families of antibiotics [Davies, 2010].

1.1.6: Antibiotic resistance spread: Genetically, antibiotic resistance spreads through bacteria populations both "vertically," when new generations inherit antibiotic resistance genes, and "horizontally," when bacteria share or exchange sections of genetic material with other bacteria. Horizontal gene transfer can even occur between different bacterial species. Environmentally, antibiotic resistance spreads as bacteria themselves move from place to place; bacteria can travel via airplane, water and wind. People can pass the resistant bacteria to others; for example, by coughing or contact with unwashed hands.

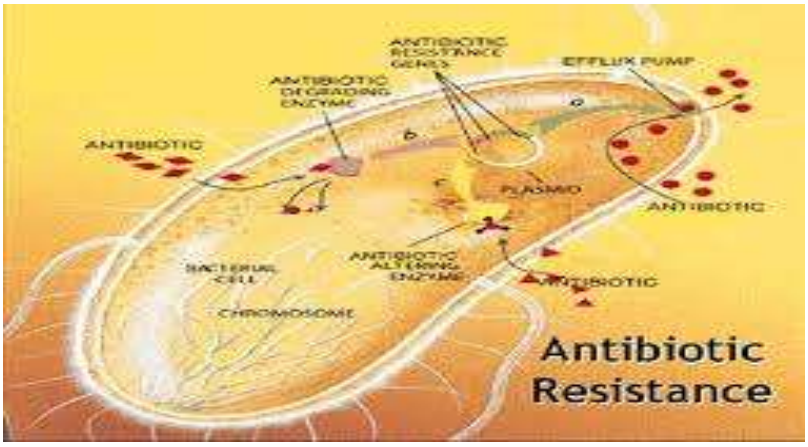

Fig 1.05: Antibiotic Resistance

1.2 Bacteria description:

1.2.1. *Pseudomonas spp.*: The genus *Pseudomonas*, of the *Pseudomonadaceae* family, are motile gram-negative aerobic bacteria, 2 – 4 μm long plump-shaped rods, with polar flagella which have an important role in pathogenicity They are non-spore forming and can produce pigments, such as pyocyanine (green-blue) and pyorubrin (yellow-green) fluorescence.

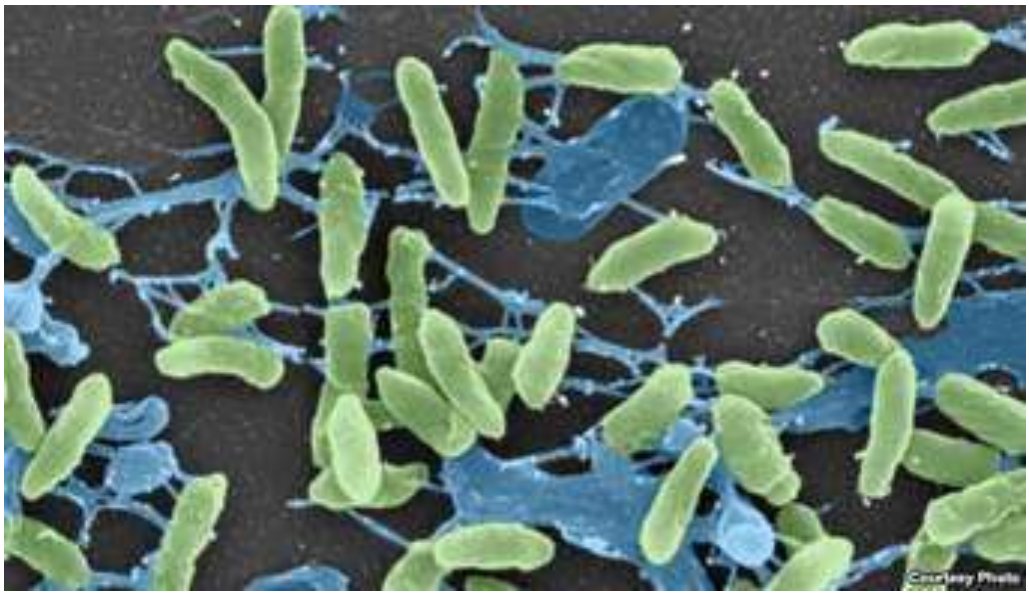

Fig.1.06: *Pseudomonas spp.*

1.2.2. Scientific classification of *Pseudomonas spp.*

Domain:	**Bacteria**
Phylum:	**Proteobacteria**
Class:	**Gammaproteobacteria**
Order :	**Pseudomonadales**
Family:	**Pseudomonadaceae**
Genus:	***Pseudomonas***
Species:	***P. aeruginosa group***
	P. chlororaphis group
	P. pertucinogena group
	P. putida group
	P. stutzeri group
	P. syringae group

1.2.3. Pathogenicity of *Pseudomonas spp.:* As opportunistic pathogens, *Pseudomonas* spp. often invades the host tissue and cause infection and bacteremia in immunocompromised hosts (e.g., HIV/AIDS, cystic fibrosis, bronchiectasis, and severe chronic obstructive pulmonary disease, burns, malignancy, or diabetes mellitus) . The common site of infection is the lower respiratory tract, and severity ranges from colonization without immunological response to severe necrotizing bronchopneumonia; such severe infection in patients with cystic fibrosis is almost impossible to eradicate once established in the airways. Pseudomonal pneumonia often develops from oro-pharyngeal contamination or secondary bacteremia, and is also a common cause of nosocomial ventilator-related pneumonia in intensive care settings. Infections also include endocarditis, osteomyelitis, urinary tract infections, gastrointestinal infections, meningitis, and, commonly, septicaemia. *P. aeruginosa* is the most common agent associated with infection and inflammation during contact lens wear. The bacteria colonize on lenses and produce proteases to kill or invade corneal cells, an infection that can lead to scarring and vision loss. The species is also the most virulent with a mortality rate of 30%, which can be higher depending on predisposing conditions. *P. aeruginosa* can also readily colonize on open burn

wounds, causing infections, abscesses, and sepsis, with edema and/or discoloration of unburned skin at wound margins and green pigment in subcutaneous fat. *P. aeruginosa* is also associated with swimmer's ear (otitis externa). Other *Pseudomonas* species are also opportunistic; however, cases of infection are rare.

1.2.4. *Acinetobacter spp.*: *Acinetobacter* are strictly aerobic, non-fermentative, Gram-negative bacilli. They show preponderantly coccobacillary morphology on nonselective agar. Rods predominate in fluid media, especially during early growth [Berezine and Towner, 1996].

Fig.1.07: *Acinetobacter spp.*

1.2.5. Scientific classification of *Acinetobacter spp.*:

Scientific classification	
Kingdom:	**Bacteria**
Phylum:	**Proteobacteria**
Class:	**Gammaproteobacteria**
Order:	**Pseudomonadales**
Family:	**Moraxellaceae**
Genus:	***Acinetobacter***
Species:	***A.ureae*** ***A.hominis***

1.2.6 Pathogenicity *of Acinetobacter spp.*: *A. ureae* and *A. hominis* have primarily been found in the sputum and tracheal secretions in patients with chronic respiratory tract diseases or pneumonia, although systemic infections have been reported. It is assumed that they also colonize the respiratory tract of healthy individuals. *A. equuli* and *A. suis* cause a variety of diseases in horses and pigs and human infection are mostly due to horse or pig bites. Both species have also been isolated from the human upper respiratory tract. *A. pleuropneumoniae* causes porcine pleuropneumonia, a highly contagious and often fatal respiratory disease of major economic importance to the pig industry. The disease, which occurs in pigs of all ages, is characterized by necrotizing, haemorrhagic bronchopneumonia and serofibrinous pleuritis.

1.2.7: *Escherichia coli:* *E. coli* is a Gram-negative, facultative anaerobic (that makes ATP by aerobic respiration if oxygen is present, but is capable of switching to fermentation or anaerobic respiration if oxygen is absent) and nonsporulating bacterium. E. coli stains Gram-negative because its cell wall is composed of a thin peptidoglycan layer and an outer membrane. During the staining process, E. coli picks up the color of the counterstain safranin and stains pink. The outer membrane surrounding the cell wall provides barriers to certain antibiotics such that E. coli is not damaged by penicillin. A strain that possesses flagella is motile. The flagella have a peritrichous arrangement [*Darnton*, 2007].

1.2.8. Scientific classification of *Escherichia coli*

Scientific classification	
Domain:	Bacteria
Phylum:	Proteoba cteria
Class:	Gammaproteobacteria
Family:	Enterobacteriaceae
Genus:	*Escherichia*
Species:	***E. coli***

Fig.1.08:*E.coli*

1.2.9. Pathogenicity of *Escherichia coli*: Patients with ETEC enteritis usually have an abrupt onset of watery diarrhea that does not contain blood, pus, or mucus (no dysenteric). The diarrhea is usually mild to moderate in severity, but some patients may have severe fluid loss. Low-grade fever, nausea, and abdominal pain may also be present. Dehydration may become severe or life threatening in neonates and children, necessitating aggressive fluid and electrolyte replacement. A self-limited course, with resolution in 2-5 days, is most common in adult travelers who acquire the disease though some strains of the organism may produce a disease lasting much longer, with a median duration of illness of 7 days.There are an estimated 800,000 deaths each year due to ETEC.

1.2.10. *Salmonella typhy*: *Salmonella i*s a genus of rod-shaped (*bacillus)* Gram-negative bacteria of the Enterobacteriaceae family. The two species of *Salmonella* are *Salmonella bongori* and *Salmonella enterica.* The full designation for *Salmonella* Typhi is *Salmonella enterica* subsp. *enterica*, serotype Typhi [David and Pollack, 2003].

1.2.11. Scientific classification of *Salmonella typhy*:

Scientific classification	
Super kingdom:	Bacteria
Kingdom:	Bacteria
Phylum:	Proteobacteria
Class:	Gammaproteobacteria
Order:	Enterobacteriales
Family:	Enterobacteriaceae
Genus:	***Salmonella***
Species	
S. bongori	
S. enterica	

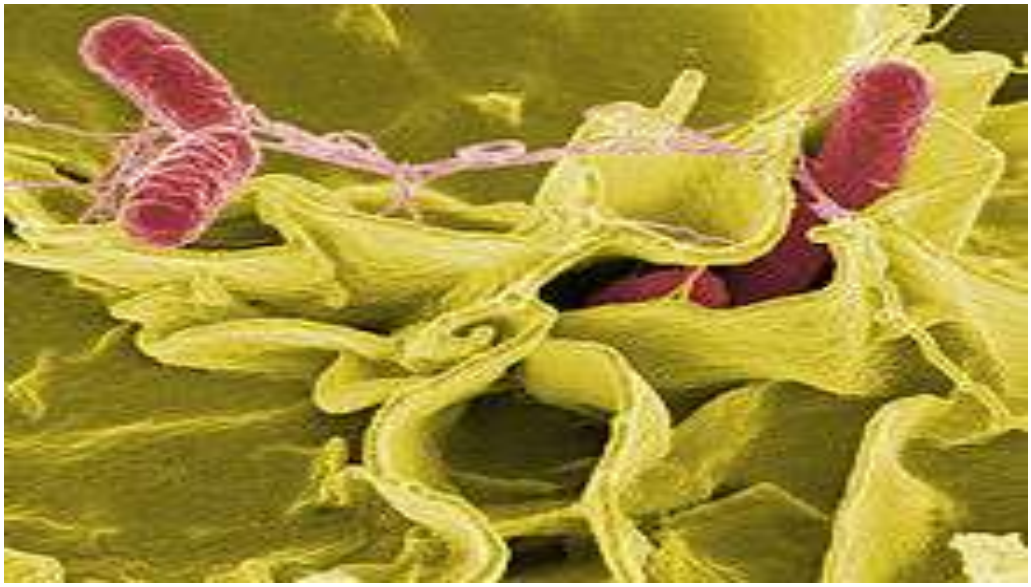

Fig1.09*: Salmonella typhy*

1.2.12. Pathogenicity of *Salmonella typhy*: *Salmonella enterica* can cause four different clinical manifestations: gastroenteritis, bacteremia, enteric fever, and an asymptomatic carrier state. It is more common in children under the age of 5, adults 20-30 year olds, and patients 70 years or older.

1.2.13. *Staphylococcus aureus:* *Staphylococcus aureus* is a gram-positive coccal bacterium that is a member of the Firmicutes, and is frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis, and food poisoning.

1.2.14. Scientific classification of *Staphylococcus aureus*:

Scientific classification	
Domain:	Bacteria
Kingdom:	Eubacteria
Phylum:	Firmicutes
Class:	Coccus
Order:	Bacillales
Family:	Staphylococcaceae
Genus:	*Staphylococcus*
Species:	***S. aureus***

Fig1.10: *Stapylococcus aureus*

1.2.15. Pathogenicity of *Staphylococcus aureus*: *Staphylococcus aureus* is an opportunistic pathogen that can cause a variety of self-limiting to life-threatening diseases in humans. The bacteria are a leading cause of food poisoning, resulting from the consumption of food contaminated with enterotoxins. Staphylococcal food intoxication involves rapid onset of nausea, vomiting, abdominal pain, cramps, and diarrhea. Symptoms usually resolve after 24 hours. Animal bites can result in local infections, cellulitis, erythema, tenderness, mild fever, adenopathy, and lymphangitis (rarely). Scalded skin syndrome is caused by exfoliative toxins secreted on the epidermis and mostly affects neonates and young children. Other skin conditions caused by Staphylococcal exfoliative toxins include blisters, skin loss, pimples, furuncles, impetigo, folliculitis, abscesses, poor temperature control, fluid loss, and secondary infection . *S. aureus* can also cause necrotizing fasciitis in immunocompromised individuals, although this is very rare. Necrotizing fasciitis is life-threatening and causes severe morbidity.

Toxic shock syndrome is associated with vaginal colonization with toxin-producing *S. aureus* during menstruation, complications with staphylococcal infection at other sites, or complications of surgical procedures. Deep infections include endocarditis, peritonitis, necrotizing pneumonia, bacteremia, meningitis, osteomyelitis, septic arthritis, and infections of bones, joints and organs

1.2.16. *Klebsiella pneumoniae*: *Klebsiella pneumoniae* is a Gram-negative, nonmotile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium [Matthew, et al, 2005].

1.2.17. Scientific classification of *Klebsiella pneumoniae*

Scientific classification	
Kingdom:	Bacteria
Phylum:	Proteobacteria
Class:	Gammaproteobacteria
Order:	Enterobacteriales
Family:	Enterobacteriaceae
Genus:	*Klebsiella*
Species:	***K. pneumonia***

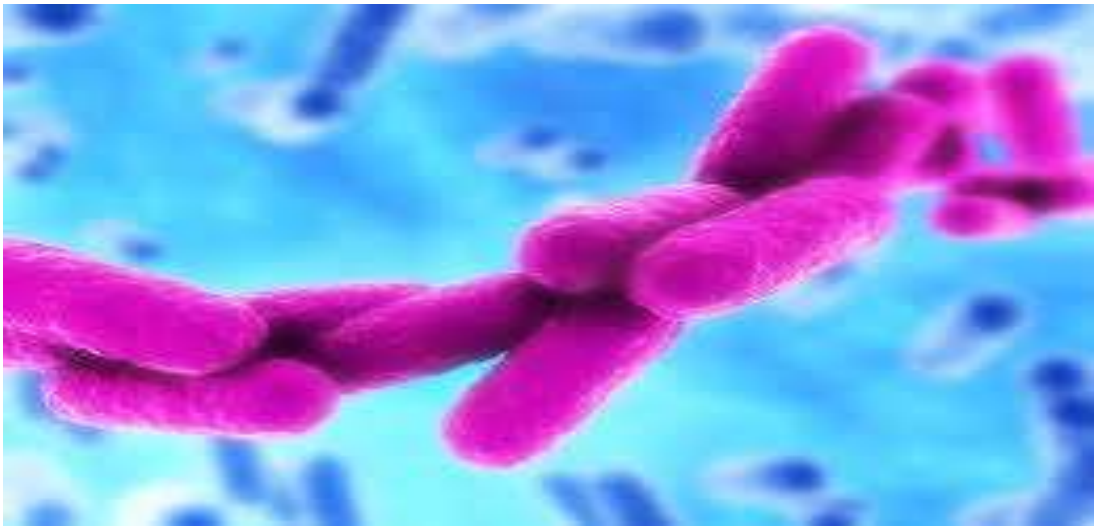

Fig1.11: *Klebsiella pneumoniae*

1.2.18. Pathogenicity of *Klebsiella pneumoniae*: *Klebsiella* spp. has been identified as important common pathogens for nosocomial pneumonia (7 to 14% of all cases), septicaemia (4 to 15%), and urinary tract infection (UTIs; 6 to 17%), wound infections (2 to 4%), intensive care unit (ICU) infections (4 to 17%), and neonatal septicaemias (3 to 20%). *Klebsiella* spp. can also cause bacteremias and hepatic infections, and have been isolated from a number of unusual infection, including endocarditis, primary gas-containing mediastinal abscess, peritonitis, acute cholecystitis, crepitant myonecrosis, pyomyositis, necrotizing fasciitis, psoas muscle abscess, fascial space infections of the head and neck, and septic arthritis. They are also important opportunistic pathogens, particularly among the immunocompromised. Pathogenicity factors of *Klebsiella* spp. include adhesins, siderophores, capsular polysaccharides (CPLs), cell surface lipopolysaccharides (LPSs), and toxins, each of which plays a specific role in the pathogenesis of these species. Depending on the type of infection and the mode of infectivity, cells of *Klebsiella* spp. may adhere and attack upper respiratory tract epithelial cells, cells in gastrointestinal tract, endothelial cells, or uroepithelial cells, followed by colonization of mucosal membranes. Common underlying conditions include alcoholism, diabetes mellitus, chronic liver disease (cirrhosis), chronic renal failure, cancer, transplants, burns, and/or use of catheters [Eisenstein, et al, 2000].

1.3. Antibiotic: A drug used to treat bacterial infections. Antibiotics have no effect on viral infections. Originally, an antibiotic was a substance produced by one microorganism that selectively inhibits the growth of another. Synthetic antibiotics, usually chemically related to natural antibiotics, have since been produced that accomplish comparable tasks.

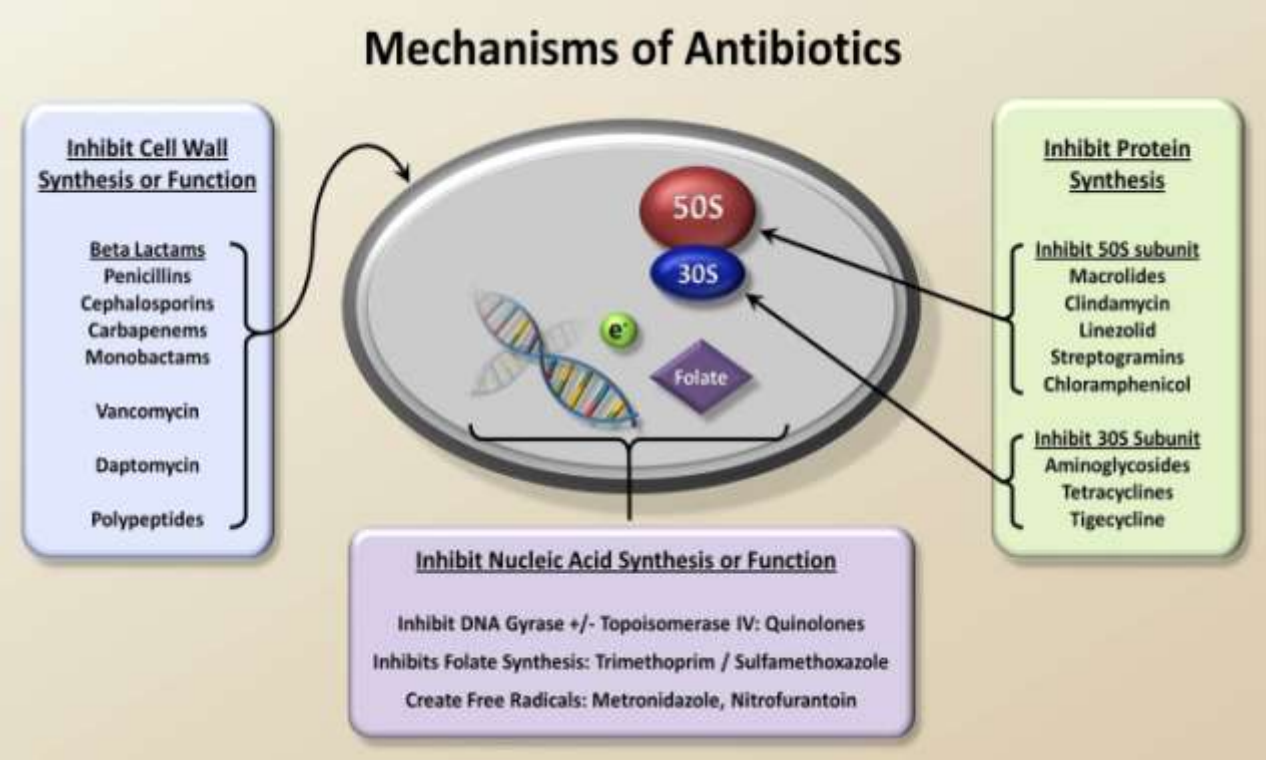

Fig1.12: Mechanism of Antibiotic

1.3.1. Ciprofloxacin: Ciprofloxacin is an antibiotic in a group of drugs called fluoroquinolones. It is an antibiotic used to treat a number of bacterial infections [Goodman et al, 2008].

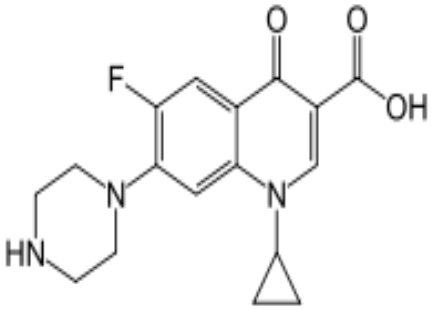

Fig1.13: structure of ciprofloxacin

1.3.2. Mode of action: DNA and RNA are keys to the replication of all living forms, including bacteria. Some antibiotics work by binding to components involved in the process of DNA or RNA synthesis, which causes interference of the normal cellular processes which will ultimately compromise bacterial multiplication and survival [Goodman et al, 2008].

1.3.3. Medical uses of Ciprofloxacin: Ciprofloxacin is used to treat a wide variety of infections, including infections of bones and joints, endocarditis, gastroenteritis, malignant otitis externa, respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, and chancroid.

1.3.4. Cephradine: Cephradine is in a group of drugs called cephalosporin antibiotics. Cephradine fights bacteria in the body. Unnecessary use or overuse of any antibiotic can lead to its decreased effectiveness.

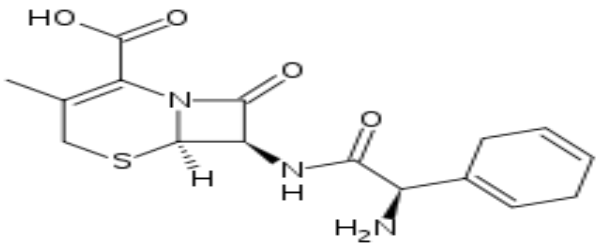

Fig1.14: structure of cephradine

1.3.5. Mode of action: Inhibitors of cell wall synthesis**.**

1.3.6. Medical uses of Cephradine: Cephradin**e** is used to treat infections caused by bacteria, including upper respiratory infections, ear infections, skin infections, and urinary tract infections.

1.3.7. Levofloxacin: Levofloxacin is in a group of antibiotics called fluoroquinolones .Levofloxacin fights bacteria in the body.

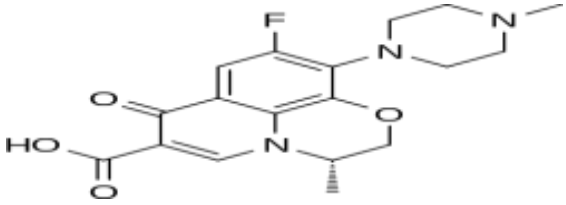

Fig1.15: structure of levofloxacin

1.3.8. Mode of action: DNA and RNA are keys to the replication of all living forms, including bacteria. Some antibiotics work by binding to components involved in the process of DNA or RNA synthesis, which causes interference of the normal cellular processes which will ultimately compromise bacterial multiplication and survival [Goodman et al, 2008].

1.3.9. Medical uses of Levofloxacin:

● Used to treat bacterial infections of the skin, sinuses, kidneys, bladder, or prostate.

●Used to treat bacterial infections that cause bronchitis or pneumonia, and to treat people who have been exposed to anthrax or plague.

1.3.10. Azithromycin: Azithromycin is an antibiotic that fights bacteria. Azithromycin. It is macrolide group.

1.3.11. Mode of action: Enzymes and cellular structures are primarily made of proteins. Protein synthesis is an essential process necessary for the multiplication and survival of all bacterial cells. Several types of antibacterial agents target bacterial protein synthesis by binding to either the 30S or 50S subunits of the intracellular ribosomes. This activity then results in the disruption of the normal cellular metabolism of the bacteria, and consequently leads to the death of the organism or the inhibition of its growth and multiplication [Goodman et al, 2008].

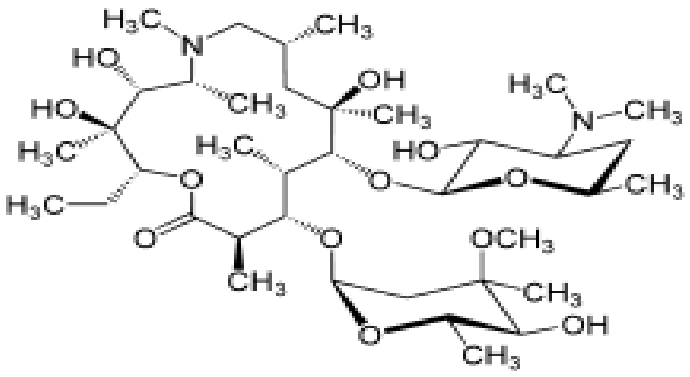

Fig1.16: Azithromycin

1.3.12. Medical uses of Azithromycin:

●Use of chronic obstructive pulmonary disease.

●Acute bacterial sinusitis .

●Use of Acute otitis media.

●Pharyngitis or tonsillitis .

●Uncomplicated skin and skin structure infections.

1.3.13. Cefuroxime Axetil: Cefuroxime is an enteral second-generation cephalosporin antibiotic. Cefuroxime is used to treat many kinds of bacterial infections, including severe or life-threatening forms [Goodman et al, 2008].

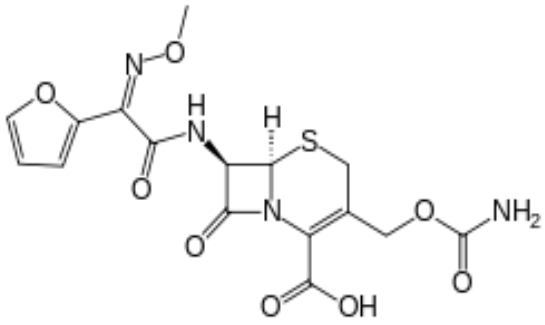

Fig1.17: structure of cefuroxime

1.3.14. Mode of action: Inhibitors of cell wall synthesis

1.3.15. Medical uses of Cefuroxime Axetil:

- The efficacy of Cefuroxime Axetil in the prevention of rheumatic fever was not established in clinical trials.
- The efficacy of Cefuroxime Axetil in the treatment of penicillin-resistant strains of Streptococcus pyogenes has not been demonstrated in clinical trials.

1.3.16. Ceftriaxone: It is a third-generation cephalosporin. Like other third-generation cephalosporins, it has broad-spectrum activity against Gram-positive bacteria and expanded Gram-negative coverage compared to second-generation agents.

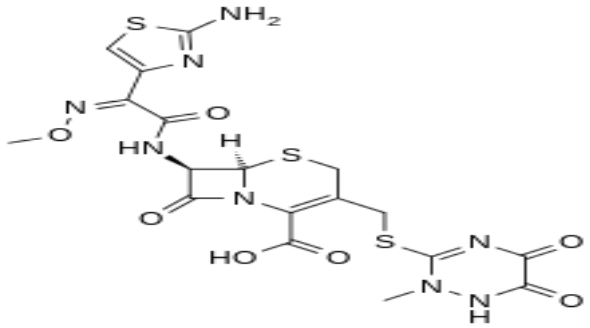

Fig1.18: structure of ceftriaxone

1.3.17. Mode of action: Inhibitors of cell wall synthesis

1.3.18. Medical use of Ceftriaxone:

- Lower respiratory tract infections
- Acute bacterial otitis media
- Skin and skin structure infections
- Urinary tract infections
- Uncomplicated gonorrhea
- Pelvic inflammatory disease
- Bacterial septicemia
- Intra-abdominal infections
- Meningitis
- Surgical prophylaxis

1.3.19. Cefixime Micronized: Cefixime is an antibiotic useful for the treatment of a number of bacterial infections. It is a third generation cephalosporin. It is on the World Health Organization's List of Essential Medicines [Jennifer, et al, 2007].

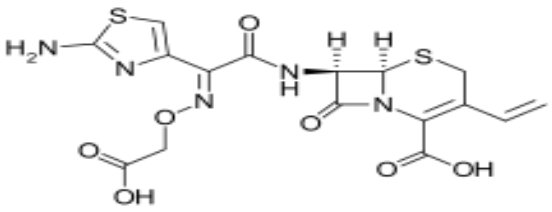

Fig1.19: structure of cefixime

1.3.20. Mode of action: Inhibitors of cell wall synthesis

1.3.21. Medical use of Cefixime Micronized:

Ear: Otitis

Sinuses:Sinusitis.

Throat:Tonsillitis,pharyngitis.

Chest and lungs: Bronchitis, pneumonia it is also used to treat typhoid fever.

1.3.22. Vancomycin: Vancomycin is used to treat an infection of the intestines caused by Clostridium difficile, which can cause watery or bloody diarrhea. It is also used to treat staph infections that can cause inflammation of the colon and small intestines.

Oral vancomycin works only in the intestines. This medicine is not normally absorbed into the body and will not treat other types of infection. An injection form of this medication is available to treat serious infections in other parts of the body [Stan, 2009].

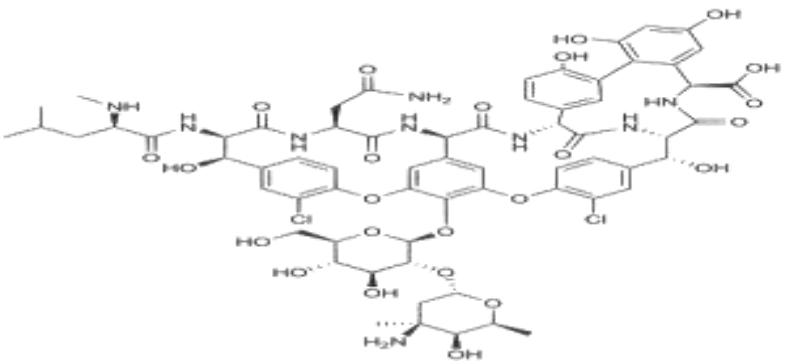

Fig1.20: structure of vancomycin

1.3.23.: Mode of action: Inhibitors of cell wall synthesis

1.3.24. Medical use of Vancomycin: Vancomycin is indicated for the treatment of serious, life-threatening infections by gram-positive bacteria unresponsive to other antibiotics.

●Uase as skin infections,

●Bloodstream infections,

● Endocarditis,

●Bone and joint infections,

●Meningitis caused by methicillin-resistant *S. aureus*.

● Used for severe *Clostridium difficile* colitis.

1.4. Determination of Minimum Inhibitory Concentration

1.4.1. Minimum Inhibitory Concentration:

Introduction: (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. The MIC or minimum inhibitory concentration test determines antimicrobial activity of a material against a specific bacterium [Jennifer, 2001].

1.4.2. Determination: The minimum inhibitory concentration is the lowest concentration of the test sample of drug at which it shows the highest activity against microorganisms. It is determined by serial dilution technique against bacteria. The serial dilution assay quantities the anti-microbial activity of compound by providing the MIC value of compound for specific susceptibility test organisms, and important consideration in the further development of bioactive compounds.

In this method a large number of autoclaved compound for specific susceptible test tubes were used and each of the test tubes containing serial nutrient broth medium in a serial dilution containing 10µl of test organisms and mixed well. The test sample in various concentrations

were applied to the nutrient medium (1ml) in each test tube and incubated at 37.5^0 c for 12-18 hour. Then the growth of organism observed.

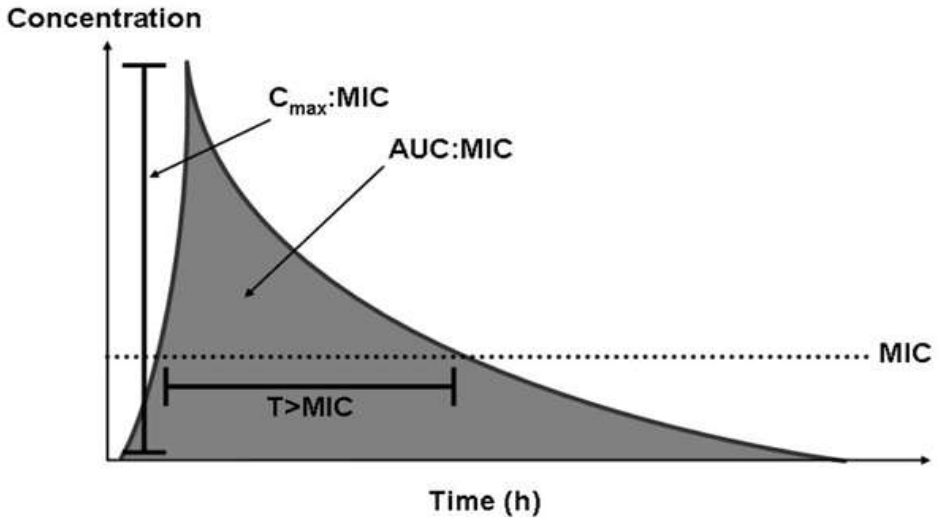

Fig1.21: curve for determining MIC value

The minimum inhibitory concentration (MIC) is necessary to achieve in vivo potency and is dependent on the pharmacokinetics of the drug.

Here-
- AUC, area under the concentration-time curve;
- C_{max}, maximum plasma concentration;
- T > MIC, time spent above the

1.4.3. Clinical significance of MIC: Clinically, the minimum inhibitory concentrations are used not only to determine the amount of antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the opportunity for microbial resistance to specific antimicrobial agents. In this experiment, the Minimum Inhibitory Concentration (MIC) of Standard sample of antibiotics (levofloxacin, Azithromycin, Ciprofloxacin, Cepharadine, Cefuroxime, Ceftriaxone, Cefixime, and Vancomycin was tested against the following organisms different strain of *E.coli, Staphylococcus aureus, Salmonella typhy, Klebsiella spp., Acinetobacter spp.*

Chapter-2

Significance and Aims of the study

Significance of the Study

Minimum inhibitory concentration provides qualitative assessments using the categories susceptible, intermediate, or resistant. In general, current testing methods provide accurate detection of common antimicrobial resistance mechanisms.

Aim of the Study

The main goals of Minimum inhibitory concentration (MIC) testing are-

- Aim is to measure susceptibility of an isolate to range of antibiotics.
- At the individual patient level for effective prescribing.
- But also to assess emerging bacterial resistance patterns.
- 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.
- It is an important consideration for further development of bioactive compounds.

Chapter-3

Literature Review

Department of Medicine, Division of Infectious Diseases, State University of New York, conducted a research on Comparison of agar dilution, microtitre broth dilution and tube macro dilution susceptibility testing of ciprofloxacin against several pathogens at two different inocula.In this study found that the susceptibility of six different genera of organisms to ciprofloxacin was determined by the tube macro dilution, broth micro dilution and the agar dilution methods. The minimal inhibitory concentrations of ciprofloxacin determined by the broth micro dilution and the agar dilution methods correlated well with each other, but in general the tube macro dilution technique gave somewhat higher results. Raising the initial inoculum of the tested organisms from 1×10^5 to 1×10^7 cfu/ml did not result in a significant increase in the minimal inhibitory or bactericidal concentrations of ciprofloxacin [Myles and Taryn, 1985].

Dow University of Health Sciences, Karachi, Pakistan careered out a research on comparative in vitro antibacterial analysis of different brands of cefixime against clinical isolates of *Staphylococcus aureus* and Escherichia coli .The objective of this study was to compare the antibacterial activity of standard and different brands of Cefixime, against standard samples and clinical isolates of *E. coli* and *S.aureus* collected from different hospitals. Standard samples and isolates of *E. coli* and *S.aureus* were separately cultured in Mueller Hinton broth. After the bacterial incubation, 5 ml solution each of standard Cefixime and its different brands were added to the test tubes containing bacterial culture. Cefixime samples were added in the concentration of 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128µg/ml to separate test tubes. The cultures were again incubated and then the culture samples were analyzed by UV-spectrophotometer, and minimum inhibitory concentrations of all samples were determined. The analysis and interpretation of results were done by single factor ANOVA. An MIC of 0.75µg/ml and 8µg/ml of standard Cefixime was found for standard E. coli and S. aureous respectively. Standard Cefixime and its six selected brands exhibited a higher MIC range for clinical isolates of S. aureus than the clinical isolates of E. coli. Higher MIC values of standard Cefixime and its brands were observed for clinical isolates of *E. coli* and *S. aureus*. Higher MIC values for the clinical isolates of *E. coli* and *S. aureus* indicated that both the organisms have developed resistance to Cefixime in comparison to standard microorganisms acquired from ATCC. [Hafiz et al, 2012].

Faculty de Medicine Veterinaries, University de Montréal, Saint-Hyacinthe, QC, Canada carried out a research they found antibiotic resistance is an ever-growing problem yet the development of new antibiotics has slowed to a trickle, giving rise to the use of combination therapy to eradicate infections. The purpose of this study was to evaluate the combined inhibitory effect of lithium fluoride (LiF) and commonly used antimicrobials on the growth of the following bacteria: *Enterococcus faecalis, Staphyloccoccus aureus, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, Serratia marcescens, and Streptococcus pneumoniae*. The in vitro activities of ceftazidime, sulfamethoxazole-trimethoprim, streptomycin, erythromycin, amoxicillin, and ciprofloxacin, doxycycline, alone or combined with LiF were performed by microdilution method. MICs were determined visually following 18–20h of incubation at 37◦C. We observed reduced MICs of antibiotics associated with LiF ranging from two-fold to sixteen-fold. The strongest decreases of MICs observed were for streptomycin and erythromycin associated with LiF against Acinetobacter baumannii and Streptococcus pneumoniae. An eight-fold reduction was recorded for streptomycin against S. pneumoniae whereas an eight-fold and a sixteen-fold reduction were obtained for erythromycin against A.baumannii and S. pneumoniae. This suggests that LiF exhibits a synergistic effect with a wide range of antibiotics and is indicative of its potential as an adjuvant in antibiotic therapy. [Syed and Ravaoarinoro, 2011].

USDA-ARS Bacterial Epidemiology and Antimicrobial Resistance Research Unit, Athens, conducted a research on Antimicrobial Susceptibility to Azithromycin among Salmonella enterica Isolates from the United States. Due to emerging resistance to traditional antimicrobial agents, such as ampicillin, trimethoprim-sulfa methoxazole, and chloramphenicol, azithromycin is increasingly used for the treatment of invasive Salmonella infections. In this study, 696 isolates of non-Typhi Salmonella collected from humans, food animals, and retail meats in the United States were investigated for antimicrobial susceptibility to azithromycin. Seventy-two Salmonella enterica serotype Typhi isolates from humans were also tested. For each isolate, MICs of azithromycin and 15 other antimicrobial agents were determined by broth microdilution. Among the non-Typhi Salmonella isolates, azithromycin MICs among human isolates ranged from 1 to 32 μg/ml, whereas the MICs among the animal and retail meat isolates ranged from 2 to 16 μg/ml and 4 to 16 μg/ml, respectively. Among Salmonella serotype Typhi

isolates, the azithromycin MICs ranged from 4 to 16μg/ml. The highest MIC observed in the this study was 32 μg/ml, and it was detected in three human isolates belonging to serotypes Kentucky, Montevideo, and Paratyphi A. Based on our findings, we propose an epidemiological cutoff value (ECOFF) for wild-type Salmonella of ≤16 μg/ml of azithromycin. The susceptibility data provided could be used in combination with clinical outcome data to determine tentative clinical breakpoints for azithromycin and Salmonella enterica [Maria, et al, 2011].

JMI Laboratories, North Liberty, Iowa,1and GlaxoSmithKline, Collegeville, Pennsylvania conducted a research on Determination of Disk Diffusion and MIC Quality Control Ranges for GSK1322322, a Novel Peptide Deformylase Inhibitor. GSK1322322 is a novel peptide deformylase inhibitor in the early phase of development for treatment of complicated bacterial skin and skin structure infection and hospitalized community-acquired pneumonia. This quality control (QC) study was performed to establish broth microdilution and disk diffusion QC ranges for strains *Staphylococcus aureus* ATCC 29213 (MIC range, 1 to 4 μg/ml), Haemophilus influenzae ATCC 49247(MIC and disk diffusion zone diameter ranges, 0.5 to 4 μg/ml and 20 to 28 mm, respectively), *Streptococcus pneumoniae* ATCC 49619 (MIC and disk diffusion zone diameter ranges, 0.12 to 0.5 μg/ml and 23 to 30 mm, respectively), and *S. aureus* ATCC 25923 (disk diffusion zone diameter range, 18 to 26 mm) [James, et al, 2011].

Department of Forestry, National Taiwan University conducted a research on Antibacterial activity of leaf essential oils and their constituents from Cinnamomum osmophloeum.The antibacterial activities of the essential oils from leaves of two Cinnamomum osmophloeum clones (A and B) and their chemical constituents were investigated in this study. The nine strains of bacteria, including *Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, methicillin-resistant Staphylococcus aureus (MRSA), Klebsiella pneumoniae, Salmonella sp., and Vibrio parahemolyticus,* were used in the antibacterial tests. Results from the antibacterial tests demonstrated that the indigenous cinnamon B leaf essential oils had an excellent inhibitory effect. The MICs (minimum inhibitory concentrations) of the B leaf oil were 500 μg/ml against both *K.pneumoniae and Salmonella spp*. and 250 μg/ml against the other seven strains of bacteria. Cinnamaldehyde possessed the strongest antibacterial activity compared to the other constituents of the essential oils. The MICs of cinnamaldehyde against the E. coli, P.

aeruginosa, E. faecalis, S. aureus, S. epidermidis,*MRSA, K. pneumoniae, Salmonella spp.,* and V. parahemolyticus were 500, 1000, 250, 250, 250, 250, 1000, 500, and 250 µg/ml, respectively. These results suggest that C.osmophloeum leaf essential oil and cinnamaldehyde are beneficial to human health, having the potential to be used for medical purposes and to be utilized as anti-bacterial additives in making paper products [Shang, et al, 2001].

Centre of Excellence for Food Safety Research, Faculty of Food Science and Technology, University Putra Malaysia, conducted a research on minimum inhibitory concentration and minimum bactericidal concentration of Nano Colloidal Silver on food-borne pathogens. In the emerging issue of increased multi-resistant properties in foodborne pathogens, silver nano particles are being used increasingly as antimicrobial agents. Thus, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Nano Colloidal Silver towards food-borne pathogens such as *Escherichia coli* O157:H7, Listeria monocytogenes, *Salmonella enterica Serovar Typhi, Vibrio cholerae, Vibrio parahaemolyticus, Bacillus cereus* and *Staphylococcus aureus* were examined in this study. The results obtained suggested that Nano Colloidal Silver exhibit a good bacteriostatic effect but poor bactericidal effect towards all food-borne pathogens tested. Nano Colloidal Silver can be a potential antimicrobial agent due to its low cost of production and high effectiveness in antimicrobial properties, which may find wide applications in various food industries to address food safety issues [Petrus, 2011].

A constituent college of Manipal University conducted a research on Antibacterial effect of neem (Azadirachta indica) oil on multidrug resistant bacteria isolated from human infections. The aim of the this study was to determine the inhibitory and killing effect of neem (Azadirachta indica) oil on multidrug resistant bacteria isolated from human infections. Twenty five strains of multidrug resistant *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa* isolated from different clinical specimens were used in the study. Time kill assay and broth macrodilution methods for determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were used to study the inhibitory and bactericidal effect of neem oil on ese multidrug resistant bacteria. Undiluted neem oil killed all strains of S. aureus within 8 h of exposure, whereas neem oil at concentratio500 µl/ml took 18 h to kill S. aureus. Undiluted neem oil killed E. coli and P.aeruginosa within 18 h of exposure. The MIC of

neem oil was 500 µl/ml. Neem oil showed bactericidal effect on both gram-positive (*S. aureus)* and gram negative (*E. coli and P.aeruginosa*) bacteria. The anti-bacterial effect of neem oil was concentration and time dependent S. aureus was more susceptible to neem oil than *E. coli and P. aeruginosa* [Divya, et al, 2010].

Patna Women's College, Patna, India conducted research on Antibiotic susceptibility and determination of Minimum Inhibitory Concentration (MIC) of potent antibiotics used against Staphylococcus spp. isolated from raw milk. This study they found the uncontrolled use of antibiotics has led to the development of multiple antibiotic resistance, there by rendering the treatment ineffective. In the present study, raw milk samples were collected from different areas of Patna. Out of the 12 isolates obtained, nine were identified as *Staphylococcus s*pecies. The isolates were examined for their susceptibilities by Bauer Kirby Disc Diffusion test against ten antibiotics. Results showed that incidence of resistance to the antibiotics was quite high, as the maximum susceptibility obtained was only about 13.19%, Rifampicin and Tetracycline being the most ineffective in vitro. Amoxicillin and Cloxacillin were the most effective in phase I exhibiting 12.13% and 11.24% efficacy, respectively, while Ampicillin + Cloxacillin was the most effective combination exhibiting 14.31% efficacy in phase II. The MIC values of two antibiotics in pure form and three in combinations were determined by agar dilution and broth dilution methods. The MIC values ranged between 0.5 – 1.0 µg/L showing comparable results throughout the dilution range. However, slightly higher values were obtained for Amoxicillin +Erythromycin and Amoxicillin + Clavulanate i.e. > 1.0 µg/L [Ankita, et al, 2011]

Department of Medical Nanotechnology, Faculty of Advanced Sciences and Technology, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS) carried out a research on Preparation and evaluation of ampicillin solid lipid nanoparticles .In this research article stated that Solid lipid nanoparticles (SLNs) have been studied as a drug-delivery system for the controlling of drug release. These systems have many important advantages, such as biocompatibility, good tolerability, and ease of scale up. Ampicillin as a β-lactam antibiotic was studied to load on SLNs for control of drug release to increase administration intervals and decrease dose of drug to increase patient compliance and decrease antibiotic resistance. The size of ampicillin loaded nanoparticles; drug loading, drug release profile, morphology and antibacterial effect were studied. The conventional broth macrodilution tube method was used to

determine the minimum inhibitory concentration (MIC) and minimum bacteriostatic concentration (MBC) of ampicillin SLNs with respect to P.aeruginosa, *E. coli and S. aureus* in vitro. Prepared particles show 150 nm of size. Drug loading efficiency was 77±3%, all prepared particles had spherical shape. After 24 hours more than 95% of loaded drug was detected in release samples. MIC and MBC of ampicillin loaded nanoparticles decreased in comparison with free ampicillin *against P.aeruginosa, E. coli* and *S. aureus* [Faezeh, et al, 2015]

Department of Pharmacology, Assam Medical College & Hospital, Dibrugarh, Assam, India, carried out a research on Antibacterial Activity of the Ethanolic Extract of Leaves of Citrus maxima (Burm.) Merr. On *Escherichia Coli and Pseudomonas Aeruginosa*. The objective of the study was to evaluate the antibacterial activity of the ethanolic extracts of leaves of Citrus maxima (Burm.) Merr. (EECM) on Escherichia coli and Pseudomonas aeruginosa. Methods: The ethanolic extract of leaves of Citrus maxima (Burm.)Merr. (EECM) was prepared by percolation method. Pathological isolates Escherichia coli and Pseudomonas aeruginosa were obtained from the Department of Microbiology, Assam Medical College & Hospital. Disc diffusion method for antimicrobial susceptibility testing was performed according to the Kirby-Bauer method. The whatman-1 filter paper discs of 6mm sizes impregnated with the plant extract were placed on Mueller-Hinton agar plates seeded with bacterial cultures of 0.5 Mc Farland standards. The antibacterial activities were assessed by the presence or absence of inhibition zones after incubating the plates at 370c for 24 hours. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of EECM for the selected pathogens were determined by broth macrodilution method. Results: Maximum zone of inhibition in antibacterial susceptibility test was shown by Pseudomonas aeruginosa. MIC value of the extract for Pseudomonas aeruginosa (0.312mg/ml) was found to be lower than *Escherichia coli* but MBC value (1.25mg/ml) was found to be the same for both the bacteria.in this study result found that the plant extract Citrus maxima (Burm.) Merr showed significant antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* [Swarnamoni, et al, 2013].

Department of Microbiology, Kurukshetra University Kurukshetra, India carried out a research on In Vitro Antimicrobial Activity and Phytochemical Studies of Terminalia Chebulaagainst the Microbes Isolated from Fruit Juices. The present work has been conducted to evaluate the antimicrobial activity of Terminalia chebula against microorganisms associated with juices.

Methanol, ethanol, acetone, and aqueous (hot and cold) extracts from fruits of T. chebula were tested for their antimicrobial activity through agar well diffusion method and minimum inhibitory concentration (MIC)/minimum bactericidal concentration (MBC) values were determined through the macrodilution broth method against *Bacillus cereus* ,*Serratia spp*. and Rhodoto rula mucilaginosa.Their total phenolic content and total tannin content were also evaluated. Organic and cold aqueous extracts displayed activity against all three tested microbes. There were highly positive relationship between antimicrobial activities and phenolic and tannin content of the tested extracts against each microorganism. Methanolic extract was found to be best against all tested microbes with lowest MIC of 0.78 mg/ml and MBC of 1.56 mg/ml and showed better antimicrobial activity than sodium benzoate. Therefore, methanolic extract of T. chebula has a bio preservative potential in fruit juices [Romika, et al, 2015].

Chapter-4

Methods and Materials

4.1: Study design

For the in vitro antimicrobial susceptibility test of different antibiotic standard powder was collected from Incepta Pharmaceutical and Asiatic Laboratory. Different strains of *E. coli*, *Pseudomonas spp.and Salmonella typhi*, *Klebsiella*, *Acinobactor*, *Staphylococus aureus* and were collected from Pathology department, Ibrahim Medical College (BIRDEM) .Then the clinical isolates of these microorganisms were sub cultured and MIC test was performed by measuring the minimum concentration value.

4.1.1: Period and place of the study

The duration of this study was 1 year and all the test was performed in the Microbiological

Laboratory of East West University.

4.1.2: List of Antibiotic standard powder Used in the Test:

Antibiotic Standard Powder	Name of Company	Potency
Levofloxacin USP	Asiatic laboratory Ltd	95.87%
Azithromycin	Incepta Pharmaceutical	99.99%
Cephradine	Asiatic laboratory Ltd	91.67%
Ciprofloxacin	Incepta Pharmaceutical	99.99%
Vancomycin HCL	Incepta Pharmaceutical	99.189%
Ceftriaxone	Incepta Pharmaceutical	99.99%
Cefuroxime Axetil	Incepta Pharmaceutical	79.51%
Cefixime Micronized	Incepta Pharmaceutical	99.99%

4.1.3: List of Microorganisms Used in the Test:

Gram Positive Bacteria	Gram negative Bacteria
Staphylococcus aureus	***E.coli***
	Pseudomonas Spp.
	Salmonella typhi
	Acinobactor
	Klebsiella

4.1.4: Apparatus & Solvent:

1. Sterile Test tubes	**16. Distilled water**
2. Inoculating loop	**17. Sterile saline solution (Sodium Chloride)**
3. Sterile forceps	**18.Sample of Antibiotics Standard Powder**
4. Sterile cotton	**19. Sample of microorganism.**
5. Sterile Petri dishes	**20.Ethanol (95%)**
6. Measuring cylinder	**21.$Bacl_2.2H_2O$**
7. Nutrient Agar media	**22. H_2SO_4 (1%)**
8. Nutrient broth media	
9. Hot air oven (FN-500, Niive)	
10. Bunsen burners	
11. Micropipettes (2-20µl)	
12. Laminar air-flow unit (ESCO,Singapore)	
13. Autoclave(HIRAYAMA,Japan)	
15. Incubator (BK 4266).	

4.1.5: Sterilization procedure:

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.

4.1.6: 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:

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

4.1.7: Preparation of inoculum:

The test organism 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.

4.1.8: Preparation of 0.5 McFarland Standards:

McFarland standards are suspensions of either barium sulfate or latex particles that allow visual comparison of bacterial density (Fig.4.01). 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 1.5×10^8 CFU/ml of *E.coli*. 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).

4.1.9: 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.

Fig. 4.01: 0.5 McFarland standards

4.1.10: Procedure of Macrodilution Minimum Inhibitory Concentration:

1. Seventeen autoclaved test tube were taken, of which fourteen were marked 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. To each of Seventeen test tubes, 1 ml of sterile nutrient broth medium was taken.

3. Then to the first test tube, 1 ml of the sample solution was added and mixed well.

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 tests 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 to observe the growth of the organism in the medium used. 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.

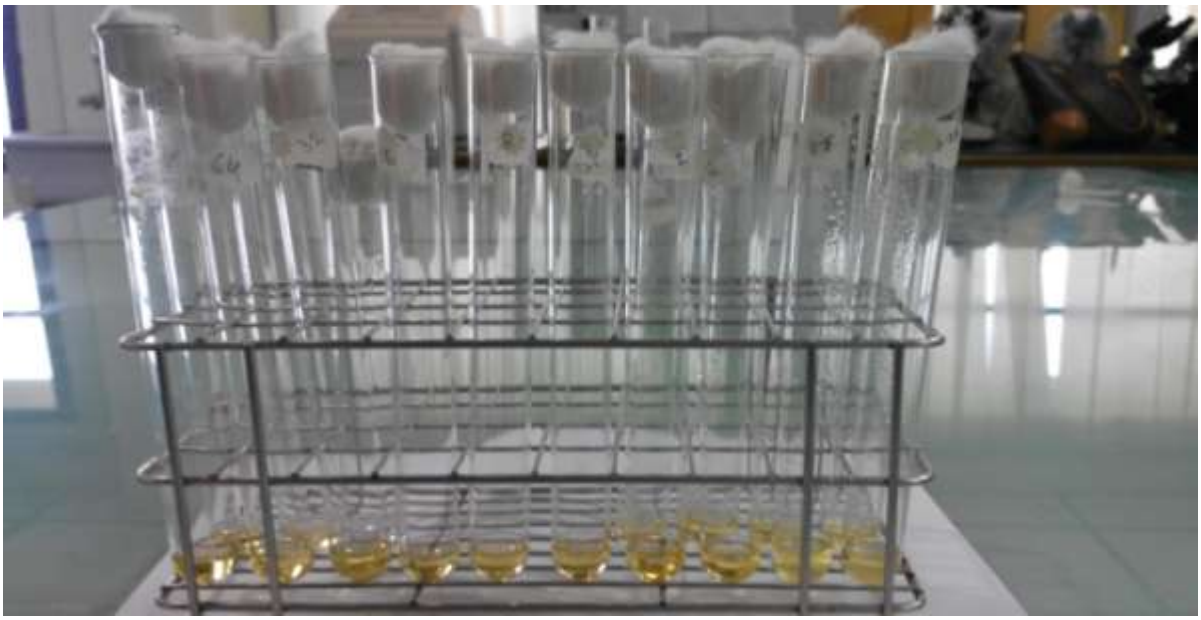

Fig4.02: Macrodiluton of MIC

Chapter-5
Result and Discussion

5.1: Result and Discussion of Minimum Inhibitory Concentration (MIC):

The Minimum Inhibitory Concentration is the lowest concentration of a test sample or drug at which it shows the highest activity against specific microorganism. The serial dilution assay quantities the anti-microbial activity of the Antibiotics standard powder by providing the MIC value of the drug for specific organism. It is an important consideration for further development of bioactive compounds.

In this method a large number of autoclaved test tubes containing sterile nutrient broth medium were used. Antibiotic standard powder were used in various concentrations (in serial dilution from 0.015625-128μg/ml) and 10μl suspension of test organisms (containing 1.5×10^6 cells per ml) were taken in the test tubes and mixed well. Three control test tubes C_S, C_M and C_I were used to perform control test containing test sample and nutrient broth medium, only nutrient broth medium and inoculums and nutrient broth medium respectively. Then the test tubes were incubated at 37.5ºc for 18-20 hrs.

After complete incubation of 18-20 hrs. Growth of organism was observed only in the C_I test tube among the control test tubes. The C_S and C_M showed no growth of organisms. The other test tubes were compared with them and the concentration up to which no growth of organism was observed was determined. This concentration is the Minimum Inhibitory Concentration of the test sample against the specific organism.

This study carried out only on the clinically isolated bacterias to observe their sensitivity pattern against API sample of antibiotics. In this study I tried to cover a some spectrum of antibiotics which includes Cephalosporins, macrolide (Azithromycin) groups of antibiotics Vancomycin a glycopeptides also included in this sensitivity studies.

A lot of research has been carried out in the field drug discovery and development regarding antibiotics as it has emergence due to development of antibiotic resistance. Cephalosporin group of drugs have drawn a lot of attention and as a result they are possessing a few generation of drugs. Here in this study we used three generations of cephalosporins. We have selected cephradine from first generation, Cefuroxime from second generation, Ceftriaxone and Cefixime from third generation. In this study we have also selected second generation quinolone eg.ciprofloxacin and third generation quinolone levofloxacin.

Sample 1: *Escherichia coli* resistance pattern:

Escherichia coli has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient (8 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range. Though E. coli is highly susceptible to resistance against most of the antibiotics, but this E. coli found sensitive against all the antibiotics used in this study except cephradine, cefuroxime, ceftriaxone, cefixime.

Here, levofloxacin (1µg/ml) which showed sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Ciprofloxacin (0.03125 µg/ml) showed very high sensitivity against this E.coli; with a MIC range ≤1µg/mL, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

All cephalosporin (cephradine, cefuroxime, ceftriaxone, cefixim) group antibiotics have not showed sensitivity effect against this E.coli.

In case of azithromycin and vancomycin the MIC values were found about 0.5µg/mL and 16µg/mL respectively but Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) stated that they might be active in vitro but not clinically effective.

Table 1: Antibacterial study of *Escherichia coli* against Standard antibiotics powder

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

Sample 2: *Escherichia coli* resistance pattern:

Escherichia coli has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient (60 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (8µg/ml) which showed not sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which not compelled to the standard value of Clinical Laboratory Standard Institute (CLSI)

Ciprofloxacin (8 µg/ml) showed not sensitivity against this E.coli; with a MIC range ≤1µg/mL, which not compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Cephradine (0.5 µg/ml) showed very high sensitivity against this E.coli; with a MIC range ≤2µg/mL, which compelled to the standard value of German institute for standardization (DIN).

Cefuroxime, ceftriaxone, cefixim group antibiotics have not showed sensitivity effect against this E.coli.

In case of azithromycin and vancomycin the MIC values were found at 8µg/mL and 16µg/mL respectively has not showed sensitivity effect against this E.coli.

The detail result of this antibacterial study has been presented in the table.

Table 2: Antibacterial study of *Escherichia coli* against Standard antibiotics powder

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

Sample 3: ***Escherichia coli* resistance pattern**

Escherichia coli has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a female patient (82 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (0.125µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Ciprofloxacin (8 µg/ml) showed not sensitivity against this E.coli; with a MIC range ≤1µg/mL, which not compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Cephradine (2µg/ml) showed sensitivity against this E.coli; with a MIC range ≤2µg/mL, which compelled to the standard value of German institute for standardization (DIN).

Cefuroxime, ceftriaxone, cefixim antibiotics have not showed sensitivity effect against this E.coli.

In case of azithromycin the MIC value at 0.5µg/mL but Clinical Laboratory Standard Institute (CLSI) stated that they might be active in vitro but not clinically effective.

A vancomycin antibiotic has not showed sensitivity effect against this E.coli.

Quinolones (Levofloxacin) is the choice of treatment for *E.coli* infections. The detail result of this antibacterial study has been presented in the table.

Table 3: Antibacterial study of *Escherichia coli* against Standard antibiotics powder

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

Sample 4: *Escherichia coli* resistance pattern:

Escherichia coli has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient (1 year old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Levofloxacin antibiotic has not showed sensitivity effect against this E.coli.

Ciprofloxacin has not showed sensitivity against this E.coli.

All cephalosporin (cephradine, cefuroxime, ceftriaxone, cefixim) group antibiotics have not showed sensitivity effect against this E.coli.

Azithromycin and vancomycin have not effect against this E.coli.

The detail result of this antibacterial study has been presented in the table.

Table 4: Antibacterial study of *Escherichia coli* against Standard antibiotics powder

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

Sample 5: *Escherichia coli* resistance pattern:

Escherichia coli has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a female patient (48 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Levofloxacin antibiotic has not showed sensitivity effect against this E.coli.

Ciprofloxacin has not showed sensitivity against this E.coli.

Cephradine (8µg/ml) showed not sensitivity against this E.coli; with a MIC range ≤2µg/mL, which not compelled to the standard value of German institute for standardization (DIN).

Cefixim and Cefuroxime, antibiotics have not showed sensitivity effect against this E.coli.

Ceftriaxone (0.0625µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤1µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Azithromycin the MIC value at 16µg/mL but Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) stated that they might be active in vitro but not clinically effective.

A vancomycin antibiotic has not showed sensitivity effect against this E.coli.

The detail result of this antibacterial study has been presented in the table.

Table 5: Antibacterial study of *Escherichia coli* against Standard antibiotics powder

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

Sample 6: *Escherichia coli* resistance pattern:

Escherichia coli has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient (55 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (0.0625μg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤2μg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Ciprofloxacin (4 μg/ml) showed not sensitivity against this E.coli; with a MIC range ≤1μg/mL, which not compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Cephradine, cefuroxime, cefixim antibiotics have not showed sensitivity effect against this E.coli.

Ceftriaxone (4 μg/ml) showed not sensitivity against this E.coli; with a MIC range ≤1μg/mL, which not compelled to the standard value of European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Azithromycin the MIC value at 8μg/mL but Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) stated that they might be active in vitro but not clinically effective.

A vancomycin antibiotic has not showed sensitivity effect against this E.coli.

The detail result of this antibacterial study has been presented in the table.

Table 6: Antibacterial study of *Escherichia coli* against Standard antibiotics powder

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

Sample 7: *Escherichia coli resistance pattern:*

Escherichia coli has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient (40 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (0.0625µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Ciprofloxacin (2µg/ml) showed not sensitivity against this E.coli; with a MIC range ≤1µg/mL, which not compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Cephradine, cefuroxime, cefixim antibiotics have not showed sensitivity effect against this E.coli.

Ceftriaxone (8µg/ml) showed not sensitivity against this E.coli; with a MIC range ≤1µg/mL, which not compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Azithromycin (0.0625µg/mL) showed high sensitivity against this E.coli but of Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) stated that they might be active in vitro but not clinically effective.

Vancomycin (16µg/mL) showed sensitivity against this E.coli but CLSI stated and EUCAST that they might be active in vitro but not clinically effective.

The detail result of this antibacterial study has been presented in the table.

Table 7: Antibacterial study of *Escherichia coli* against Standard antibiotics powder

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

Sample 8: *Escherichia coli* resistance pattern:

Escherichia coli has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient (39 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (0.5µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Ciprofloxacin antibiotic has not showed sensitivity effect against this E.coli.

All cephalosporin (cephradine, cefuroxime, ceftriaxone, cefixim) group antibiotics have not showed sensitivity effect against this E.coli.

Azithromycin (4µg/mL) showed sensitivity against this E.coli but standard value of Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) stated that they might be active in vitro but not clinically effective.

A vancomycin antibiotic has not showed sensitivity effect against this E.coli.

The detail result of this antibacterial study has been presented in the table.

Table 8: Antibacterial study of *Escherichia coli* against Standard antibiotics powder

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

Sample 9: *Escherichia coli* resistance pattern:

Escherichia coli has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient (43 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (0.015625µg/ml) which showed highest sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Ciprofloxacin antibiotic has not showed sensitivity effect against this E.coli.

Cephradine (4µg/ml) showed not sensitivity against this E.coli; with a MIC range ≤2µg/mL, which not compelled to the standard value of German institute for standardization (DIN).

Cefixim and Cefuroxime and ceftriaxone antibiotics have not showed sensitivity effect against this E.coli.

Azithromycin (2µg/mL) showed sensitivity against this E.coli but Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) stated that they might be active in vitro but not clinically effective.

Vancomycin (16µg/mL) showed sensitivity against this E.coli but Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) stated that they might be active in vitro but not clinically effective.

The detail result of this antibacterial study has been presented in the table.

Table 9: Antibacterial study of *Escherichia coli* against Standard antibiotics powder

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

Sample 10*: Klebsiella spp*.resistance pattern:

Klebsiella spp. has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient (40 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (0.0625µg/ml) which showed highest sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Ciprofloxacin (0.25µg/ml) showed high sensitivity against this *Klebsiella sp*.; with a MIC range ≤1µg/mL, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Cephradine (2µg/ml) showed sensitivity against this *Klebsiella spp*; with a MIC range ≤2µg/mL, which compelled to the standard value of German institute for standardization (DIN).

Cefixim and Cefuroxime antibiotics have not showed sensitivity effect against this *Klebsiella sp.*

Ceftriaxone (32µg/ml) showed not sensitivity against this *Klebsiella sp.*; with a MIC range ≤1µg/mL, which not compelled to the standard value of European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Azithromycin (2µg/mL) showed sensitivity against this *Klebsiella spp.* but Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) stated that they might be active in vitro but not clinically effective.

Vancomycin (8µg/mL) showed sensitivity against this *Klebsiella spp.* but CLSI and EUCAST stated that they might be active in vitro but not clinically effective.

Table 10: Antibacterial study of *Klebsiella* against Standard antibiotics powder

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

Sample 11*: Klebsiella spp*.resistance pattern

Klebsiella spp.has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient (34 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (0.25µg/ml) which showed sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Ciprofloxacin (2µg/ml) showed not sensitivity against this *Klebsiella spp*.; with a MIC range ≤1µg/mL, which not compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

All cephalosporin (cephradine, cefuroxime, ceftriaxone, cefixim) group antibiotics have not showed sensitivity effect against this *Klebsiella spp*.

Azithromycin (16µg/mL) showed sensitivity against this *Klebsiella spp.* but CLSI and EUCAST stated that they might be active in vitro but not clinically effective.

Vancomycin (8µg/mL) showed sensitivity against this *Klebsiella spp.* but CLSI and EUCAST stated that they might be active in vitro but not clinically effective.

Table 11: Antibacterial study of *Klebsiella* against Standard antibiotics powder

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

Sample 12: *Klebsiella spp*.resistance pattern

Klebsiella spp.has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient (57 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (0.25µg/ml) which showed high sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Ciprofloxacin (1µg/ml) showed sensitivity against this *Klebsiella sp.*; with a MIC range ≤1µg/mL, which not compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

All cephalosporin (cephradine, cefuroxime, ceftriaxone, cefixim) group antibiotics have not showed sensitivity effect against this *Klebsiella sp*.

Azithromycin (16µg/mL) showed sensitivity against this *Klebsiella spp.* but Clinical Laboratory Standard Institute (CLSI) stated that they might be active in vitro but not clinically effective.

A vancomycin antibiotic has not showed sensitivity effect against this *Klebsiella spp*.

Table 12: Antibacterial study of *Klebsiella* against Standard antibiotics powder

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

Sample 13: *Klebsiella spp*.resistance pattern

Klebsiella spp.has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a female patient (85 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (0.125µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which compelled to the standard value of CLSI.

Ciprofloxacin has not showed sensitivity against this *Klebsiella spp.*

Cephradine, Cefixim and Cefuroxime, antibiotics have not showed sensitivity effect against this *Klebsiella spp*.

Ceftriaxone (8µg/ml) which showed not sensitivity against this clinical isolate; with a MIC range ≤1µg/ml, which not compelled to the standard value of CLSI.

Azithromycin (0.0625µg/mL) showed high sensitivity against this *Klebsiella spp.* but CLSI and EUCAST stated that they might be active in vitro but not clinically effective.

A vancomycin antibiotic has not showed sensitivity effect against this *Klebsiella spp*.

Table 13: Antibacterial study of *Klebsiella* against Standard antibiotics powder

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

Sample 14: *Staphylococcus aureus* Resistance pattern:

Staphylococcus aureus has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the throat sore of a male patient (21 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (0.03125µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤1µg/ml, which compelled to the standard value of but Clinical Laboratory Standard Institute (CLSI).

Ciprofloxacin has not showed sensitivity against this *Staphylococcus aureus.*

Cephradine (0.125µg/ml) showed very high sensitivity against this *Staphylococcus aureus*; with a MIC range ≤2µg/mL, which compelled to the standard value of German institute for standardization (DIN).

A Cefixim antibiotic has not showed sensitivity effect against this *Staphylococcus aureus.*

Cefuroxime (0.5µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤4µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Ceftriaxone (0.5µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤8µg/ml, which compelled to the standard value of CLSI

Azithromycin (0.0625µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Vancomycin (16µg/ml) which showed not sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which not compelled to the standard value of CLSI.

Table 14: Antibacterial study of *Staphylococcus aureus* against Standard antibiotics powder

Name of Antibiotic	Sample MIC Value (µg/mL)	Standard MIC Range (µg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	0.03125	≤ 1	2	≥ 4
Ciprofloxacin	--	≤ 1	2	≥ 4
Cephradine	0.125	≤ 2	--	≥ 4
Cefixim	--	≤ 1	--	≥ 2
Cefuroxime	0.5	≤ 4	8-16	≥ 32
Ceftriaxone	0.5	≤ 8	16-32	≥ 64
Azithromycin	0.0625	≤ 2	4	≥ 8
Vancomycin	16	≤ 2	4-8	≥ 16

Sample 15: *Staphylococcus aureus* Resistance pattern

Staphylococcus aureus has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient (45years old). The sample

was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (0.0625µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤1µg/ml, which compelled to the standard value of CLSI and EUCAST.

Ciprofloxacin has not showed sensitivity against this *Staphylococcus aureus.*

Cephradine, Cefixim, Cefuroxime antibiotic has not showed sensitivity effect against this *Staphylococcus aureus.*

Ceftriaxone (32µg/ml) which showed not sensitivity against this clinical isolate; with a MIC range ≤8µg/ml, which not compelled to the standard value of CLSI and EUCAST.

Azithromycin (0.0625µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which compelled to the standard value of CLSI and EUCAST.

Vancomycin (32µg/ml) which showed not sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which not compelled to the standard value of CLSI and EUCAST.

Table 15: Antibacterial study of *Staphylococcus aureus* against Standard antibiotics powder

Name of Antibiotic	Sample MIC Value (µg/mL)	Standard MIC Range (µg/mL)		
		Sensitivity	Intermediate	Resistance
Levofloxacin	0.0625	≤ 1	2	≥ 4
Ciprofloxacin	--	≤ 1	2	≥ 4
Cephradine	--	≤ 2	--	≥ 4
Cefixim	--	≤ 1	--	≥ 2
Cefuroxime	--	≤ 4	8-16	≥ 32
Ceftriaxone	32	≤ 8	16-32	≥ 64
Azithromycin	16	≤ 2	4	≥ 8
Vancomycin	32	≤ 2	4-8	≥ 16

Sample 16: Resistance pattern of *Acinetobector*

Acinetobector has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the trachial of a female patient (41 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (0.5µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Ciprofloxacin has not showed sensitivity against this *Acinetobector*.

Cephradine (32µg/ml) has showed sensitivity against this *Acinetobector*.

A Cefixim antibiotic has not showed sensitivity effect against this *Acinetobector*.

Cefuroxime antibiotic has not showed sensitivity effect against this *Acinetobector*

Ceftriaxone (4µg/ml) which showed sensitivity against this clinical isolate; with a MIC range ≤8µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Azithromycin antibiotic has not showed sensitivity effect against this *Acinetobector*

Vancomycin (64µg/mL) showed sensitivity against this *Acinetobector* but CLSI and EUCAST stated that they might be active in vitro but not clinically effective.

Table 16: Antibacterial study of *Acinetobector against* Standard antibiotics powders

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

Sample 17: Resistance pattern of *Pseudomonas spp.*

Pseudomonas has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a female patient (6 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin antibiotic has not showed sensitivity effect against this *Pseudomonas spp.*

Ciprofloxacin (0.03125µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤1µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Cephradine, Cefixim and cefuroxime antibiotics have not showed sensitivity effect against this *Pseudomonas spp.*

Ceftriaxone (32µg/ml) which showed sensitivity against this clinical isolate.

Azithromycin (1µg/mL) showed sensitivity against this *Pseudomonas* but Clinical Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) stated that they might be active in vitro but not clinically effective.

A vancomycin antibiotic has not showed sensitivity effect against this *Pseudomonas.*

Table 17: Antibacterial study of *Pseudomonas spp* against Standard antibiotics 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	-	≤ 2		≥ 4
Cefixim	-	≤ 1	--	≥ 2
Cefuroxime	-	--	--	--
Ceftriaxone	32	--	--	--
Azithromycin	1	--	--	--
Vancomycin	-	--	--	--

Sample 18: Resistance pattern of *Pseudomonas spp.*

Pseudomonas has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient (30year old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Levofloxacin antibiotic has not showed sensitivity effect against this *Pseudomonas.*

Ciprofloxacin has not showed sensitivity against this *Pseudomonas.*

All cephalosporin (cephradine, cefuroxime, ceftriaxone, cefixim) group antibiotics have not showed sensitivity effect against this *Pseudomonas.*

Azithromycin and vancomycin have not effect against this *Pseudomonas spp.*

Table 18: Antibacterial study of *Pseudomonas spp.* against Standard antibiotics 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
Cefixim	-	≤ 1	--	≥ 2
Cefuroxime	-	--	--	--
Ceftriaxone	-	--	--	--
Azithromycin	-	--	--	--
Vancomycin	-	--	--	--

Sample 19: Resistance pattern of *Pseudomonas spp.*

Pseudomonas has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the pus of a male patient (37year old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Levofloxacin (1µg/ml) which showed sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Ciprofloxacin has not showed sensitivity against this *Pseudomonas spp.*

All cephalosporin (cephradine, cefuroxime, ceftriaxone, cefixim) group antibiotics have not showed sensitivity effect against this *Pseudomonas spp.*

Azithromycin and vancomycin have not effect against this *Pseudomonas spp.*

Table 19: Antibacterial study of *Pseudomonas spp.* against Standard antibiotics powder

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

Sample 20: Resistance pattern of *Salmonella typhy*

Salmonella typhy has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the blood of a female patient (28 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (0.25µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Ciprofloxacin (0.125µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤1µg/ml, which compelled to the standard value of CLSI.

Cephradine (0.25 µg/ml) showed very high sensitivity against this Salmonella typhy; with a MIC range ≤2µg/mL, which compelled to the standard value of DIN.

Cefixim, Cefuroxime and ceftriaxone antibiotics have not showed sensitivity effect against this *Salmonella typhy.*

Azithromycin (8µg/mL) showed sensitivity against this *Salmonella typhy* but CLSI and EUCAST stated that they might be active in vitro but not clinically effective.

Vancomycin (4µg/mL) showed sensitivity against this *Salmonella typhy* but CLSI and EUCAST stated that they might be active in vitro but not clinically effective.

Table 20: Antibacterial study of *Salmonella typhy* against Standard antibiotics powder

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

Sample 21: Resistance pattern of *Salmonella typhy*

Salmonella typhy has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the blood of a male patient (30 years old). The

sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (0.0625µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Ciprofloxacin (0.25µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤1µg/ml, which compelled to the standard value of Clinical Laboratory Standard Institute (CLSI).

Cephradine Cefixim and Cefuroxime antibiotics have not showed sensitivity effect against this *Salmonella typhy.*

Ceftriaxone (1µg/ml) which showed sensitivity against this clinical isolate; with a MIC range ≤1µg/ml, which compelled to the standard value of CLSI.

Azithromycin (16µg/mL) showed sensitivity against this *Salmonella typhy* but CLSI and EUCAST stated that they might be active in vitro but not clinically effective. Vancomycin antibiotic has not showed sensitivity effect against this *Salmonella typhy.*

Table 21: Antibacterial study of *Salmonella typhy* against Standard antibiotics powder

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

Sample 22: Resistance pattern of *Salmonella typhy*

Salmonella typhy has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the blood of a female patient (17 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (4µg/ml) which showed not sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which not compelled to the standard value of CLSI.

Ciprofloxacin (0.125µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤1µg/ml, which compelled to the standard value of CLSI.

Cephradine (0.25µg/ml) showed sensitivity against this *Salmonella typhy*; with a MIC range ≤2µg/mL, which compelled to the standard value of German institute for standardization (DIN).

Cefixim and Cefuroxime antibiotics have not showed sensitivity effect against this *Salmonella typhy*.

Ceftriaxone (32µg/ml) which showed not sensitivity against this clinical isolate; with a MIC range ≤1µg/ml, which not compelled to the standard value of CLSI.

Azithromycin (8µg/mL) showed sensitivity against this *Salmonella typhy* but CLSI and EUCAST stated that they might be active in vitro but not clinically effective

A vancomycin antibiotic has not showed sensitivity effect against this *Salmonella typhy*.

Table 22: Antibacterial study of *Salmonella typhy* against Standard antibiotics powder

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

Sample 23: Resistance pattern of *Salmonella typhy*

Salmonella typhy has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the blood of a male patient (7 years old). The sample was subjected to sensitivity test against standard antibiotic powder and the result was measured by observing minimum inhibitory concentration (MIC) standard range.

Here, levofloxacin (0.5µg/ml) which showed sensitivity against this clinical isolate; with a MIC range ≤2µg/ml, which compelled to the standard value of CLSI.

Ciprofloxacin (0.5µg/ml) which showed very high sensitivity against this clinical isolate; with a MIC range ≤1µg/ml, which compelled to the standard value of CLSI.

Cephradine (32µg/ml) showed not sensitivity against this *Salmonella typhy*; with a MIC range ≤2µg/mL, which not compelled to the standard value of German institute for standardization (DIN).

Cefixim and Cefuroxime antibiotics have not showed sensitivity effect against this *Salmonella typhy*.

Ceftriaxone (2µg/ml) which showed sensitivity against this clinical isolate; with a MIC range ≤1µg/ml, which compelled to the standard value of CLSI.

Azithromycin (0.25µg/mL) showed sensitivity against this *Salmonella typhy* but CLSI and EUCAST stated that they might be active in vitro but not clinically effective.

A vancomycin antibiotic has not showed sensitivity effect against this *Salmonella typhy*.

Table 23: Antibacterial study of *Salmonella typhy* against Standard antibiotics powder

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

Chapter-6

Conclusion

Conclusion

From my research work, I find out that pathogenic bacteria have sensitivity effect and also resistant effect. Here, *E.coli* has total 9 strains and gives resistant activity against antibiotics standard powder but majority of the strains sample gives highest sensitivity MIC values that are Levofloxacin (0.015625µg/ml) for sample 9, Ciprofloxacin (0.03125µg/ml) for sample 1, Cepharadin (0.5µg/ml) for sample 2, Ceftriaxone (0.0625µg/ml) for sample 5 & Azithromycin (0.0625µg/ml) for sample 7. *E.coli* sample 4 is resistant against all antibiotics. Here, *Klebsiella spp.* total 4 strain sample some gives highest sensitivity MIC values that are Levofloxacin (0.0625µg/ml) for sample 10, Ciprofloxacin (0.25µg/ml) for sample 10, Cepharadin (2µg/ml) for sample 10 & Azithromycin (0.0625µg/ml) for sample 13. *Staphylococcus aureus* total 2 strains some gives highest sensitivity MIC values that are Levofloxacin (0.0625µg/ml), Cepharadine (0.125µg/ml), Cefuroxime (0.5µg/ml), Ceftriaxone (0.5µg/ml) & Azithromycin (0.0625µg/ml) for sample 14. *Acinetobector spp*. has 1 strain gives a highest sensitivity MIC values that are Levofloxacin (0.5µg/ml) & Ceftriaxone (4µg/ml) for sample 16. *Pseudomonas spp*. total 3 strains some gives highest sensitivity MIC values that are Levofloxacin (1µg/ml) for sample 19, Ciprofloxacin (0.03125µg/ml) for sample 17 & Azithromycin (1µg/ml) for sample 17. *Pseudomonas spp*. sample 18 is resistant against all antibiotics. *Salmonella typhy* total 4 strains some gives highest sensitivity MIC values that are Levofloxacin (0.0625µg/ml) for sample 21, Ciprofloxacin (0.125µg/ml) for sample 20 & 22, Cepharadin (0.25µg/ml) for sample 20 &22, Ceftriaxone (1µg/ml) for sample 21 & Azithromycin (0.25µg/ml) for sample 23.

It may be concluded from the study that the Fluoroquinolone group antibiotic Levofloxacin showed sensitivity effect against Gram positive and Gram negative bacteria. Macrolide group antibiotic Azithromycin showed sensitivity and resistant effect against Gram positive and Gram negative bacteria. β-lactam group antibiotics cephalosporins showed sensitivity and resistant effect against Gram positive and Gram negative bacteria. Glycopeptides group antibiotic Vancomycin showed resistant effect against Gram positive bacteria.

Chapter-7

Reference

Reference

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