In Vitro Minimum Inhibitory Concentration (MIC) of Antibiotics Against Clinically Isolated Bacteria

Submitted By

Zulfia Nafsin

ID: 2013-03-79-038

Supervisor

Dr.Shamsun Nahar Khan

Chairperson& Associate Professor

Department of Pharmacy

East West University,Dhaka

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

DEDICATION

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

Declaration by the Research Candidate

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

_____

Zulfia Nafsin

ID: 2013-03-79-038

Department of Pharmacy

East West University

Date: 09.02.2016

Certificate by the Supervisor

This is to certify that the dissertation entitled **"In Vitro Minimum Inhibitory Concentration (MIC) of Antibiotics Against Clinically Isolated Bacteria"** submitted to the department of pharmacy, East West University in partial fulfilment of the requirements for the degree of Masters of Pharmacy was carried out by **Zulfia Nafsin (ID: 2013-03-79-038)** under your guidance and supervision and that no part of the research has been submitted for any other degree. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

_____

Dr. Shamsun Nahar Khan

Chairperson & Associate Professor ,

Department of Pharmacy,

East West University ,

Certificate by the Chairperson

This is to certify that the thesis entitled "**In Vitro Minimum Inhibitory Concentration (MIC) of Antibiotics Against Clinically Isolated Bacteria"** 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 is a record of original and genuine research work carried out by **Zulfia Nafsin** 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

MIC is the lowest concentration of an antibiotic required to inhibit the growth of an organism. MIC was done against eight conventional antibiotics e.g. Cephradine, Cefuroxime, Ceftriaxone, Cefixime, Vancomycin , Azithromycin , Ciprofloxacin, Levofloxacin. Enterococci sp, E.coli , Pseudomonus, Klebsiella, were collected from hospital . Antibiotics were subjected to sensitivity test against this clinical isolates. Seventeen autoclaved test tube were taken, of which nine were marked 1 to 14 and the rest three were assigned as C_M (medium), C_S (medium+ sample) and C_I (medium+ inoculums). Then 10µl of the diluted inoculums of organism (1.5×10^6cells/ml) was added to each of the fourteen test tubes and mixed well. The terbidity of Inoculums must be same as McFarland Standard. The results compelled to the standard value of CLSI, EUCAST . Most of the time cephalosporine group antibiotics showed resistance against clinically isolated bacteria , but Ceftriaxone from this group with MIC range 0.5mg/L ,compelled to the standard value of CLSI, against *E.coli* sample 3 , so it is considered effective against this strain of *E.coli* . The highest MIC value for levofloxacin was 0.0625 mg/L against E.coli sample 3. levofloxacin also effective against *E .coli* sample 4, sample5 & sample 1 with MIC value 0.25mg/L ,0.25 mg/L & 2 respectively . The highest MIC value for ciprofloxacin against *E.coli* sample 4 & sample 5 was 0.5 mg/L The highest value of pseudomonas against Levofloxacin & Ciprofloxacin was 0.5 mg/L & 0.0625 mg/L respectively for sample 1. The highest value for Klebsiella sample 1 & sample 4 against Levofloxacin was 0.5 mg/L & 0.25 respectively. The highest MIC value for Klebsiella sample 1 & sample 4 against Ciprofloxacin was o.25 mg/L & 1 mg/L respectively .Ceftriaxone also gives activity with MIC range 0.5 mg/L against Klebsiella sample 1 , otherwise Macrolide group antibiotic Azithromycin & cell wall synthesis inhibitors Vancomycin is totally resistance against all strains of klebsiella & *E.coli* . Enterococci & Acinobactor sp. was totally resistance against our used antibiotics.

Key words : Antibiotics, Clinical isolates, Dilution test, Sensitivity , MIC, Bacteria

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CHAPTER 1
INTRODUCTION

Historically, most in vitro susceptibility testing was performed by disk diffusion (Kirby-Bauer) method. The size of the growth-free zone determined whether the bacterium was considered to be susceptible, resistant or intermediate to a particular antibiotic. While used as a guide to select an effective antibiotic, Kirby-Bauer testing could not tell the clinician the exact concentration of antibiotic needed to achieve a therapeutic result. Now, by a quantitative method of susceptibility testing known as the minimum inhibitory concentration (MIC), the precise concentration of antibiotic required to inhibit growth of a pathogen can be determined. Most antibiograms will include MICs in order to determine the most effective antibiotic that will result in effective treatment. This guide provides a detailed explanation of the following concepts important in implementing the MIC:

• The MIC number is the lowest concentration (in μg/ml) of an antibiotic that inhibits the growth of a given strain of bacteria.

• An MIC number for one antibiotic cannot be compared to the MIC number for another antibiotic.

• The choice of antibiotic should be based on the MIC number, the site of infection and an antibiotic's breakpoint. Consider safety, ease of use and cost when determining the optimum antibiotic .

1.1 Mechanism of drug resistance:

The development and spread of resistance to currently available antibiotics is a worldwide concern. Bacterial resistance is an increasing threat to the successful treatment of infectious diseases. As bacterial resistance continues to evolve, some pathogens that were once considered routine to treat are developing, or have developed, resistance to almost every antibacterial agent currently available. Several mechanisms have evolved in microorganisms, which confer them with antimicrobial resistance.

Three mechanisms predominate in antimicrobial resistance:

1) Enzymatic inactivation of the antimicrobial agent,

2) Substitutions, amplifications or modifications of the drug target reducing the affinity of the drug to the target or

3) Reduced access of the antimicrobial agents to the target by means of permeability barriers or efflux pumps.

These mechanisms can either chemically modify the antibiotic, or it becomes inactive through physical removal from the cell, or modify target site so not recognized by the antibiotics. (Chanda .S, *et.al*, 2010)

Examples include:

Methicillin-resistant staphylococci, pneumococci resistant to penicillin and macrolides, vancomycin-resistant enterococci as well as multi-drug resistant Gram-negative organisms and fungi. (Chanda .S, *et.al*, 2010)

1.2 Role of pathogens in infection:

S. aureus is a facultative anaerobic organism, which causes food poisoning and usually grows on the nasal membrane and skin. It causes boils, abscesses, wound infection, pneumonia, toxic shock syndrome and other diseases.

Klebsiella species cause diseases such as pneumonia, urinary and respiratory tract infections.

K. pneumoniae are widely distributed in hospitals and are increasingly being isolated from community-acquired infections.

S. typhi is a serious public health problem in developing countries and represents a constant concern for the food industry.

P. mirabilis is a secondary invader of ulcers, pressure sores, septicemia and occasionally meningitis and chest infections.

C. albicans is the agent of candidisis; is one of the most pervasive pathogenic fungi, especially infecting immune compromised hosts, in which it can invade various tissues.

C. tropicalis is one of the non-albicans candida strains that are emerging in fungal infections.

C. glabrata is a highly opportunistic pathogen of urogenital tract and of the blood stream. It is especially prevalent in HIV positive people. (Chanda .S, *et.al*, 2010)

1.3 Approaches towards natural drugs:

In recent years, multiple drug resistance in human pathogenic microorganisms developing due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation has necessitated a search for new antimicrobial compounds and for this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against pathogenic microbial strains.The emergence of antibiotic resistance is further complicated by the fact that bacteria and their resistant genes are traveling faster and further. We are facing not only epidemics but pandemics of antibiotic resistance. Existing antibiotics are losing their effect at an alarming rate, but development of new antibiotics is declining. There is a tremendous need for novel antimicrobial agents from different sources. Screening of plants with validated methods can lead to identify potentially useful molecules against infectious disease. Medicinal plants produce a large number of secondary metabolites with antimicrobial effects on pathogens. All parts of plants individually or in combination show antimicrobial properties. A significant part of the chemical diversity produced by plants is thought to protect plants against microbial pathogens. Many medicinal plants remain unexplored; screening of antibiotic resistance modifying compounds from plants sources are expected to provide the basis for identifying leads for the isolation of therapeutically useful compounds. The antimicrobial constituents are present in all parts of the plant viz. bark, stalks, leaves, fruits, roots, flowers, pods, seeds, stems, latex, hull and fruit rind . Recent research has revealed that fruit peels and seeds, such as grape seeds and peels pomegranate peel, wampee peel and mango seed kernel may potentially possess antimicrobial property. (Chanda .S, *et.al*, 2010)

1.3.1 Combination Therapy:

Antibiotics are frequently used in combination for the following reasons: (1) to treat a life-threatening infection; (2) to prevent emergence of bacterial resistance; (3) to treat mixed infections of aerobic and anaerobic bacteria; (4) to enhance antibacterial activity (synergy); and

(5) to use lower doses of a toxic drug. Combined treatment is reasonable when the precise agents of a serious infection are unknown. Use of two or more drugs to prevent the emergence of resistance is effective for tuberculosis and for therapy of some chronic infections. The use of combinations to achieve synergy is more complicated. Synergy occurs when a combination of two drugs causes inhibition or killing when used at a fourfold-lower concentration than that of either component drug used separately.

1.4 Structure of the Bacteria:

1.4.1 Gram-Positive Cell Structure:

• The Gram-positive cell wall is thick and consists of 90% peptidoglycan

• Teichoic acids link various layers of peptidoglycan together. Teichoic acids also regulate the autolysin activity in this complex equilibrium.

• The cytoplasmic membrane (which defines the intracellular space) consist of:

- a lipid bilayer
- intrinsic proteins which are hydrophobic (mostly enzymes involved in respiration and transmembrane transport)
- extrinsic proteins which are hydrophilic

• Penicillin-Binding Proteins (PBPs): periplasmic space proteins involved in peptidoglycan synthesis (glycosyltransferase, transpeptidase and carboxypeptidase activities).

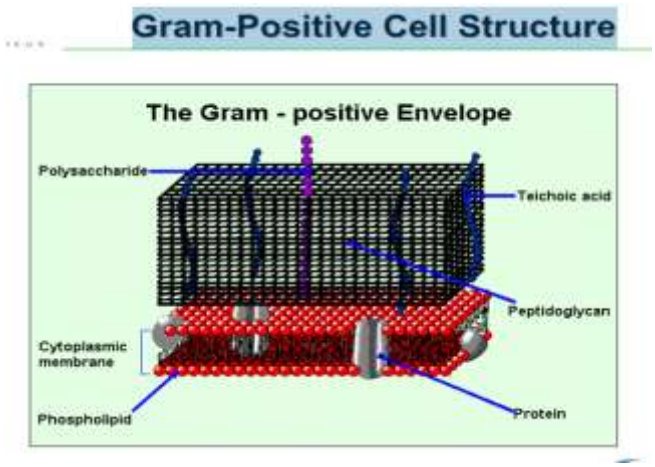

Fig-1.1 Image of gram positive bacteria

1.4.2 Gram-Negative Cell Structure:

• The outer membrane is made up of:

- phospholipids
- endotoxin or lipopolysaccharide (LPS) - plays an important role in the antibiotic entry into the cell
- proteins including the porins (complexes of three proteins) form aqueous channels that provide a route across the outer membrane for all the water-soluble compounds needed by the bacterium

• The periplasmic space contains:

- peptidoglycan – 5-20% of cell wall
- various enzymes (in particular, ß-lactamases)

• The cytoplasmic membrane (which defines the intracellular space) consists of:

- a lipid bilayer
- intrinsic proteins which are hydrophobic (mostly enzymes involved in respiration and transmembrane transport)
- extrinsic proteins which are hydrophilic

• Penicillin-Binding Proteins (PBPs) - periplasmic space proteins involved in peptidoglycan synthesis (glycosyltransferase, transpeptidase and carboxypeptidase activities).

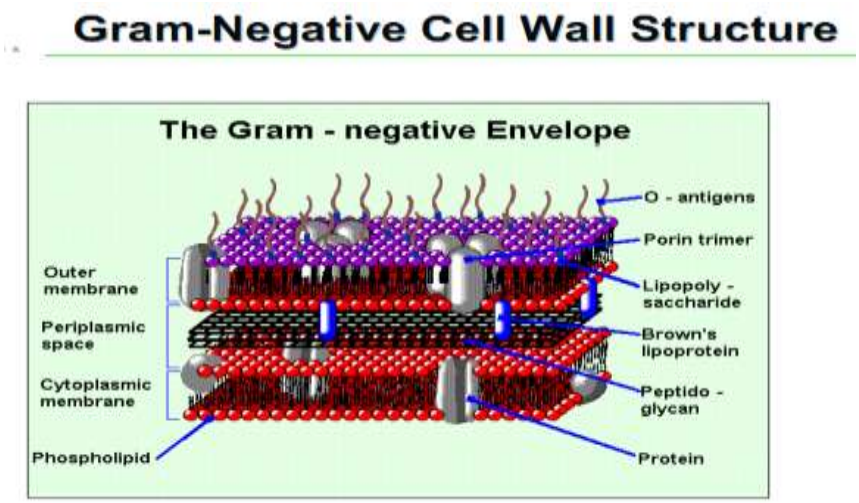

Fig-1.2 Image of gram negative bacteria

1.5 Description of the Bacteria:

Escherichia coli:

Escherichia coli (also known as *E.coli*) is a Gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strainsare harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. Yet, *E. coli* is an essential organism to human. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, and preventing colonization of the intestine with pathogenic bacteria. *E. coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards.

Fig-1.3 Microscopic picture of E.coli

Pseudomonas:

Pseudomonas is a genus of Gram-negative, aerobic gammaproteobacteria, belonging to the family Pseudomonadaceae containing 191 validly described species.The members of the genus demonstrate a great deal of metabolic diversity, and consequently are able to colonize a wide range of niches.Their ease of culture *in vitro* and availability of an increasing number of *Pseudomonas* strain genomesequences has made the genus an excellent focus for scientific

research; the best studied species include *P. aeruginosa* in its role as an opportunistic human pathogen, the plant pathogen *P. syringae*, the soil bacterium *P. putida*, and the plant growth-promoting *P. fluorescens*.

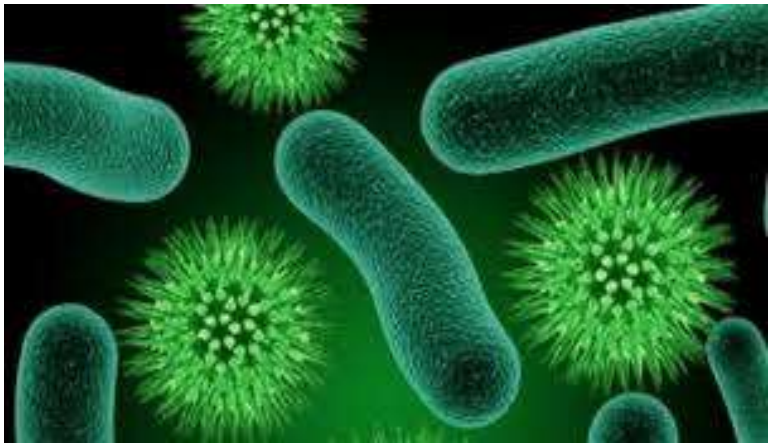

Fig-1.4 Microscopic picture of Pseudomonus

Salmonella:

Salmonella is a genus of rod-shaped Gram-negative bacteria ofthe Enterobacteriaceae family. The two species of *Salmonella* are *Salmonella bongori* and *Salmonella enterica*. *Salmonella enterica* is further divided into six subspecies and over 2500 serovars.Salmonellae are found worldwide in both cold-blooded and warm-blooded animals, and in the environment. Strains of *Salmonella*cause illnesses such as typhoid fever, paratyphoid fever, and food poisoning.

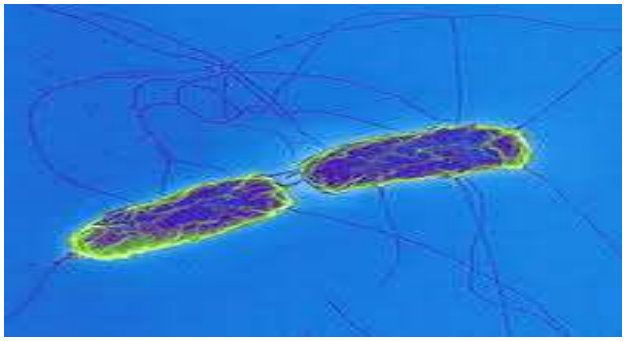

Fig-1.5 Microscopic picture of Salmonella typhi

Klebsiella:

Klebsiella is a genus of nonmotile, Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule. It is named after the German microbiologist Edwin

Klebs (1834–1913).*Klebsiella* species are found everywhere in nature. This is thought to be due to distinct sublineages developing specific niche adaptations, with associated biochemical adaptations which make them better suited to a particular environment. They can be found in water, soil, plants, insects, animals, and humans.

Fig-1.6 Microscopic picture of Klebsiella

Acinetobacter:

Acinetobacter is a genus of Gram-negative bacteria belonging to the wider class of Gammaproteobacteria. *Acinetobacter* species are oxidase-negative and non-motile, and occur in pairs under magnification.They are important soil organisms, where they contribute to the mineralization of, for example, aromatic compounds.*Acinetobacter* species are a key source of infection in debilitated patients in the hospital, in particular the species *Acinetobacter baumannii*.

Fig-1.7 Microscopic picture of Acinobector

Enterococcus:

Enterococcus is a large genus of lactic acid bacteria of the phylum Firmicutes. Enterococci are Gram-positive cocci that often occur in pairs (diplococci) or short chains, and are difficult to distinguish from streptococci on physical characteristics alone.Two species are common commensal organisms in the intestines of humans: *E. faecalis* (90-95%) and *E. faecium* (5-10%). Rare clusters of infections occur with other species, including *E. casseliflavus*, *E. gallinarum*, and *E. raffinosus*.

Fig-1.8 Microscopic picture of Enterococcus

1.6 Antibiotic Classification:

Grouped by Structure and Function

Five functional groups cover most antibiotics

1. Inhibitors of cell wall synthesis

2. Inhibitors of protein synthesis

3. Inhibitors of membrane function

4. Anti-metabolites

5. Inhibitors of nucleic acid synthesis

1.6.1 Inhibitors of Cell Wall Synthesis:

Beta-lactams

- Penicillins
- Cephalosporins
- Monobactams
- Carbapenems

Glycopeptides

Fosfomycins

Penicillins:

Penicillin is a class of drugs with a characteristic ring (β-lactamring). Penicillin inhibits its target protein by mimicking D-alanine.

Fig-1.9 Penicillin

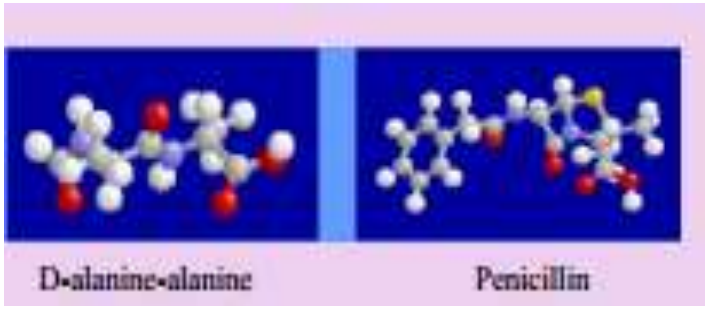

Fig-1.10 Comparison of Penicillin and D-alanine-alanine

Mechanism of action:

Penicillin kills bacteria by inhibiting the proteins which cross-link peptidoglycans in the cell wall. When a bacterium divides in the presence of penicillin, it cannot fill in the "holes" left in its cell wall.

Cephalosporins:

The cephalosporins are derivatives of 7-amino-cephalosporanic acid and are closely related in structure to penicillin. They have a beta-lactam ring. They are relatively stable in dilute acid and are highly resistant to penicillinase.

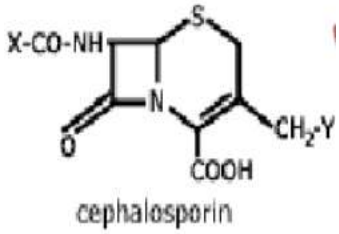

Fig-1.11 Image of cephalosporin

Mechanism of action:

Cephalosporins inhibit the peptido-glycan synthesis of bacterial cell wall in a manner similar to that of penicillin and are considered bactericidal.

Other inhibitiors of cell wall synthesis:

Vancomycin:

Vancomycin is an antibiotic produced by Streptococcus orientalis.

Mechanism of action:

- Binds to precursor units of bacterial cell walls, inhibiting cell wall synthesis, also inhibits RNA synthesis
- bactericidal antibiotic for gram-positive bacteria in concentration of 0.5-10 µg/mL.

Pharmacologic effects:

1. Vancomycin is very effective against most staphylococci including those producing beta-lactamases and other G+ cocci such as streptococcus viridans, enterococci, and pneumococcus.

2. It is also active against clostridium species, Corynebacterium diphtheriae, and Bacillus anthracis.

1.6.2 Protein Synthesis Inhibitors:

• Macrolides - Lincosamides

• Aminoglycosides

• Tetracyclines

• Chloramphenicol

• Oxazolidinones

• Streptogramins

Mechanism of Protein Synthesis inhibitors:

Protein synthesis is a complex, multi-step process involving many enzymes as well as conformational alignment. However, the majority of antibiotics that block bacterial protein synthesis interfere with the processes at the 30S subunit or 50S subunit of the 70S bacterial ribosome. The aminoacyl-tRNA synthetases that activate each amino acid required for peptide synthesis are not antibiotic targets. Instead, the primary steps in the process that are attacked are (1) the formation of the 30S initiation complex (made up of mRNA, the 30S ribosomal subunit, and formyl-methionyl-transfer RNA), (2) the formation of the 70S ribosome by the 30S initiation complex and the 50S ribosome, and (3) the elongation process of assembling amino acids into a polypeptide.

Tetracyclines, including doxycycline, prevent the binding of aminoacyl-tRNA by blocking the A (aminoacyl) site of the 30S ribosome. They are capable of inhibiting protein synthesis in both 70S and 80S (eukaryotic) ribosomes, but they preferentially bind to bacterial ribosomes due to structural differences in RNA subunits. Additionally, tetracyclines are effective against bacteria by exploiting the bacterial transport system and increasing the concentration of the antibiotic

within the cell to be significantly higher than the environmental concentration.

Aminoglycoside antibiotics have an affinity for the 30S ribosome subunit. Streptomycin, one of the most commonly used aminoglycosides, interferes with the creation of the 30S initiation complex. Kanamycin and tobramycin also bind to the 30S ribosome and block the formation of the larger 70S initiation complex.

Erythromycin, a macrolide, binds to the 23S rRNA component of the 50S ribosome and interferes with the assembly of 50S subunits. Erythromycin, roxithromycin, and clarithromycin all prevent elongation at the transpeptidation step of synthesis by blocking the 50S polypeptide

export tunnel. Elongation is prematurely terminated after a small peptide has been formed, but cannot move past the macrolide roadblock.

Peptidyl transferase is a key enzyme involved in translocation, the final step in the peptide elongation cycle. Lincomycin and clindamycin are specific inhibitors of peptidyl transferase, while macrolides do not directly inhibit the enzyme. (Washington JA.1996)

1.6.3 Inhibitors of membrane function:

Mechanism:

Bacterial Cytoplasmic Membranes

Biologic membranes are composed basically of lipid, protein, and lipoprotein. The cytoplasmic membrane acts as a diffusion barrier for water, ions, nutrients, and transport systems. Most workers now believe that membranes are a lipid matrix with globular proteins randomly distributed to penetrate through the lipid bilayer. A number of antimicrobial agents can cause disorganization of the membrane. These agents can be divided into cationic, anionic, and neutral agents. The best-known compounds are polymyxin B and colistemethate (polymyxin E). These high-molecular-weight octapeptides inhibit Gram-negative bacteria that have negatively charged lipids at the surface. Since the activity of the polymyxins is antagonized by Mg^{2+} and Ca^{2+}, they probably competitively displace Mg^{2+} or $Ca^{2+}$from the negatively charged phosphate groups on membrane lipids. Basically, polymyxins disorganize membrane permeability so that nucleic acids and cations leak out and the cell dies. The polymyxins are of virtually no use as systemic agents since they bind to various ligands in body tissues and are potent toxins for the kidney and

nervous system. Gramicidins are also membrane-active antibiotics that appear to act by producing aqueous pores in the membranes. They also are used only topically.

Example:Cyclic Lipopeptides,Daptomycin

1.6.4 Inhibitors of nucleic acid synthesis:

Mechanism:

Antimicrobial agents can interfere with nucleic acid synthesis at several different levels. They can inhibit nucleotide synthesis or interconversion; they can prevent DNA from functioning as a proper template; and they can interfere with the polymerases involved in the replication and transcription of DNA.

Interference with Nucleotide Synthesis:

A large number of agents interfere with purine and pyrimidine synthesis or with the interconversion or utilization of nucleotides. Other agents act as nucleotide analogs that are incorporated into polynucleotides.

Flucytosine (5-fluorocytosine) is an antifungal agent that inhibits yeast species. It is converted in the fungal cell to 5-fluorouracfl, which inhibits thymidylate synthetase resulting in a deficit of thymine nucleotides and impaired DNA synthesis. Adenosine arabinoside inhibits viruses. It is phosphorylated in virus-infected cells and acts as a competitive analog of DATP, inhibiting the incorporation of DATP into DNA. Acyclovir is a nucleoside analog that, after being converted to a triphosphate, inhibits the thymidine kinase and DNA polymerase of herpes viruses. Zidovudine (AZT) inhibits human immunodeficiency virus (HM replication by interfering with viral RNA-dependent DNA polymerase (reverse transcriptase).

Agents That Impair the Template Function of DNA:

A number of substances bind to DNA by intercalation. None of them is useful as an antibacterial agent; however, chloroquine and miracil D (lucanthone) inhibit plasmodia and schistosomes, respectively. These agents are thought to intercalate into the DNA and thereby to inhibit further nucleic acid synthesis. Acridine dyes such as proflavine act by this intercalation mechanism, but because they are toxic and carcinogenic in mammals they cannot be used as antibacterial agents.

Inhibition of DNA-Directed DNA Polymerase:

Rifamycins are a class of antibiotics that inhibit DNA-directed RNA polymerase. Polypeptide chains in RNA polymerase attach to a factor that confers specificity for the recognition of promoter sites that initiate transcription of the DNA. Rifampin binds no covalently but strongly to a subunit of RNA polymerase and interferes specifically with the initiation process. However, it has no effect once polymerization has begun.

Inhibition of DNA Replication:

DNA gyrase and topoisomerase I act in concert to maintain an optimum supercoiling state of DNA in the cell. In this capacity, DNA gyrase is essential for relieving torsional strain during replication of circular chromosomes in bacteria. The enzyme is a tetrameric protein composed of two A and two B subunits. A transient, covalent bond between the A subunit and DNA occurs during the double strand passage reaction catalyzed by gyrase. Quinolones such as nalidixic acid, bind to the cleavage complex composed of DNA and gyrase during this strand passage. This interaction of quinolone acts to stabilize the cleavage intermediate which has a detrimental effect on the normal DNA replication process. The effects of this inhibition result in the death of the bacterial cell. The newer fluoroquinolones such as ciprofloxacin, norfloxacin, and ofloxacin also interact with DNA gyrase and possess a broad spectrum of antimicrobial activity.(Neu .HC ,*et al.*,1996).

Classification:

Classification of Inhibitors of nucleic acid synthesis

	Common Name
Quinolones 1st Generation – Narrow Spectrum	Nalidixic Acid Cinoxacin

Fluoroquinolones	Ciprofloxacin Enoxacin Garenoxacin Levofloxacin Lomefloxacin Norfloxacin Ofloxacin Gatifloxacin Moxifloxacin

1.6.5 Anti metabolites:

Mechanism:

A drug may be classified by the chemical type of the active ingredient or by the way it is used to treat a particular condition. Each drug can be classified into one or more drug classes.

Antimetabolites are drugs that interfere with one or more enzymes or their reactions that are necessary for DNA synthesis. They affect DNA synthesis by acting as a substitute to the actual metabolites that would be used in the normal metabolism (for example antifolates interfere with the use of folic acid).

Antimetabolites are drugs used in cancer chemotherapy. Cancer cells divide more rapidly compared to normal cells so antimetabolites affect cancer cell replication more than they affect normal cell replication. (Neu HC, *et al*, 1996)

Example:

Folate Pathway Inhibitors:

Sulfonamides

- ✓ Bacteriostatic
- ✓ Introduced in 1930's – first effective systemic antimicrobial agent

- ✓ Used for treatment of acute, uncomplicated UTI's

Trimethoprim/Sulfamethoxazole

- ✓ TMP/SXT is bactericidal
- ✓ Broad spectrum
- ✓ Synergistic action

Spectrum of Action: Prescribed for treatment of certain UTI's, otitis media in children, chronic bronchitis in adults, enteritis and Travelers' Diarrhea. (Neu HC, *et al*., 1996)

1.7 MIC(Minimum Inhibitory Concentration):

The MIC, or minimum inhibitory concentration, is the lowest concentration (in µg/ml) of an antibiotic that inhibits the growth of a given strain of bacteria. A quantitative method of susceptibility testing, an MIC helps determine which class of antibiotic is most effective. This information can lead to an appropriate choice of an antibiotic that will increase chances of treatment success and help in the fight to slow antibiotic resistance.("Microbiology Guide To Interpreting Minimum Inhibitory Concentration(MIC)".

1.8 Technique of Reporting MIC:

Next to each antibiotic is the susceptibility interpretation: S (sensitive), I (intermediate) or R (resistant), followed by the MIC in µg/ml. Sensitive implies that the organism is inhibited by the serum concentration of the drug that is achieved using the recommended dosage; intermediate includes isolates with MIC's that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates; and implies clinical efficacy in body sites where the drug is physiologically concentrated or when a higher than normal dosage of the drug can be used; and resistant implies that the organisms are resistant to the usually achievable serum drug levels. These interpretive standards have been established by the Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards (NCCLS).

1.9 Reasons of Not Performing MIC:

MICs are *not* performed when:

• The growth requirements of some organisms require the sensitivity testing to be performed by another method.

• Interpretive criteria is not available from CLSI. In these cases, recommended antibiotics will usually be reported based on clinical efficacy studies.

• The drug is known to be clinically ineffective against the organism, regardless of the in vitro results.

CHAPTER 2

Literature Review

Table-22 Some Literature Review

Sl No	Article Title	Investigation/ Result	References
1	Determination of Minimum Inhibitory Concentrations	This journal provide Standardized methods for determining MICs and MBCs are described in this paper. Like all standardized procedures, the method must be adhered to and may not be adapted by the user. The method gives information on the storage of standard antibiotic powder, preparation of stock antibiotic solutions, media, preparation of inoculam, incubation conditions, and reading and interpretation of results. .	Andrews,,J.(2001)Determination of *Minimum Inhibitory Concentration* Vol(48). Journal of antimicrobial chemotherapy. [Accessed:on 2001]
2	Establishing MIC breakpoints and the interpretation of in vitro susceptibility	This paper attempts to summarize the philosophy of the British Society for AntimicrobialChemotherapy(BSAC) Working Party in conjunction with EUCAST in its approach to setting	MacGowan,P.A&Wise,R *EstablishingMIC breakpoints and the interpretation of in vitro susceptibility tests.* Vol

	tests.	breakpoint and to update the activities of the Working Party since it initially published breakpoints.	(48). [Accessed:on 2001]
3	MicrobiologyGuide to InterpretingMinimum Inhibitory Concentration (MIC) .	This guide provides a detailed explanation of the following concepts important in implementing the MIC: • The MIC number is the lowest concentration (in µg/ml) of an antibiotic that inhibits the growth of a given strain of bacteria. (See the "What Is an MIC?" section.) • An MIC number for one antibiotic CANNOT be compared to the MIC number for another antibiotic. (See the "How Are MICs Used?" section.) • The choice of antibiotic should be based on the MIC number, the site of infection and an antibiotic's breakpoint. Consider safety, ease of use and cost when determining the optimum antibiotic	"Microbiology Guide To InterpretingMinimum InhibitoryConcentration (MIC)". (2013).*UK206-0613 / UK-MAR-EXT-3381* 1-14. Web. 2013.

4.	Rapid Broth Macrodilution Method for Determination of MICs for Mycobacterium avium Isolates.	A multicenter study was done to investigate the accuracy and reproducibility of a method for determining the MICs of antimicrobial agents against the Myco bacterium avium complex in 7H12 broth with the BACTEC system.	SIDDIQI, SALMAN H. et al. (1993)"Rapid Broth Macro dilution Method For Determination Of Mics For Mycobacterium Avium Isolates". *JOURNAL OF CLINICALMICROBIOLOGY* , Vol. 31.No. 9 (1993): 2332-2338. Web. 29 May 1993.
5.	A new method for determining the minimum inhibitory concentration of essential oils.	A new micro dilution method has been developed for determining the minimum inhibitory concentration (MIC) of oil-based compounds. The redox dye resazurin was used to determine the MIC of a sample of the essential oil of*Melaleuca alternifolia* (tea tree) for a range of Gram-positive and -negative bacteria.	Mann, C. and Markham, J. (1998). A new method for determining the minimum inhibitory concentration of essential oils. *Journal of Applied Microbiology*, [online] 84(4), pp.538–544. [Accessed 23 May 1997].

6.	Fruit and vegetable peels – strong natural source of antimicrobics.	The antimicrobial activity was evaluated by agar well diffusion method. The Mangifera indica peel showed best and promising antimicrobial activity. This study will definitely open, scope for future utilization of the waste products for therapeutic purpose.	Chanda .S, *et al*.,(2010) 'Fruit and vegetable peels – strong natural source of antimicrobics', Technology and education topics in applied microbiology and microbial biotechnology, A.Mendez-Vilas(ED). A Accessed on2010
7.	Antibacterial activity of selected medicinal plants against multiple antibiotic resistant uropathogens.	Antibacterial activity	Narayanan A.S *et.al* Toxicol Ind Health. 2012 Apr;28(3):238-44. doi: 10.1177/0748233711410911. Epub 2011 Jul 1.

8	Minimum inhibitory and minimum bactericidal concentrations of boron compounds against several bacterial strains	Boron compounds are essential micronutrients for many organisms. However, they negatively affect plant, soil, and water microflora if excessive amounts exist in irrigation water. Therefore, this study aimed to define the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of boric acid and borax by selecting the bacteria that can survive in all environments.	Tolga YILMAZ, Murat. "Minimum Inhibitory And Minimum Bactericidal Concentrations Of Boron Compounds Against Several Bacterial Strains". *Turk J Med Sci* 42.(Sup.2) (2012): 1423-1429. Web. 18 Jan. 2012.

CHAPTER 3

OBJECTIVE OF THE STUDY

Clinical significance

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. Applying MIC testing to a number of bacterial strains in the same species provides an estimate of the concentration that inhibits 50% (MIC_{50}) and 90% (MIC_{90}) of bacterial isolates and can indicate shifts in the susceptibility of bacterial populations to antibiotics, MICs are therefore often the starting point for larger preclinical evaluations of novel antimicrobial agents.

Aim of the study

- A lower MIC value indicates that less drug is required for inhibiting growth of the organism therefore, drugs with lower MIC scores are more effective antimicrobial agents.
- By identifying appropriate drugs and their effective concentrations, MIC scores aid in improving outcomes for patients and preventing evolution of drug-resistant microbial strains.
- An MIC helps determine which class of antibiotic is most effective. This information can lead to an appropriate choice of an antibiotic that will increase chances of treatment success and help in the fight to slow antibiotic resistance.

CHAPTER 4

METHODS & MATERIALS

Minimum inhibitory concentration is determined when a patient does not respond to treatment thought to be adequate, relapses while being treated or when there is immune suppression.

4.1 Dilution methods:

1. Broth dilution and Agar Dilution Method

Broth dilution testing allows the option of providing both quantitative (MIC) and qualitative (category interpretation) results.MIC can be helpful in establishing the level of resistance of a particular bacterial strain and can substantially affect the decision to use certain antimicrobial agents.

Broth Dilution can again be performed by 2 ways

1. Macro dilution: Uses broth volume of 1ml in standard test tubes .
2. Microdilution: Uses about 0.05 to 0.1 ml total broth volume and can be performed in a microtiter plate or tray .

The procedure for both macro and microdilution are same except the volume of the broth.

1. Agar dilution

MIC of an antibiotic is determined by using the following procedure

1. Preparation of antibiotic stock solution
2. Preparation of antibiotic dilution range
3. Preparation of agar dilution plates
4. Preparation of inoculum
5. Inoculation
6. Incubation
7. Reading and interpreting results

4.2 Study design

For the in vitro antimicrobial susceptibility test of different Active Pharmaceutical Ingredient (API) was collected from Incepta Pharmaceutical and Asiatic Laboratory. Different strains of E. coli, Pseudomonas spp.and Salmonella typhi, Klebsiella, Acinobactor,Staphylococusaureus and Enterococci were collected from Pathology department, Ibrahim Medical College (Birdem) Then the clinical isolates of these microorganisms were subcultured and MIC test was performed by measuring the minimum concentration value.

4.3 Period and place of the study

The duration of this study was 1 years and all the test was performed in the microbiological laboratory of East West University.

Table-20 List of Sample Used in the Test:

API Ingredient	Name of	Potency	Shelf
Levofloxacin USP	Asiatic laboratory Ltd	95.87%	June 2016
Azithromycin	Incepta Pharmaceutical	99.99%	January 2016
Cephradine	Asiatic laboratory Ltd	91.67%	June 2015
Ciprofloxacin	Incepta Pharmaceutical	99.99%	
Vancomycin HCL	Incepta Pharmaceutical	99.189%	
Ceftriaxone	Incepta Pharmaceutical	99.99%	

Cefuroxime Axetil	Incepta Pharmaceutical	79.51%
CefixImeMicronased	Incepta Pharmaceutical	99.99%

Table-21 List of Microorganisms Used in the Test

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

Apparatus & Solvent:

1. Sterile Test tubes
2. Inoculating loop
3. Sterile forceps
4. Sterile cotton
5. Sterile Petri dishes
6. Measuring cylinder
7. Distilled water
8. Sterile saline solution (Sodium Chloride)
9. Hot air oven (FN-500, Niive)
10. Bunsen burners
11. Micropipettes (2-20µl)
12. Sterile Micropipette tips
16. Nutrient Agar media
17. Nutrient broth media
18. Sample of Active Pharmaceutical Ingredient
19. Sample of microorganism
20. Ethanol(95%)

13. Laminar air-flow unit (ESCO, Singapore)
14. Autoclave(HIRAYAMA, Japan)
15. Incubator (BK 4266).

4.4 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.

Fig- 1.12 Autoclave

4.5 Preparation of Solution:

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

Weight of powder (mg) =

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

4.6 Preparation of inoculum:

The turbidity of inoculums should be same as McFarland standard.

- At first nutrient agar & nutrient broth is weighted& Autoclaved.
- Then, nutrient agar is poured into desired amount of Petri dishes& wait for drying.
- Then,1 loop full bacterial culture is transferred into Petri dishes &spread.
- Then, those Petri dishes are incubated over night for growth.
- After over night incubation, bacterial culture from Petri dish is transformed to the testube through loop(1loop full),which are already filled with nutrient broth.
- Then ,testtubes are incubated over night for bacterial growth.
- After , incubation we will get our desired inoculums.

Fig- 1.13 Laminar Airflow

Fig-1.14 Incubator

4.7 McFarland Standard:

McFarland standards are suspensions of either barium sulfate or latex particles that allow visual comparison of bacterial density (Fig. 1). 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 containingbetween 1×10^8 and 2×10^8 CFU/ml of E.coli.

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

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

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

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

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

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

Fig- 1.15 McFarland standards 0.5

FIG.- 1.16.McFarland standards (left to right) 0.5, 1.0, 2.0, 3.0, positioned in front of a Wickerham card.

4.8 Procedure:

1.Seventeen autoclaved test tube were taken, of which 14th 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 uniforml-y and again 1 ml of this mixture transferred to the third test tube. This process of serial dilution was continued up to the ninth test tube.

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

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

7.10µl of the inoculums (1.5×10^6cells/ml) was added to the control test tube C_I 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.

CHAPTER 5

RESULT & DISCUSSION

We, have compared our results with British Society For Antimicrobial Chemotherapy suggested EUCAST&CLSI MIC Breakpoint.

- EUCAST Is , European Committee on Antimicrobial Susceptibility Testing formed in 1997, restructured into present form in 2002. It divides Microorganism into three categories , susceptible, Resistant , Intermediate on the basis of concentration of an antimicrobial.

  - Susceptible : A micro organism is defined as susceptible of inhibited invitro by a concentration of an antimicrobial agent that is associated with a high likelihood of therapeutic success.

  - Resistant : A microorganism is defined as resistant if inhibited in vitroby a concentration of an antimicrobial agent that isassociated with a high likelihood of therapeutic failure

  - Intermediate : A micro organism is defined as intermediate by a level of antimicrobial agent activity associated with uncertain effect.

Breakpoint

A breakpoint is a chosen concentration (mg/L) of an antibiotic which defines whether a species of bacteria is susceptible or resistant to the antibiotic. If the MIC is less than or equal to the susceptibility breakpoint the bacteria is considered susceptible to the antibiotic. If the MIC is greater than this value the bacteria is considered intermediate or resistant to the antibiotic.

Isolate

Isolate is a pure culture of bacteria, all the same species and strain.

MIC

MIC is the Minimum Inhibitory Concentration. This is the lowest concentration of an antibiotic required to inhibit the growth of an organism. To identify the MIC the bacteria are added to plates containing varying concentrations of the antibiotic. The concentration of antibiotic is doubled in each successive plate and the MIC is found by identifying the first plate in which there is no visible colony after an incubation period.

Table1:Antibacterial study of *Enterococci*(55 BIRDEM EWU) against conventional antibiotics

		MIC break point			
Name of Antibiotic	**Sample sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistenc (mg/L)**	**Zone of inhibition cm**
Levofloxacin	**8**	**≤ 2**	**4**	**> 8**	**2.4/2.5**
Ciprofloxacin	**0.0625**	**≤ 1**	**2**	**> 4**	**R**
Azithromycin	**R**	**-------**	**--------**	**--------**	**2.2/2.3**
Vancomycin	**R**	**---------**	**---------**	**---------**	**1.7/1.8**
Cephradine	**R**	**----------**	**----------**	**----------**	**R**
Cefuroxime	**32mg/l**	**----------**	**----------**	**----------**	**1.8/1.9**
Ceftriaxone	**R**	**≤ 1**	**2**	**> 4**	**1.4/1.5**
Cefixime	**R**	**---------**	**---------**	**---------**	**R**

Discussion for the sample collected from 45 years old female patient pus

we have compared our MIC ranges with EUCAST suggested MIC breakpoint(These breakpointshave been used extensively to interpret MIC results). *Enterococci* has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the pas of a female patient(45 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.

According to, CLSI Cefuroxime, Ceftriaxone, Cefixime, Ceftriaxone , cephradine which belongs to Cephalosporine group is not clinically effective against Enterococci.

BSCI guideline stated that if the MIC is less than or equal to suscebility breakpoint bacteria is considered susceptible to the antibiotic, so,levofloxacin & Azithromycin with MIC range 8&0.0625 respectively fulfill this criteria, so those drugs are susceptible to conventional antibiotics.

levofloxacin & Ciprofloxacin which belongs to Fluoroquinolone group did not show any activity against conventional antibiotics.

Antibacterial study of Pseudomonas

Table 2:Antibacterial study of Pseudomonas(53 BIRDEM EWU)against conventional antibiotics

			MIC break point		
Name of Antibiotic	**Sample sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistance (mg/L)**	**Zone of inhibition of cm**
Levofloxacin	**R**	**≤ 2**	**4**	**≥ 8**	**R**
Ciprofloxacin	**R**	**≤ 1**	**2**	**≥ 4**	**R**
Azithromycin	**R**	--------	--------	--------	**R**
Vancomycin	**R**	---------	---------	--------	**R**
Cephradine	**R**	----------	----------	----------	**R**
Cefuroxime	**R**	---------	----------	---------	**R**
Ceftriaxone	**R**	---------	---------	---------	**R**
Cefixime	**R**	---------	---------	----------	**R**

Discussion is given bellow for sample 1 collected from 53 years old male patient urine

We have compared our MIC ranges with CLSI break point& (These breakpoints have been used extensively to interpret MIC results). *Pseudomonas* has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a female patient(53 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 & Ciprofloxacin, which belongs to Fluoroquinolone group did not show any activity against Pseudomonus species.

Azithromycin& Vancomycin from macrolide group did not show any activity against Pseudomonus species.

Cephradine,. Cefuroxime, Ceftriaxone, Cefixime Ceftriaxone from Cephalosporine group, are not effective against Pseudomonus species .

Table 3:Antibacterial study of Pseudomonas(58 BIRDEM EWU) against conventional antibiotics

			MIC break point		
Name of Antibiotics	**Sample sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistance (mg/L)**	**Zone of inhibition cm**
Levofloxacin	**0.5**	**≤ 1**	**2**	**>2**	**2.4/2.6**
Ciprofloxacin	**0.0625**	**≤ 2**	**1**	**>1**	**--------**
Azithromycin	**16**	**--------**	**-------**	**--------**	**2.0/2.1**
Vancomycin	**R**	**---------**	**---------**	**---------**	**1.5/1.6**
Cephradine	**R**	**----------**	**----------**	**---------**	**R**
Cefuroxime	**R**	**----------**	**----------**	**----------**	**0.7/0.8**
Ceftriaxone	**R**	**---------**	**---------**	**---------**	**1.2/1.3**
Cefixime	**R**	**---------**	**---------**	**---------**	**0.9/1.0**

Discussion is given bellow for sample 2 collected from 66 years old male patien's urine

We have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint(These breakpoints have been used extensively to interpret MIC results).). *Pseudomonas* has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient(58year 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.

Cephradine,. Cefuroxime, Ceftriaxone, Cefixime from Cephalosporine group, are not effective against Pseudomonus species .

Macrolide grouped Azithromycin is effective through our invitro procedure,but it is not clinically effective by following CLSI break point& EUCAST MIC breakpoint data.

According, to BSCI guideline if the MIC is less than or equal to suscebility breakpoint bacteria is considered susceptible to the antibiotic, so,levofloxacin & Ciprofloxacin fulfill this criteria, so those drugs are susceptible.

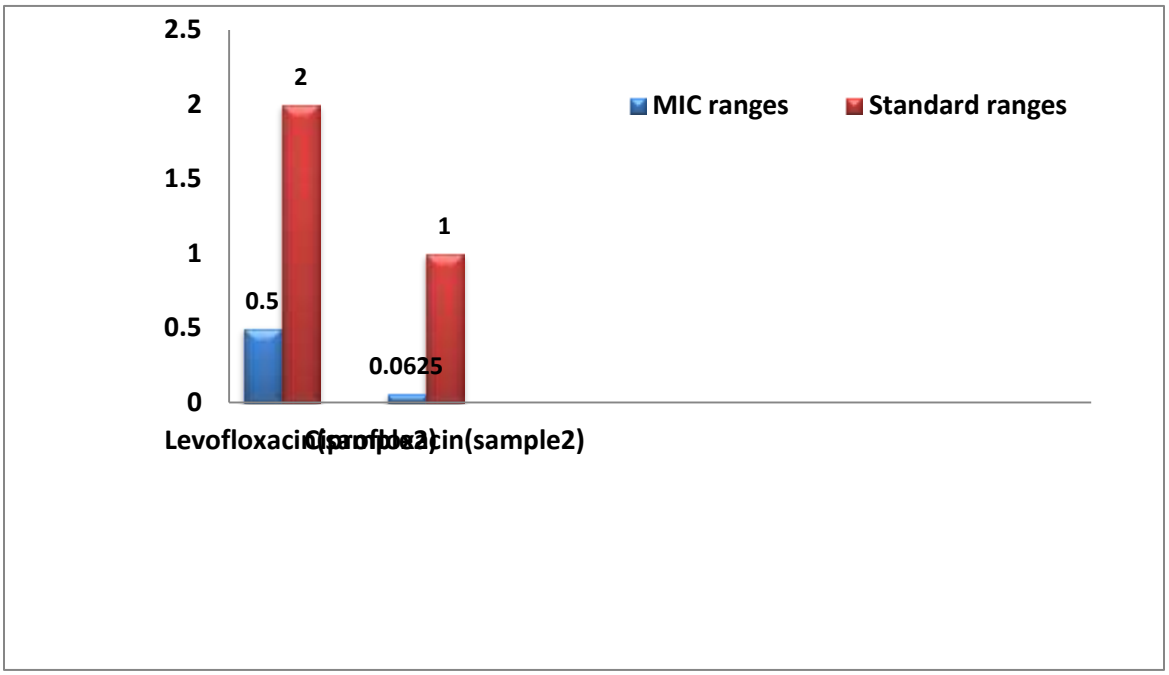

Fig 1.17: **Comparison of sensitivity between Antibiotics for pseudomonas**

Antibacterial study of E.coli

Table4:Antibacterial study of E.coli (47BIRDEM EWU) against conventional Antibiotics

				MIC break point	
Name of Antibiotics	**Sample sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistence (mg/L)**	**Zone of inhibition cm**
Levofloxacin	**2**	**≤ 2**	**4**	**≥ 8**	**1.5/1.6**
Ciprofloxacin	**4**	**≤ 1**	**2**	**≥ 4**	**2.0/2.1**
Azithromycin	**R**	**-------**	**---------**	**--------**	**R**
Vancomycin	**R**	**-------**	**---------**	**--------**	**4/1.5**
Cephradine	**32**	**≤ 2**	**---------**	**≥ 4**	**1.6/1.7**
Cefuroxime	**R**	**≤ 4**	**8-16**	**≥ 32**	**0.8/0.9**
Ceftriaxone	**32**	**≤ 1**	**--------**	**≥ 2**	**1.3/1.4**
Cefixime	**R**	**1**	**2**	**≥ 4**	**R**

Discussion is given bellow for sample 1collected from 51 years old male patien's urine

We have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint(These breakpoints have been used extensively to interpret MIC results).*E.coli* has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient(51year 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,Fluoroquinolone group, levofloxacin which showed sensitivity against this clinical isolate, with a MIC range ≤2µg/ml, which compelled to the standard value of CLSI,but another Fluoroquinolone groupe Ciprofloxacin is resistant against clinical isolates.

Cephradine, Cefuroxime, Ceftriaxone, Cefixime from Cephalosporine group showed resistant pattern which compelled to the standard value of CLSI against this clinical isolate

Macrolide grouped Azithromycin did not show any activity against this clinical isolate.

Table5 :Antibacterial study of E.coli (48BIRDEM EWU) against conventional antibiotic

			MIC break point		
Name of Antibiotics	**Sample sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistance (mg/L)**	**Zone of inhibition cm**
Levofloxacin	**R**	**≤ 1**	**2**	**> 2**	**R**
Ciprofloxacin	**R**	**≤ 0.5**	**R**	**> 1**	**0.7/0.8**
Azithromycin	**R**	**---------**	**--------**	**-------**	**R**
Vancomycin	**R**	**≤ 2**	**--------**	**> 4**	**R**
Cephradine	**R**	**≤ 2**	**--------**	**> 4**	**R**
Cefuroxime	**R**	**≤ 8**	**-------**	**> 8**	**R**
Ceftriaxone	**R**	**≤ 1**	**2**	**> 2**	**R**
Cefixime	**R**	**1**	**--------**	**1**	**R**

Discussion is given bellow for sample 2collected from 56 years old male patien's urine

we have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint(These breakpoints have been used extensively to interpret MIC results). *E.coli* has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient(51year 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 &Ciprofloxacin from Fluoroquinolone group is resistant against *E.coli* .

Here, Azithromycin& Vancomycin from Macrolide group is resistant against *E.coli* .

Cephradine, Cefuroxime, Ceftriaxone, Cefixime from Cephalosporine group showed resistant pattern which compelled to the standard value of CLSI against this clinical isolates .

Table6 :Antibacterial study of E.coli (49BIRDEM EWU) against conventional antibiotics

		MIC break point			
Name of Antibiotics	**Sample sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistence (mg/L)**	**Zone of inhibition cm**
Levofloxacin	**0.0625**	**≤ 2**	**4**	**≥ 8**	**2.3/2.4**
Ciprofloxacin	**R**	**≤ 1**	**2**	**≥ 4**	**R**
Azithromycin	**R**	**--------**	**-------**	**------**	**1.3/1.4**
Vancomycin	**R**	**--------**	**--------**	**-------**	**R**
Cephradine	**32**	**≤ 2**	**--------**	**≥ 4**	**R**
Cefuroxime	**R**	**≤ 4**	**8-16**	**≥ 32**	**1.0/1.1**
Ceftriaxone	**0.5**	**≤ 1**	**-------**	**≥ 2**	**1.4/1.5**
Cefixime	**R**	**≤ 1**	**2**	**≥ 4**	**R**

Discussion is given bellow for sample 3collected from 33 years old female patien's urine

We have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint(These breakpoints have been used extensively to interpret MIC results). *E.coli* has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a female patient(33year 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, with MIC range (0.0625 mg/L), from Fluoroquinolone group showed sensitivity against this clinical isolates. which compelled to the standard value of CLSI,but another Fluoroquinolone groupe Ciprofloxacin is resistant against clinical isolates.

Azithromycin& Vancomycin from Macrolide group is resistant against *E.coli* .

Cephradine, Cefuroxime, Cefixime from Cephalosporine group showed resistant pattern which compelled to the standard value of CLSI against this clinical isolate,but ceftriaxone from Cephalosporine group, with MIC range(0.5 mg/L), group showed sensitivity against this clinical isolates. which compelled to the standard value of CLSI.

Table7 :Antibacterial study of E.coli (51BIRDEM EWU) against conventional Antibiotics

			MIC break point		
Name of Antibiotics	**Sample sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistance (mg/L)**	**Zone of inhibition cm**
Levofloxacin	**0.25**	**≤ 1**	**2**	**> 2**	**2.2/2.4**
Ciprofloxacin	**0.5**	**≤ 0.5**	**1**	**> 2**	**2.0/2.1**
Azithromycin	**16**	**---------**	**--------**	**-------**	**0.8/0.9**
Vancomycin	**R**	**----------**	**--------**	**-------**	**2.2/2.4**
Cephradine	**R**	**≤ 2**	**---------**	**>4**	**1.8/1.9**
Cefuroxime	**R**	**≤ 8**	**--------**	**>8**	**0.9/1.0**
Ceftriaxone	**R**	**≤ 1**	**2**	**>2**	**1.92/2.0**
Cefixime	**R**	**1**	**--------**	**1**	**0.7/0.8**

Discussion is given bellow for sample 4 collected from 45 years old female patien's urine:

we have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint(These breakpoints have been used extensively to interpret MIC results). *E.coli* has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a female patient(45year 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, Ciprofloxacin which belongs to Fluoroquinolone group with MIC range 0.25mg/L & 0.5 mg/L respectively are effective aginst clinical isolates, which compelled to the standard value of CLSI.

Macrolide group Azithromycin showed its activity through invitro test ,but it is clinically ineffective according to CLSI break point& EUCAST MIC breakpoint. Macrolide group vancomycin did not show any activity aginst clinical isolates Cephradine, Cefuroxime, Ceftriaxone, Cefixime from cephalosporine group did not show any activity aginst clinical isolates .

Table8 :Antibacterial study of E.coli (52BIRDEM EWU) against conventional Antibiotics

			MIC break point		
Name of Antibiotics	**Sample sensitivity (mg/L)**	**Standard sensitivity**	**Intermediate (mg/L)**	**Resistance (mg/L)**	**Zone of inhibition**
Levofloxacin	**0.125**	**≤ 1**	**2**	**>2**	**2.1/2.2**
Ciprofloxacin	**0.5**	**≤ 0.5**	**1**	**>1**	**2.4/2.5**
Azithromycin	**2**	**-------**	**--------**	**-------**	**2.1/2.2**
Vancomycin	**32**	**-------**	**--------**	**---------**	**1.6/1.7**
Cephradine	**R**	**≤ 2**	**--------**	**> 4**	**1.2/1.3**
Cefuroxime	**R**	**≤ 8**	**--------**	**> 8**	**0.9/1.0**
Ceftriaxone	**R**	**≤ 1**	**2**	**> 2**	**R**
Cefixime	**R**	**1**	**--------**	**1**	**R**

Discussion is given bellow for sample 5collected from 39years old male patien's urine:

we have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint(These breakpoints have been used extensively to interpret MIC results). *E.coli* has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient(39 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.

Here,Levofloxacin, Ciprofloxacin from Fluoroquinolone group with MIC range 0.125mg/L&0.5mg/L respectively are effective against clinical isolates, which compelled to the standard value of CLSI.

Vancomycin& Azithromycin, from macrolide group has no clinically effectiveness, but it has invitro effectiveness.

Cephradine, Cefuroxime, Ceftriaxone, Cefixime are not effective aginst this clinical isolates.

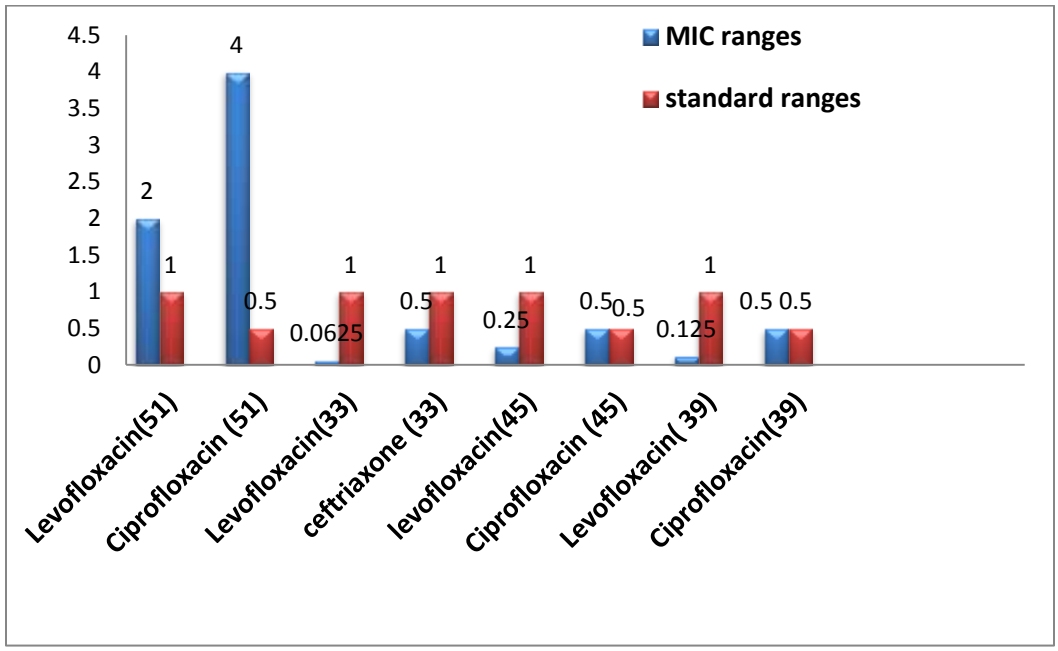

Fig 1.18: Comparison of sensitivity between antibiotics against E.coli

Table9 :Antibacterial study of E.coli (61BIRDEM EWU) against conventional Antibiotics

			MIC break point		
Name of Antibiotics	**Sample sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistance (mg/L)**	**Zone of inhibition cm**
Levofloxacin	**R**	**≤ 1**	**2**	**> 2**	**R**
Ciprofloxacin	**R**	**≤ 0.5**	**1**	**> 1**	**R**
Azithromycin	**R**	**--------**	**--------**	**------**	**R**
Vancomycin	**R**	**-------**	**--------**	**-------**	**R**
Cephradine	**R**	**≤ 2**	**---------**	**> 4**	**R**
Cefuroxime	**R**	**≤ 8**	**---------**	**> 8**	**R**
Ceftriaxone	**R**	**≤ 1**	**2**	**> 2**	**R**
Cefixime	**R**	**1**	**--------**	**1**	**R**

Discussion is given bellow for sample 6collected from 45years old male patien's urine:

We have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint(These breakpoints have been used extensively to interpret MIC results). *E.coli* has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a male patient(45 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.

Here, Levofloxacin &Ciprofloxacin from Fluoroquinolone group is resistant against *E.coli* .

Here, Azithromycin& Vancomycin from Macrolide group is resistant against *E.coli* .

Cephradine, Cefuroxime, Ceftriaxone, Cefixime from Cephalosporine group showed resistant pattern which compelled to the standard value of CLSI against this clinical isolate.

Table10 :Antibacterial study of E.coli (62BIRDEM EWU) against conventional Antibiotics

			MIC break point		
Name of Antibiotics	**Sample sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistance (mg/L)**	**Zone of inhibition cm**
Levofloxacin	**32**	**≤ 1**	**2**	**> 2**	**2.1/2.2**
Ciprofloxacin	**R**	**≤ 0.5**	**1**	**> 1**	**2.3/2.5**
Azithromycin	**16**	**---------**	**---------**	**--------**	**R**
Vancomycin	**R**	**--------**	**---------**	**--------**	**R**
Cephradine	**R**	**≤ 2**	**---------**	**> 4**	**R**
Cefuroxime	**R**	**≤ 8**	**--------**	**> 8**	**R**
Ceftriaxone	**R**	**≤ 1**	**2**	**> 2**	**R**
Cefixime	**R**	**1**	**---------**	**1**	**R**

Discussion is given bellow for sample 7 collected from 37years old female patien's urine:

we have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint(These breakpoints have been used extensively to interpret MIC results). *E.coli* has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the

urine of a female patient(37 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.

Here, Levofloxacin &Ciprofloxacin from Fluoroquinolone group is resistant against *E.coli* .

Macrolide group Azithromycin showed its activity through invitro test ,but it is clinically ineffective according to CLSI break point& EUCAST MIC breakpoint. Macrolide group vancomycin did not show any activity aginst clinical isolates .

Cephradine, Cefuroxime, Ceftriaxone, Cefixime are not effective aginst clinical isolates.

Table11 :Antibacterial study of E.coli (63BIRDEM EWU) against conventional Antibiotics

			MIC break point		
Name of Antibiotics	**Sample sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistance (mg/L)**	**Zone of inhibition cm**
Levofloxacin	**R**	**≤ 2**	**4**	**≥ 8**	**1.1/1.2**
Ciprofloxacin	**R**	**≤ 1**	**2**	**≥ 4**	**R**
Azithromycin	**R**	**---------**	**----------**	**-------**	**1.1/1.2**
Vancomycin	**R**	**---------**	**----------**	**--------**	**R**
Cephradine	**R**	**≤ 2**	**---------**	**≥ 4**	**R**
Cefuroxime	**R**	**≤ 4**	**8-16**	**≥ 32**	**R**
Ceftriaxone	**R**	**≤ 1**	**--------**	**≥ 2**	**R**
Cefixime	**R**	**≤ 1**	**2**	**≥ 4**	**R**

Discussion is given bellow for sample 8 collected from 71years old female patient's urine

we have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint(These breakpoints have been used extensively to interpret MIC results). *E.coli* has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a female patient(71 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.

Here, Levofloxacin &Ciprofloxacin from Fluoroquinolone group is resistant against *E.coli* .

Here, Azithromycin& Vancomycin from Macrolide group is resistant against *E.coli* .

Cephradine, Cefuroxime, Ceftriaxone, Cefixime from Cephalosporine group showed resistant pattern against *E.coli* .which compelled to the standard value of CLSI against this clinical isolate.

Table12 :Antibacterial study of E.coli (66BIRDEM EWU) against conventional Antibiotics

			MIC break point		
Name of Antibiotics	**Samples sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistance (mg/L)**	**Zone of inhibition cm**
Levofloxacin	**1**	**≤ 2**	**4**	**≥ 8**	**1.6/1.8**
Ciprofloxacin	**R**	**≤ 1**	**2**	**≥ 4**	**R**
Azithromycin	**16**	**--------**	**-------**	**-------**	**R**
Vancomycin	**64**	**---------**	**--------**	**-------**	**R**
Cephradine	**R**	**≤ 2**	**--------**	**≥ 4**	**R**
Cefuroxime	**R**	**≤ 4**	**8-16**	**≥ 32**	**1.2/1.3**
Ceftriaxone	**R**	**≤ 1**	**-------**	**≥ 2**	**R**
Cefixime	**R**	**1**	**2**	**≥ 4**	**R**

Discussion is given bellow for sample 9collected from 38years old female patien's urine

We have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint (These breakpoints have been used extensively to interpret MIC results). *E.coli* has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a female patient(38 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.

Here, Only Levofloxacin(≤1) from Flouroquinolone group is effective against bacteria according, to BSCI guideline, but ciprofloxacin from Flouroquinolone group did not show any activity. Azithromycin & Vancomycin from macrolide group was resistance against this clinical isolate.

Cephradine, Cefuroxime, Ceftriaxone, Cefixime from cephalosporine group did not effective against this bacteria.

Table13 :Antibacterial study of E.coli (59BIRDEM EWU)against conventional Antibiotics

		MIC break point			
Name of Antibiotics	**Sample sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistance (mg/L)**	**Zone of inhibition cm**
Levofloxacin	**16**	**≤ 2**	**4**	**≥ 8**	**1.2/1.3**
Ciprofloxacin	**16**	**≤ 1**	**2**	**≥ 4**	**1.6/1.7**
Azithromycin	**16**	**----------**	**---------**	**-------**	**1.9/2.1**
Vancomycin	**R**	**---------**	**---------**	**---------**	**R**
Cephradine	**R**	**≤ 2**	**……..**	**≥ 4**	**R**
Cefuroxime	**R**	**≤ 4**	**8-16**	**≥ 32**	**R**
Ceftriaxone	**R**	**≤ 1**	**-------**	**≥ 2**	**R**
Cefixime	**R**	**≤ 1**	**2**	**≥ 4**	**R**

Discussion is given bellow for sample 10 collected from 28years old female patien's urine

we have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint(These breakpoints have been used extensively to interpret MIC results).). *E.coli* has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a female patient(28 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, Ciprofloxacin from Flouroquinolone group are resistance aginst clinical isolates.

Azithromycin, Vancomycin from macrolide group did not show any activity.

Cephradine, Cefuroxime, Ceftriaxone, Cefixime from cephalosporine group did not show any activity against clinical isolates

Antibacterial study of Acinobector

Table14 :Antibacterial study of Acinobector (65BIRDEM EWU)against conventional Antibiotics

			MIC break point		
Name of Antibiotics	**Samples sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistance (mg/L)**	**Zone of inhibition cm**
LevofloxacinCiprofloxacin	**R**	**≤ 1**	**2**	**>2**	**0.9/1.12**
Azithromycin	**32**	**≤ 1**	**---------**	**>1**	**2.2/2.3**
Vancomycin	**R**	**----------**	**---------**	**>1**	**R**
Cephradine	**R**	**----------**	**----------**	**-------**	**R**
Cefuroxime	**R**	**----------**	**----------**	**--------**	**R**
Ceftriaxone	**R**	**----------**	**----------**	**--------**	**R**
Cefixime	**R**	**----------**	**----------**	**--------**	**R**
	R	**----------**	**---------**	**--------**	**R**

Discussion is given bellow for 65 BIRDEM EWUcollected from 28years old female patien's trachial:

We have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint(These breakpoints have been used extensively to interpret MIC results). *Acinobactor* has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a female patient(28 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.

Azithromycin, Cephradine, Vancomycin, Cefuroxime, Cefixime are not effective as same as CLSI break point& EUCAST MIC breakpoint. Ceftriaxone is also not effective aginst our bacteria

collected from BIRDEM.(by compairing our data with CLSI break point& EUCAST MIC breakpoint).

Antibacterial study of Klebsiella

Table15 :Antibacterial study of Klebsiella (50BIRDEM EWU)against conventional Antibiotics

			MIC break point		
Name of Antibiotics	**Samples sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistance**	**Zone of inhibition cm**
Levofloxacin	**o.5**	**≤ 1**	**2**	**> 2**	**2.5/2.6**
Ciprofloxacin	**1**	**≤ 0.5**	**---------**	**> 1**	**2.5/2.6**
Azithromycin	**16**	**--------**	**---------**	**--------**	**2.2/2.3**
Vancomycin	**32**	**≤ 2**	**---------**	**> 4**	**1.8/1.9**
Cephradine	**4**	**---------**	**---------**	**-------**	**0.8/0.9**
Cefuroxime	**R**	**≤ 8**	**--------**	**> 8**	**1.1/1.2**
Ceftriaxone	**o.5**	**≤ 1**	**---------**	**> 2**	**1.0/1.1**
Cefixime	**R**	**1**	**---------**	**1**	**1.4/1.5**

Discussion is given bellow for sample 1 collected from 52years old female patien's pus

we have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint(These breakpoints have been used extensively to interpret MIC results).). klebsiella has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a female patient(28 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, from Flouroquinolone group with MIC range 0.5mg/L which compel to EUCAST MIC break point , so it has sensitivity againt clinical isolate, but Ciprofloxacin from Flouroquinolone group resistance againt clinical isolate

Azithromycin, Vancomycin from macrolide group resistance againt clinical isolate

Cephradine, Cefuroxime, Cefixime from cephalosporine group did not show any activity against clinical isolates , but ceftriaxone with MIC range ≤0.5 which compel to EUCAST MIC break point , so it has sensitivity againt clinical isolate.

Table16 :Antibacterial study of Klebsiella (54BIRDEM EWU)against Conventional Antibiotics

			MIC break point		
Name of Antibiotics	**Samples sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistance (mg/L)**	**Zone of inhibition cm**
Levofloxacin	**4**	**≤ 1**	**2**	**>2**	**2.4/2.5**
Ciprofloxacin	**1**	**≤ 0.5**	**……**	**>1**	**2.5/2.7**
Azithromycin	**0.125**	**--------**	**…….**	**--------**	**1.8/2.0**
Vancomycin	**8**	**-------**	**…….**	**--------**	**1.9/2.0**
Cephradine	**16**	**≤ 2**	**……..**	**>4**	**1.8/1.9**
Cefuroxime	**R**	**≤ 8**	**…….**	**>8**	**1.1/1.2**
Ceftriaxone	**16**	**≤ 1**	**…….**	**>2**	**1.6/1.7**
Cefixime	**R**	**1**	**……**	**1**	**R**

Discussion is given bellow for sample 2 collected from 55years old female patien's urine

we have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint(These breakpoints have been used extensively to interpret MIC results).). klebsiella has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the pus of a female patient(55 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, & ciprofloxacin from Flouroquinolone group with MIC range 4mg/L & 1mg/L respectively which did not compell to EUCAST sensitivity MIC break point , so it has resistance againt clinical isolate.

Azithromycin, Vancomycin from macrolide group showed sensitivity through invitro test.

Cephradine, Cefuroxime, Cefixime,ceftriaxone from cephalosporine group did not show any activity against clinical isolates .

Table17 :Antibacterial study of Klebsiella (56BIRDEM EWU)against conventional Antibiotics

			MIC break point		
Name of Antibiotics	**Sample sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistance (mg/L)**	**Zone of inhibition cm**
Levofloxacin	**16**	**≤ 2**	**4**	**≥ 8**	**2.0/2.1**
Ciprofloxacin	**16**	**≤ 1**	**2**	**≥ 4**	**2.6/2.7**
Azithromycin	**16**	**---------**	**---------**	**--------**	**2.0/2.1**
Vancomycin	**R**	**---------**	**---------**	**--------**	**R**
Cephradine	**R**	**≤ 2**	**--------**	**≥ 4**	**R**
Cefuroxime	**R**	**≤ 4**	**8-16**	**≥ 32**	**R**
Ceftriaxone	**R**	**≤ 1**	**-------**	**≥ 2**	**R**
Cefixime	**R**	**1**	**2**	**≥ 4**	**R**

Discussion is given bellow for sample 3 collected from 55years old female patien's urine

we have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint(These breakpoints have been used extensively to interpret MIC results).). klebsiella has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a female patient(55 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, & ciprofloxacin from Flouroquinolone group is resistance againt clinical isolate.

Azithromycin, Vancomycin from macrolide group is resistance againt clinical isolate.

.Cephradine, Cefuroxime, Cefixime,ceftriaxone from cephalosporine group did not show any activity against clinical isolates .

Table18 :Antibacterial study of Klebsiella (60BIRDEM EWU)against Conventional Antibiotics

			MIC break point		
Name of Antibiotics	**Sample sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistance (mg/L)**	**Zone of inhibition cm**
Levofloxacin	**0.25**	**≤ 2**	**4**	**≥ 8**	**R**
Ciprofloxacin	**0.25**	**≤ 1**	**2**	**≥ 4**	**R**
Azithromycin	**16**	**---------**	**---------**	**--------**	**R**
Vancomycin	**R**	**---------**	**---------**	**--------**	**R**
Cephradine	**R**	**≤ 2**	**--------**	**≥ 4**	**R**
Cefuroxime	**R**	**≤ 4**	**8-16**	**≥ 32**	**R**
Ceftriaxone	**R**	**≤ 1**	**--------**	**≥ 2**	**R**
Cefixime	**R**	**1**	**2**	**≥ 4**	**R**

Discussion is given bellow for sample 4 collected from 58 years old female patien's urine

we have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint(These breakpoints have been used extensively to interpret MIC results).). klebsiella has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the urine of a female patient(55 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, & ciprofloxacin from Flouroquinolone group with MIC range 0.25mg/L & 0.25mg/L respectively which did not compell to EUCAST sensitivity MIC break point , so it has resistance againt clinical isolate.

Azithromycin, Vancomycin from macrolide group showed sensitivity through invitro test.

Cephradine, Cefuroxime, Cefixime,ceftriaxone from cephalosporine group did not show any activity against clinical isolates .

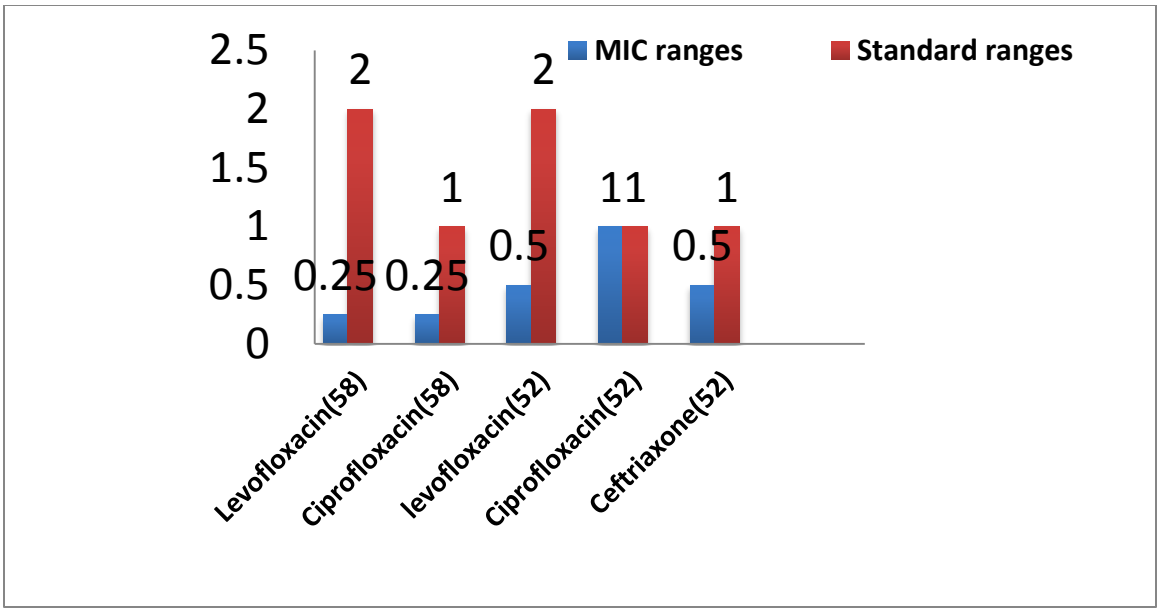

Fig 1.19: Comparison of sensitivity between antibiotics against Klebsiella

Table19 :Antibacterial study of Klebsiella (64BIRDEM EWU)against standard powder

				MIC break point	
Name of Antibiotics	**Sample sensitivity (mg/L)**	**Standard sensitivity (mg/L)**	**Intermediate (mg/L)**	**Resistance (mg/L)**	**Zone of inhibition cm**
Levofloxacin	**R**	**≤ 2**	**4**	**≥ 8**	**1.6/1.8**
Ciprofloxacin	**R**	**≤ 1**	**2**	**≥ 4**	**R**
Azithromycin	**R**	**--------**	**-------**	**-------**	**R**
Vancomycin	**R**	**--------**	**--------**	**--------**	**R**
Cephradine	**R**	**≤ 2**	**--------**	**≥ 4**	**R**
Cefuroxime	**R**	**≤ 4**	**8-16**	**≥ 32**	**1.2/1.3**
Ceftriaxone	**R**	**≤ 1**	**-------**	**≥ 2**	**R**
Cefixime	**R**	**1**	**2**	**≥ 4**	**R**

Discussion is given bellow for sample5 collected from 38years old female patien's trachia

we have compared our MIC ranges with CLSI break point& EUCAST MIC breakpoint(These breakpoints have been used extensively to interpret MIC results).). klebsiella has been subjected to the evaluation of sensitivity against conventional antibiotics. The sample was collected from the trachia of a female patient(38 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, & ciprofloxacin from Flouroquinolone group is resistance againt clinical isolate.

Azithromycin, Vancomycin from macrolide group is resistance againt clinical isolate.

.Cephradine, Cefuroxime, Cefixime,ceftriaxone from cephalosporine group did not show any activity against clinical isolates .

CHAPTER 6

CONCLUSION:

After reviewing this study we can see that the most of the time Cephradine, Cefixime, Cefuroxime & Ceftriaxone frome Cephalosporine group did not show any activity against *Clinical isolates*, but Ceftriaxone from this group with MIC range 0.5mg/L which compelled to the standard value of CLSI, against *E.coli* sample 3 , so it is considered effective against this strains of *E.coli* . Ciprofloxacin & Levofloxacin from Fluoroquinolone group showed highest sensitivity against *E.coli* . The highest MIC value for levofloxacin was 0.0625 mg/L against E.coli sample 3. levofloxacin also effective against E .coli sample 4, sample5 & sample 1 with MIC value 0.25mg/L ,0.25 mg/L & 2 respectively . The highest MIC value for ciprofloxacin against *E.coli* sample 4 & sample 5 was 0.5 mg/L . *E.coli* sample 6, sample7, sample 8 & sample 9 was completely resistance against our used antibiotics. From Macrolide group, Antibiotic Azithromycin & cell wall synthesis inhibitors Vancomycin is totally resistance against all strains of *E.coli* . The highest value of pseudomonas against Levofloxacin & Ciprofloxacin was 0.5 mg/L & 0.0625 mg/L respectively for sample 1. All Antibiotics from Cephalosporine group, Macrolide group, Antibiotics Azithromycin & cell wall synthesis inhibitors Vancomycin is totally resistance against all strains of t this clinical isolates. The highest value for Klebsiella sample 1 & sample 4 against Levofloxacin was 0.5 mg/L & 0.25 respectively. The highest MIC value for Klebsiella sample 1 & sample 4 against Ciprofloxacin was o.25 mg/L & 1 mg/L respectively .Ceftriaxone also gives activity with MIC range 0.5 mg/L against Klebsiella sample 1 , otherwise Macrolide group antibiotic Azithromycin & cell wall synthesis inhibitors Vancomycin is totally resistance against all strains of klebsiella . Enterococci & Acinobactor sp. was totally resistance against our used antibiotics.

CHAPTER 7

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