Broth Microdilution Method for Determining Resistance Pattern of Clinically Isolated Common Pathogenic Microorganisms against Conventional Antibiotics

A Dissertation submitted to the Department of Pharmacy, East West University, Bangladesh, in partial fulfillment of the requirements for the Degree of Bachelor of Pharmacy

Submitted by

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IN THE NAME OF ALLAH

THE MOST GRACIOUS

THE MOST MERCIFUL

Declaration by the Research Candidate

I, Zannatul Ferdous, ID: 2012-1-70-028, hereby declare that the dissertation entitled "In Vitro Minimum Inhibitory Concentration of Antibiotics Standard Powder against Clinically Isolated Microorganisms by Broth Microdilution" submitted by me to the Department of Pharmacy, East West University and in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, under the supervision and guidance of Dr. Shamsun Nahar Khan, Associate Professor and Chairperson, Department of Pharmacy, East West University, Dhaka.

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Certificate by the Supervisor

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Certificate by the Chairperson

This is to certify that the thesis entitled “In Vitro Minimum Inhibitory Concentration of Antibiotics Standard Powder against Clinically Isolated Microorganisms by Broth Microdilution” submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy is a bonafide record of original and genuine research work carried out by Zannatul Ferdous, ID: 2012-1-70-028 in 2015.

Dr. Shamsun Nahar Khan

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Dedication

This Research Paper is dedicated to My Beloved Parents.

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Abstract

Antibiotic resistance is a common phenomenon over the years in the world. Gradually more MIC of Fluoroquinolones, Macrolides, Cephalosporins and Vancomycin against 7 isolates of *E.coli* and *Klebsiella*, 2 isolates of *Salmonella typhi*, 1 isolates of *Staphylococcus aureus*, Pseudomonas and *Acinetobacter*. At different concentration of drug, organisms were added to 96 well containing microtiter plate. After incubation,optical densities were determined by microtiter plate reader and Minnimum Inhibitory Concentration end point was determined. Fluoroquinolones were sensitive against most isolates of organisms and they showed resistance against gram positive bacteria (Staphylococcus aureus) as Levofloxacin is considered to be active against gram positive bacteria so further study should be carried out. In case of, MIC of Azithromycin against most isolates of gram negative bacteria were sensitive however, Azithromycin showed resistance against some isolates of *E.coli* so further study should be carried out.

Keywords: *MIC, sensitive, resistance, gram positive bacteria, gram negative bacteria,*

1.1 Overview:

Antibiotics are used in the treatment and prevention of bacterial infection. They may either kill or inhibit the growth of bacteria. Several antibiotics are also effective against many fungi and protozoans, and some are toxic to humans and animals, even when given in therapeutic dosage. Antibiotics are not effective against viruses such as the common cold or influenza, and may be harmful when taken inappropriately.

Antibiotic sensitivity or antibiotic susceptibility is the susceptibility of bacteria to antibiotics. Because susceptibility can vary even within a species.

Antibiotic / Antimicrobial resistance is the ability of microbes to resist the effects of drugs – that is, the germs are not killed, and their growth is not stopped. Although some people are at greater risk than others, no one can completely avoid the risk of antibiotic-resistant infections. Infections with resistant organisms are difficult to treat, requiring costly and sometimes toxic alternatives.

Bacteria will inevitably find ways of resisting the antibiotics developed by humans, which is why aggressive action is needed now to keep new resistance from developing and to prevent the resistance that already exists from spreading (CDC, 2016)

There are several methods for determining antibiotic susceptibility i.e. disk diffusion test, E-test, Broth macrodilution, Broth microdilution test etc. In this study the main objective was to determine MIC of 6 different antibiotics which are: Azithromycin, Levofloxacin, Ciprofloxacin, Ceftriaxone, Cephradine, Vancomycin on 6 different strains of microorganism which are *Acinetobacter, E. coli, Salmonella typhi, Klebsiella, Pseudomonas, Staphylococcus aureus* using broth microdilution test. According to their MIC their susceptibility or resistance were determined which is important. Whether they are susceptible or resistant were determined using their standard MIC value against obtained MIC value. Different antibiotic has different standard MIC range based on microorganism. MIC value is expressed in microgram/ml or mg/L (David and Franklin, 2012).

1.2 Microorganisms:

Microorganism invariably refers to the minute living body not perceptible to the naked eyes, especially a bacterium or protozoon. Microorganism can be carried by the one host to another by animal source, air borne, contact infections, food borne, human carriers, insects, soil borne (Kar, 2008).

Microorganisms used in this study are:

- *Acinetobacter*
- *E. coli*
- *Salmonella typhi*
- *Klebsiella*
- *Pseudomonas*
- *Staphylococcus aureus*

1.2.1 E. coli:

E. coli is a bacterium that lives in the digestive tracts of humans and animals. There are over 700 strains of E. coli, and many of them are harmless. However, certain E. coli stains, referred to as enterohemorrhagic E. coli (EHEC), can cause bloody diarrhea, severe anemia, urinary tract infection, or kidney failure, which could ultimately lead to death.

People become infected with E. coli when they ingest food or water that has been contaminated by feces with the infectious E. coli strains.

Initial symptoms of E. coli usually appear within three to five days after ingestion of the bacterium; however, symptoms may appear anywhere from one to ten days. Symptoms include:

• Nausea

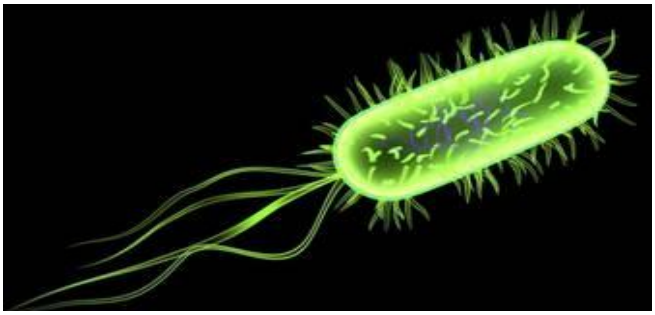
• Vomiting

• Stomach cramps

• Diarrhea, typically bloody

• Urinary tract infections

• Kidney failure etc. (Kaper, 2005).



1.2.1 Fig*: E. coli* (CDC, 2016)

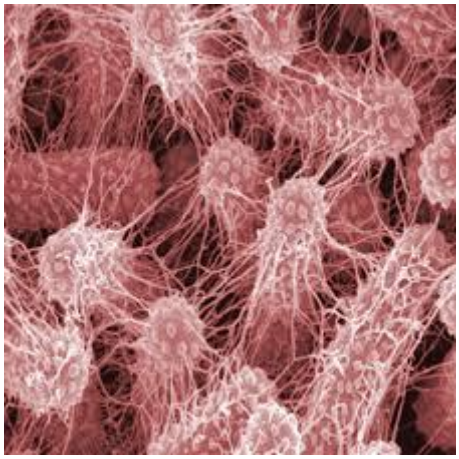
1.2.2 Klebsiella:

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

Klebsiella bacteria tend to be rounder and thicker than other members of the Enterobacteriaceae family. They typically occur as straight rods with rounded or slightly pointed ends. They can be found singly, in pairs, or in short chains. Diplobacillary forms are commonly found in vivo.

Klebsiella species are routinely found in the human nose, mouth, and gastrointestinal tract as normal flora; however, they can also behave as opportunistic human pathogens. Klebsiella species are known to also infect a variety of other animals, both as normal flora and opportunistic pathogens.

Klebsiella organisms can lead to a wide range of disease states, notably pneumonia, urinary tract infections, septicemia, meningitis, diarrhea, and soft tissue infections (Zheng et al., 2014)



1.2.2 Fig*: Klebsiella* (CDC, 2016)

1.2.3 Acinetobacter:

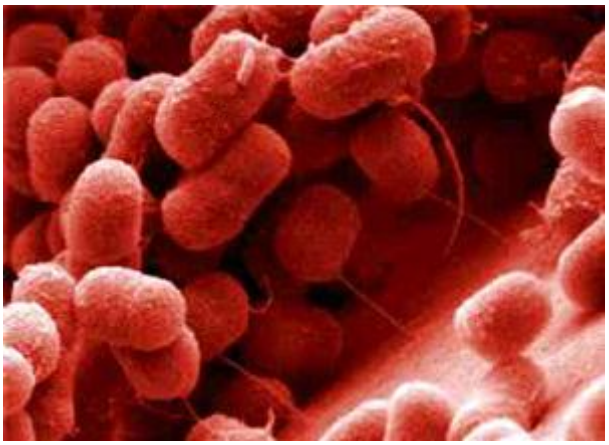
Acinetobacter is a gram-negative coccobacillus. Acinetobacter was first described in 1911 as Micrococcus calco-aceticus. Since then, it has had several names, becoming known as acinetobacter in the 1950s. Acinetobacter species are oxidase-negative and non-motile

Its natural habitats are water and soil, and it has been isolated from foods, arthropods, and the environment. In humans, *acinetobacter* can colonize skin, wounds, and the respiratory and gastrointestinal tracts. Some strains of *acinetobacter* can survive environmental desiccation for weeks, a characteristic that promotes transmission through fomite contamination in hospitals.

Acinetobacter species have low virulence but are capable of causing infection in organ transplants and febrile neutropenia. Most Acinetobacter isolates recovered from hospitalized patients, particularly those recovered from respiratory secretions and urine, represent colonization rather than infection.

They are resistant to many including penicillin, chloramphenicol, and often aminoglycosides. Resistance to fluoroquinolones has been reported during therapy, which has also resulted in increased resistance to other drug classes mediated through active drug efflux. A dramatic increase in antibiotic resistance in Acinetobacter strains has been reported by the CDC, and the carbapenems are recognized as the gold-standard and treatment of last resort.

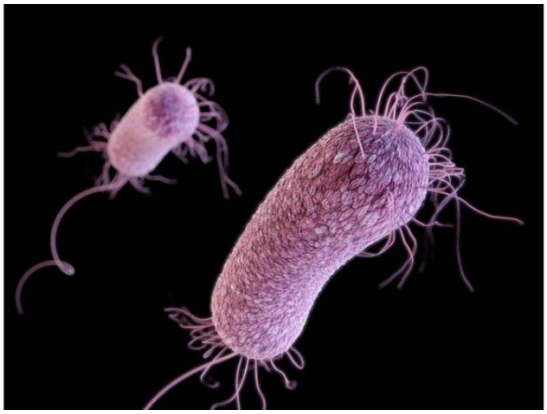
Acinetobacter species are unusual in that they are sensitive to sulbactam; sulbactam is most commonly used to inhibit bacterial beta-lactamase, but this is an example of the antibacterial property of sulbactam itself (NEJM, 2008).



1.2.3 Fig: Acinetobacter (CDC, 2016)

1.2.4 Pseudomonas:

Pseudomonas aeruginosa (pyocyanea) a gram negative rods, has in recent years, assumed the role of a dangerous pathogen. It has long been a troublesome cause of secondary infection of wounds, especially burns, but is not necessarily pathogenic. With the advent of immunosuppressive therapy following organ transplant,systemic infections including pneumonia have resulted from infection by this organism. It has also been implicated in eye infections resulting in the loss of sight.Pseudomonas aeruginosa is resistant to many antibacterial agents and is biochemically very versatile, being able to use many disinfectants as food sources (Hugo and Russel, 1998)



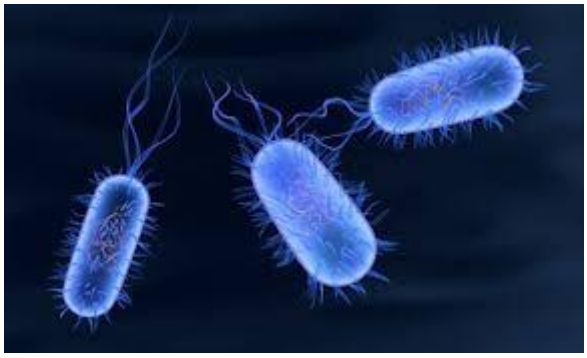
1.2.4Fig: *Pseudomonas* (CDC, 2016)

1.2.5 Salmonella typhi:

Salmonella typhi is a subspecies of Salmonella enterica, the rod-shaped, flagellated, aerobic, Gram-negative bacterium. Salmonella typhi causes typhoid fever; paratyphoid fever is caused by S. paratyphi,

The bacterium usually enters the body through the mouth by the ingestion of contaminated food or water, penetrates the intestinal wall, and multiplies in lymphoid tissue; it then enters the bloodstream and causes bacteremia.

Salmonella Typhi lives only in humans. Persons with typhoid fever carry the bacteria in their bloodstream and intestinal tract. In addition, a small number of persons, called carriers, recover from typhoid fever but continue to carry the bacteria. Both ill persons and carriers shed Salmonella Typhi in their feces (stool) (CDC, 2016).

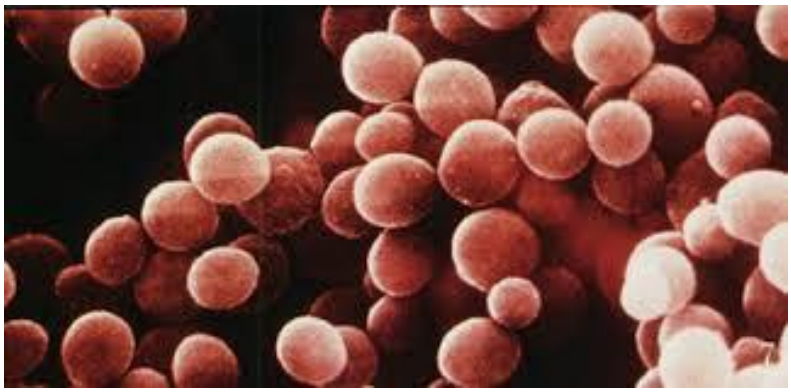


1.2.5 Fig: *Salmonella typhi* (CDC, 2016)

1.2.6 Staphylococcus aureus:

Staphylococcus aureus is a gram-positive coccal bacterium, diameters of 0.5 – 1.5 µm and characterized by individual cocci, 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 also nitrate reduction. Staphylococcus was first identified in 1880 in Aberdeen, Scotland, the staphylococci are non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation.

Staphylococcus aureus is a major pathogen of increasing importance due to the rise in antibiotic resistance (Harris, Foster and Richards 2002).



1.2.6 Fig: *Staphylococcus aureus* (CDC, 2016)

1.3 Antibiotics:

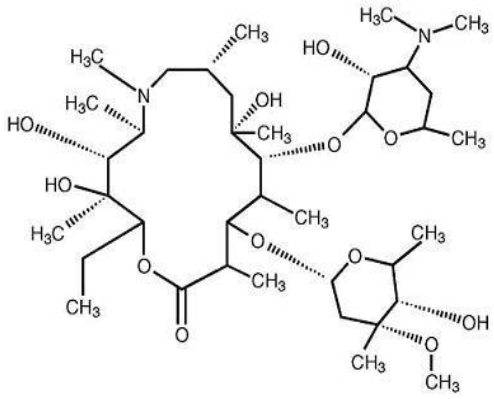
6 antibiotics were used in this study are:

- Azithromycin
- Ciprofloxacin
- Levofloxacin
- Ceftriaxone
- Vancomycin
- Cephradine

1.3.1 Azithromycin:

Azithromycin is a macrolide antibacterial drug,

1.3.1.1 chemical name: (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-dideoxy-3-C-methyl3-O-methyl-α-L-ribo-hexopyranosyl) oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.



1.3.1 Fig: Azithromycin

1.3.1.2 Indication:

- Urinary pain, burning, urgency and frequency associated with urinary tract infections.
- Sexually Transmitted Diseases.
- Acute bacterial exacerbations of chronic bronchitis and acute bacterial sinusitis due to Haemophilus influenzae, or Streptococcus pneumoniae.

1.3.1.3 Mechanism of Action:

Azithromycin binds to the 50S subunit of the 70S bacterial ribosomes, and therefore inhibits RNA-dependent protein synthesis in bacterial cells.

1.3.1.4 Pharmacokinetics:

Absorption

The absolute bioavailability of azithromycin 250 mg capsules is 38%.

In a two-way crossover study in which 12 healthy subjects received a single 500 mg dose of azithromycin (two 250 mg tablets) with or without a high fat meal, food was shown to increase Cmax by 23% but had no effect on AUC.

When azithromycin oral suspension was administered with food to 28 adult healthy male subjects, Cmax increased by 56% and AUC was unchanged.

Distribution

The serum protein binding of azithromycin is variable in the concentration range approximating human exposure, decreasing from 51% at 0.02 µg/mL to 7% at 2 µg/mL.

The antibacterial activity of azithromycin is pH related and appears to be reduced with decreasing pH, however, the extensive distribution of drug to tissues may be relevant to clinical activity.

Azithromycin has been shown to penetrate into human tissues, including skin, lung, tonsil, and cervix. Extensive tissue distribution was confirmed by examination of additional tissues and fluids (bone, ejaculum, prostate, ovary, uterus, salpinx, stomach, liver, and gallbladder).

Metabolism

In vitro and in vivo studies to assess the metabolism of azithromycin have not been performed

Elimination

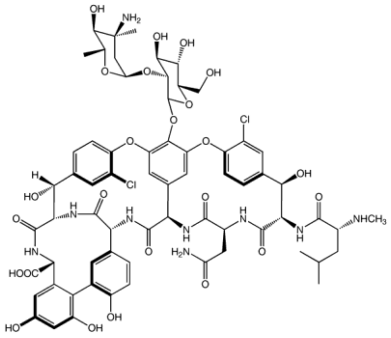
Biliary excretion of azithromycin, predominantly as unchanged drug, is a major route of elimination. Over the course of a week, approximately 6% of the administered dose appears as unchanged drug in urine (Singlas, 2016).

1.3.2 Vancomycin:

A branched tricyclic glycosylated peptide with bactericidal activity against most organisms and bacteriostatic effect on enterococci.

1.3.2.1 Chemical name: (1S,2R,18R,19R,22S,25R,28R,40S)-48-{[(2S,3R,4S,5S,6R)-3-{[(2S,4S,5S,6S)-4-amino-5-hydroxy-4,6-dimethyloxan-2-yl] oxy}-4,5-dihydroxy-6-(hydroxymethyl) oxan-2-yl]oxy}-22-(carbamoylmethyl)-5,47-dichloro-2,18,32,35,37-pentahydroxy-19-[(2R)-4-meth yl-2-

(methylamino)pentanamido]-20,23,26,42,44-pentaoxo-7,13-dioxa-21,24,27,41,43-pentaazaoctacyclo[26.14.2.2^{3},^{6}.2^{14},^{17}.1^{8},^{12}.1^{29},^{33}.0^{10},^{25}.0^{34},^{39}]pentaconta-3,5,8,10,12(48),14,16,29(45),30,32,34,36,38,46,49-pentadecaene-40-carboxylic acid.



1.3.2 Fig: vancomycin

1.3.2.2 Indication:

For the treatment of serious or severe infections caused by susceptible strains of methicillin-resistant (beta-lactam-resistant) staphylococci.

1.3.2.3 Mechanism of action:

The bactericidal action of vancomycin results primarily from inhibition of cell-wall biosynthesis. Specifically, vancomycin prevents incorporation of N-acetylmuramic acid (NAM)- and N-acetylglucosamine (NAG)-peptide subunits from being incorporated into the peptidoglycan matrix; which forms the major structural component of Gram-positive cell walls. The large hydrophilic molecule is able to form hydrogen bond interactions with the terminal D-alanyl-D-alanine moieties of the NAM/NAG-peptides. Normally this is a five-point interaction. This binding of vancomycin to the D-Ala-D-Ala prevents the incorporation of the NAM/NAG-peptide subunits into the peptidoglycan matrix. In addition, vancomycin alters bacterial-cell-membrane permeability and RNA synthesis. There is no cross-resistance between vancomycin and other antibiotics. Vancomycin is not active in vitro against gram-negative bacilli, mycobacteria, or fungi.

1.3.2.4 Pharmacokinetics:

Poorly absorbed from gastrointestinal tract, however systemic absorption (up to 60%) may occur following intraperitoneal administration.

Serum protein bound is approximately 55%.

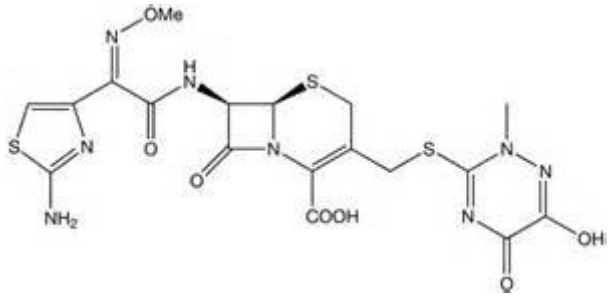
In the first 24 hours, about 75% of an administered dose of vancomycin is excreted in urine by glomerular filtration.

Half-life in normal renal patients is approximately 6 hours (range 4 to 11 hours). In anephric patients, the average half-life of elimination is 7.5 days (NCI, 2015)

1.3.3 Ceftriaxone:

A broad-spectrum cephalosporin antibiotic with a very long half-life and high penetrability to meninges, eyes and inner ears.

1.3.3.1 Chemical name: (6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino) acetamido]-3-{[(2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl) sulfanyl] methyl}-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid.



1.3.3 Fig: Ceftriaxone

1.3.3.2Indication:

For the treatment of the infections (respiratory, skin, soft tissue, UTI, ENT) caused by S. pneumoniae, H. influenzae, staphylococci, S. pyogenes (group A beta-hemolytic streptococci), E. coli, P. mirabilis, Klebsiella sp, coagulase-negative staphylococcus it is used.

1.3.3.3 Mechanism of action:

Ceftriaxone works by inhibiting the mucopeptide synthesis in the bacterial cell wall. The beta-lactam moiety of Ceftriaxone binds to carboxypeptidases, endopeptidases, and transpeptidases in the bacterial cytoplasmic membrane. These enzymes are involved in cell-wall synthesis and cell division. By binding to these enzymes, Ceftriaxone results in the formation of of defective cell walls and cell death.

1.3.3.4Pharmacokinetics:

Volume of distribution is 5.78 to 13.5L. It shows 95% protein binding

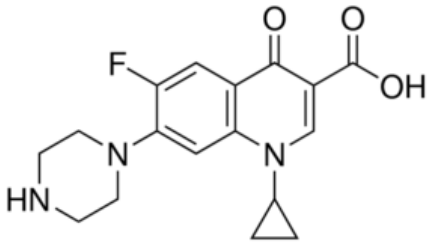
Thirty-three percent to 67% of a ceftriaxone dose was excreted in the urine as unchanged drug and the remainder was secreted in the bile and ultimately found in the feces as microbiologically inactive compounds.

Elimination half-life is 5.8-8.7 hours. And clearance is 0.58 – 1.45 L/h (Greyerz *et al.*, 2001)

1.3.4 Ciprofloxacin:

Ciprofloxacin is a synthetic broad spectrum fluoroquinolone antibiotic. Ciprofloxacin binds to and inhibits bacterial DNA gyrase, an enzyme essential for DNA replication.

1.3.4.1Chemical name: 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.



1.3.4 Fig: ciprofloxacin

1.3.4.2 Indication:

For the treatment of the following infections caused by susceptible organisms urinary tract infections, acute uncomplicated cystitis, chronic bacterial prostatitis, lower respiratory tract infections, acute sinusitis, skin and skin structure infections, bone and joint infections, complicated intra-abdominal infections (used in combination with metronidazole), infectious diarrhea, typhoid fever (enteric fever), uncomplicated cervical and urethral gonorrhea, and inhalational anthrax.

1.3.4.3 Mechanism of action:

The bactericidal action of ciprofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, strand supercoiling repair, and recombination.

1.3.4.4 Pharmacokinetics:

Rapidly and well absorbed from the GIT after oral administration. The absolute bioavailability is approximately 70% and does not have any substantial loss by first pass metabolism.

20 to 40% protin bound property.

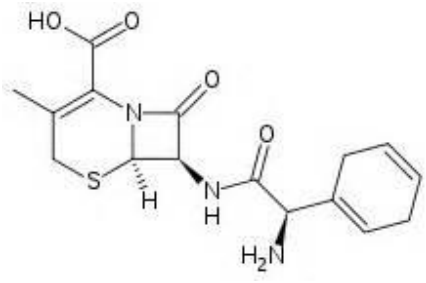
Metabolism is mainly hepatic. Four metabolites have been identified in human urine which together account for approximately 15% of an oral dose. The metabolites have antimicrobial activity, but are less active than unchanged ciprofloxacin.

40 to 50% of orally administered dose is excreted as unchanged drug in the urine t ½ 4 hours (Drusano, 1986).

1.3.5 Cephradine:

This compound belongs to the class of organic compounds known as cephalosporins

1.3.5.1 Chemical name :(6R, 7R)-7-[(2R)-2-amino-2-(cyclohexa-1, 4-dien-1-yl) acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid



1.3.5 Fig: Cephradine

1.3.5.2 Indication:

- Cephradine /is/ indicated in the treatment of bacterial urinary tract infections caused by susceptible organisms.
- In the treatment of bacterial pharyngitis
- In the treatment of skin and soft tissue infections
- In the treatment of otitis media
- In the treatment of bronchitis

1.3.5.3 Mechanism of action:

Cefradine is a first generation cephalosporin antibiotic with a spectrum of activity similar to Cefalexin. Cefradine, like the penicillins, is a beta-lactam antibiotic. By binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, it inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that Cefradine interferes with an autolysin inhibitor.

1.3.5.4 Pharmacokinetics:

Absorption: Well absorbed from the GI tract.

Distribution: Distributed widely into most body tissues and fluids, including the gallbladder, liver, kidneys, bone, sputum, bile, and pleural and synovial fluids; CSF penetration is poor. Cephradine crosses the placental barrier and is 6% to 20% protein-bound.

Metabolism: Not metabolized.

Excretion: Excreted primarily in urine by renal tubular and glomerular filtration; small amounts of drug appear in breast milk. Elimination half-life is about 1/2 to 2 hours in normal renal function; end-stage renal disease prolongs half-life to 8 to 15 hours. Hemodialysis or peritoneal dialysis removes drug (Neiss, 1973).

1.3.6 Levofloxacin:

A synthetic fluoroquinolone antibacterial agent that is the optically active L-isomer of ofloxacin which inhibits the super coiling activity of bacterial DNA gyrase, halting DNA replication.

1.3.6.1 Chemical name: (2S)-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo [7.3.1.0] trideca-5(13),6,8,11-tetraene-11-carboxylic acid.

1.3.6 Fig: Levofloxacin

1.3.6.2 Indication:

- For the treatment of bacterial conjunctivitis caused by susceptible strains of the following organisms: Corynebacterium species, Staphylococus aureus, Staphylococcus epidermidis.
- is used for the treatment of acute bacterial sinusitis caused by susceptible Streptococcus pneumoniae
- Levofloxacin is used for the treatment of mild to moderate uncomplicated urinary tract infections caused by susceptible E. coli, K. pneumonia
- Levofloxacin is used for the treatment of community-acquired pneumonia caused by susceptible S aureus, S. pneumonia.

1.3.6.3 Mechanism of action:

Levofloxacin inhibits bacterial type II topoisomerases, topoisomerase IV and DNA gyrase. Levofloxacin, like other fluoroquinolones, inhibits the A subunits of DNA gyrase, two subunits encoded by the gyrA gene. This results in strand breakage on a bacterial chromosome, supercoiling, and resealing; DNA replication and transcription is inhibited.

1.3.6.4 Pharmacokinetics:

Absorption of ofloxacin after single or multiple doses of 200 to 400 mg is predictable, and the amount of drug absorbed increases proportionately with the dose.

24-38% binds to plasma protein. Undergoes limited metabolism in humans.

Mainly excreted as unchanged drug (87%) through urine (Lavin, 2012).

1.4 Broth Microdilution:

This method is called "microdilution" because it involves the use of small volumes of broth dispensed in sterile, plastic microdilution trays that have round or conical bottom wells.

Broth microdilution is a method used to test the susceptibility of bacteria to antibiotics. It is the most commonly used method to perform this test

During testing, multiple microtiter plates are filled with a broth varying concentrations of the antibiotics and the bacteria to be tested are then added to the plate. The plate is then placed into a non-CO2 incubator and heated at thirty-five degrees Celsius for sixteen to twenty hours. Following the allotted time, the plate is removed and checked for bacterial growth. If the broth became cloudy or a layer of cells formed at the bottom, then bacterial growth has occurred. The results of the broth microdilution method are reported in Minimum Inhibitory Concentration (MIC), or the lowest concentration of antibiotics that stopped bacterial expansion.

Microtiter plate reader machine is used to determine the optical density passing through a specific length of light. After that percentage of growth is calculated through which MIC is determined (David and Franklin, 2012).

1.5 Microtiter Plate Reader:

Pharmaceutical and biotechnology research requires instrumentation to be both functional and versatile. In the HTS and Drug Discovery environments, micro plate-based assays are developed to make determinations on large numbers of samples. Regardless of the assay protocol, the end result is the measurement by some sort of

detection device. ELISA utilizes two independent sets of optics to provide uncompromised performance. For absorbance measurements, there is a xenon-flash lamp with a monochromator for wavelength selection and photodiode detection. This allows the selection of any wavelength for endpoint or kinetic measures from 200 nm to 999 nm in 1 nm increments, as well as spectral scans. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 µL per well.

Traditional visible wavelength fluorescence measurements are made using a tungsten-halogen lamp with interference filters (excitation and emission) for wavelength selection and photomultiplier (PMT) detection. If time-resolved or UV excitation fluorescence measurements are required, it automatically integrates then xenon-flash-monochromator excitation with the interference emission filter and PMT detection. Typical applications include antibody-antigen binding, receptor-liquid binding, ELISA, nucleic acid quantitation using fluorescent dyes or direct UV analysis and determines optical density (Held, 2003).

1.6 Minimum inhibitory concentrations (MIC):

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation, and minimum bactericidal concentrations (MBCs) as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media.

MICs are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the in vitro activity of new antimicrobials,

There are many standardized methods for determining MICs. 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, and preparation of inoculum, incubation conditions, and reading and interpretation of results (Wiegand, Hilpert and Hancock, 2008).

2 Aim and significance of the Study

2.1 Significance of the Study

- The minimum inhibitory concentration (MIC) is the concentration at which an antibacterial agent experiences the complete inhibition of organism growth or MIC is defined as the minimum concentration of antibiotic which will inhibit the growth of the isolated microorganism. There are many ways to measure the MIC, including: Broth dilution, Agar dilution, E-test. Determination of MIC is a common tool to confirm drug resistance but most often as a research tool to determine in vitro activity of antimicrobial agents. The MIC is expressed in mg⁄L (Vipra *et al*., 2013).
- Antibiotics are considered as lifesaving drug but the real wonder is the rise of antibiotic resistance in hospitals, communities, and the environment concomitant with their use. The extraordinary genetic capacities of microbes have benefitted from man's overuse of antibiotics to exploit every source of resistance genes and every means of horizontal gene transmission to develop multiple mechanisms of resistance for each and every antibiotic introduced into practice clinically (Davis and Davis, 2010).
- An important task of the clinical microbiology laboratory is the performance of antimicrobial susceptibility testing of significant bacterial isolates. The most widely used testing methods include broth micro dilution or Agar dilution or disk diffusion test. All of them can be used for testing antimicrobial susceptibility (Jorgensen and Ferraro, 2009).
- Onychomycosis is a common adult human mycosis, and dermatophytes of the Trichophyton genera are the most common causative agent. Many antimycotic agents are safe and highly effective for the treatment of dermatophytosis. The aim of this work was to determine the MICs of four antifungal drugs (fluconazole, itraconazole, terbinafine and griseofulvin) recognized for ungual dermatophytosis treatment caused by Trichophyton species. MICs were determined using a broth microdilution method in accordance with Clinical and Laboratory Standards Institute approved standard (Barros, Santos and Hamdan, 2007).
- Cryptococcus neoformans and Cryptococcus gattii are aetiological agents of cryptococcosis. Using the broth microdilution method, both species were found to be susceptible to the antifungals tested except for two clinical C. neoformans var. grubii isolates that were resistant to 5-flucytosine. However, no statistically significant difference in susceptibility of the two Cryptococcus species was observed against amphotericin B and 5-flucytosine. Furthermore, the environmental C. Neoformans var. grubii isolates were significantly less susceptible to fluconazole, itraconazole and 5-flucytosine (Chowdhary *et al*, 2011).
- To understand the mechanisms contributing to the variability in carbapenem MICs, 20 clinical isolates, all belonging to either of two clonal groups of KPC-possessing K. pneumonia endemic to New York City, were examined. For one clonal group, carbapenem MICs increased with decreasing expression of ompK36. A second clonal group also had carbapenem MICs that correlated

with ompK36 expression. However, all of the isolates in this latter group continued to produce OmpK36, suggesting that porin configuration may affect entry of carbapenems. In conclusion, isolates of KPC-possessing K. pneumonia that express ompK36 tend to have lower MICs to carbapenem (Landman, Bratu and Quale, 2009).

- Fungaemia caused by Malassezia spp.in hospitalized patient requires prompt and appropriate therapy. In this study, the in vitro susceptibility of Malassezia furfur from bloodstream infections (BSIs) to amphotericin B (AMB), fluconazole (FLC), itraconazole (ITC), posaconazole (POS) and voriconazole (VRC) was assessed using the broth microdilution method. itraconazole, posaconazole and voriconazole displayed lower MICs than fluconazole and amphotericin B. (Iatta *et al*, 2014).
- A collection of 48 clinical Cryptococcus neoformans isolates from Croatia was investigated retrospectively using in vitro antifungal susceptibility testing. These isolates were obtained from 15 patients: ten were human immunodeficiency virus (HIV)-negative (66.7 %) and five were HIV-positive (33.3 %). Antifungal susceptibility was tested by a broth microdilution method (Missoni *et al*, 2011).
- The MICs of 24 antimicrobials for 26 Leptospira spp. serovars were determined using a broth microdilution technique. The MICs at which 90% of isolates tested were inhibited (MIC90s) of cefepime, imipenem-cilastatin, erythromycin, clarithromycin, and telithromycin were all<0.01 microgram/ml. The MIC90s of amoxicillin, aztreonam, cefdinir, chloramphenicol, and penicillin G were>3.13 microgram/ml. Many antimicrobials have excellent in vitro activity against Leptospira (Murray and Hospenthal, 2004).

- The Epsilometer test (E test; AB Biodisk, Solna, Sweden), a new quantitative technique for the determination of antimicrobial susceptibility, was compared to reference methods (agar dilution and broth microdilution) for the antimicrobial susceptibility testing of Helicobacter pylori. Seventy-one H. pylori strains isolated from patients with duodenal ulcers were tested against 20 antimicrobial agents. E test compared to the results obtained by reference methods. Excellent agreement between E-test, agar dilution, and broth microdilution results was found for resistance to erythromycin (8%), clarithromycin (6%), and tetracycline (6%) (Piccolomini, 1997).

- Antimicrobial susceptibility of 65 isolates of Bacillus anthracis (50 historical and 15 recent U.S. clinical isolates) were tested to nine antimicrobial agents using the National Committee for Clinical Laboratory Standards (NCCLS) broth microdilution reference method. Approximately 78% of the isolates showed reduced susceptibility to ceftriaxone. All B. anthracis isolates were susceptible to chloramphenicol (MICs<8 microgram/ml), ciprofloxacin (MICs<1microgram/ml), clindamycin (MICs<0.5 microgram/ml), rifampin (MICs<0.5microgram/ml), tetracycline (MICs<0.06 microgram/ml), and vancomycin (MIC<2 microgram/ml) (Mohammed *et al*, 2002).

- The in vitro activity of voriconazole was compared with those of fluconazole and itraconazole against 270 clinical isolates of yeasts from the mouths of patients receiving palliative care for advanced cancer. A broth micro-dilution assay as described by the National Committee for Clinical Laboratory Standards was employed for determination of MICs. Of the 270 isolates, 206 (76 %) were fluconazole sensitive and 64 were fluconazole resistant. Voriconazole showed more potent activity than either fluconazole or itraconazole, including against some isolates resistant to both fluconazole and itraconazole. However, for fluconazole-resistant isolates, the MICs of itraconazole and voriconazole were proportionally higher than for the fluconazole-susceptible isolates, suggesting cross-resistance. Voriconazole may be a useful additional agent for the management of oral fungal infections caused by strains resistant to fluconazole and itraconazole (Bagg, 2005).
- The natural susceptibility of 54 Yersinia enterocoliticia like strains were tested to 69 antibiotics. MICs were determined using broth microdilution method. All Yersinia were tested showed uniform MIC to most antibiotics and were naturally sensitive or intermediate to aminoglycoside, several cephalosporins and penicillins, carbapenems, quinolones, tetracyclines, antifoltes and nitrofurantoin and naturally resistant to rifampicin, benylpenicillin, oxacillin, glycopeptides, all macrolides except azithromycin and fusidic acid. Significant differences in susceptibility affecting clinical assessment criteria were seen with aminopenicillins (in the presence and absence of β-lactamase inhibitors), some cephalosporins (e.g., cefoxitin) and fosfomycin (Stock, 2002).

- A test was carried on in Korea. One hundred and twenty-one isolates of Stenotrophomonas maltophilia complex were collected from seven Korean hospitals. Using broth microdilution method antimicrobial susceptibility was tested. Antimicrobial resistance rates varied among species or groups of S. maltophilia complex. The finding of high antimicrobial resistance rates, particularly to TMP/SMX, among S. maltophilia complex isolates from Korea, and the existence of distinct groups among the isolates, with differences in antimicrobial resistance rates, suggests consideration of alternative agents to TMP/SMX to treat S. maltophilia infections and indicates the importance of accurate identification for appropriate selection of treatment options (Rhee *et al*, 2013).
- An experiment was carried on over a 10 year period in china to investigate the susceptibility of hospital-associated (HA) and community-associated (CA) Escherichia coli and Klebsiella pneumonia isolated from patients with intra-abdominal infections. MIC were determined for 12 antibiotics against 3074 E. coli and 1025 K.pneumoniae using broth microdilution method. During the 10 year study period, ertapenem, imipenem, amikacin and piperacillinn tazobactam retained high and stable activity against E. coli and K. pneumonia. However, the susceptibility of E. coli to cephalosporin and ampicillin-sulbactam decreased dramatically during the 10 years (Yang *et al*, 2013).

- Broth microdilution was used to examine the antimicrobial susceptibility of animal and human isolates of Clostridium difficile to 30 antimicrobials. When comparing the prevalence of antimicrobial resistance, the isolates of animal origin were significantly more often resistant to oxacillin, gentamicin and trimethoprim/sulfamethoxazole. The most significant difference between the animal and human populations was found in the level of imipenem resistance, with a prevalence of 53.3 % in isolates of human origin and 28.1 % in isolates of animal origin. Overall, the results show similar MICs for the majority of tested antimicrobials for isolates from human and animal sources (Pirs *et al*, 2013).

- The optimal method for the determination of the MIC of antimicrobials against Helicobacter pylori has not been established. This journal has compared the result of broth dilution and epsilometer agar diffusion gradient test (E-test). The MICs for ampicillin and clarithromycin determined by broth microdilution were highly reproducible. The correlation between the MICs determined by E-test and broth micro dilution was excellent for both ampicillin and clarithromycin (Hachem *et al*, 1996).

- Antifungal susceptibility tests can also be performed through broth micro dilution, on fungi that cause disease, especially if they belong to a species exhibiting resistance to commonly used antifungal agents. Antifungal susceptibility testing is also important for resistance surveillance, for epidemiological studies and for comparing the in-vitro activity of new and existing agents. European Committee for Antimicrobial Susceptibility Testing (EUCAST) has given reference methods for antifungal susceptibility tests to establish the activity of a new antifungal agent or for susceptibility test (Tudela *et al*, 2008).

2.2 Aim of the Study

The main goals of this study were to determine MIC of different antibiotics for different microorganism using broth microdilution method and to know their susceptibility pattern in recent time.

3. Method and Material

3.1 Test organisms:

Test organisms were clinical isolations collected from BIRDEM hospital.

3.2 Antimicrobial agent:

Standard antimicrobial agents are collected directly from manufacturer. These antimicrobial agents are labelled properly with generic name, lot number, and potency and expiration date and stored as directed by respective manufacturer.

3.3 List of Antibiotic Standard powder

Antibiotic Powder	Name of the Company	Potency
Levofloxacin USP	Asiatic Laboratory Ltd	95.87%
Azithromycin	Incepta Pharmaceuticals	99.99%
Cephradine	Asiatic Laboratory Ltd	91.67%
Ciprofloxacin	Incepta Pharmaceuticals	99.99%
Vancomycin HCL	Incepta Pharmaceuticals	99.189%
Ceftriaxone	Incepta Pharmaceuticals	99.99%

3.3 Fig: List of Antibiotic Standard powder used in the test

3.4 Preparation of stock solution:

The amount of required antimicrobial agents were calculated by using following formula-

$$W= (V \times C)/P$$

Where,

W= weight of the antibiotic (mg) to dissolve in Volume (ml)

V= volume required (ml)

C= final concentration of antibiotic (mg/L)

P= potency given by manufacturer (mg/g)

After weighing antibiotics, these agents were dissolved in suitable solvent such as Azithromycin need to dissolve in ethanol rather than water (Andrews J.M, 2001).

3.5 Preparation of dilution range of antibiotics:

Each and every time a stock drug solution of 256 mg/ml was prepared from which a serial dilution of 2 times was carried out having concentration of 128 mg/ml, 64 mg/ml, 32 mg/ml, 16 mg/ml, 8 mg/ml, 4 mg/ml, 2 mg/ml, 1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml, 0.0625 mg/ml, 0.03125 mg/ml, 0.015625 mg/ml.

Based on the result of disk diffusion process for specific organisms, (16 – 0.0156) mg/ml range has been used for micro dilution method.

3.6 Inoculum Preparation for test

Turbidity Standard for Inoculum Preparation

A $BaSO_4$ turbidity standard was prepared equivalent to 0.5 McFarland standard to standardize the inoculum density which were used for susceptibility test.

3.6.1 Inoculum preparation

- **3.6.1.1 Subculture of organisms**
  In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24 hours at 370C for their optimum growth. These fresh cultures were used for the sensitivity test.

- **3.6.1.2 Inoculum for susceptibility test**
  Single colony was transferred from bacteria subculture into broth media to prepare colony suspension whose turbidity further standardized by using the absorbance value of 0.5 Mcfarland $BaSO_4$ solution by adding saline.

3.7 Reagents and Apparatus:

- ✓ Autoclave
- ✓ Microtitre plates (96 well)
- ✓ Aluminium foil
- ✓ UV spectrometer
- ✓ Microplate reader
- ✓ Nutrient Broth Medium
- ✓ Laminar air flow hood
- ✓ Petri dishes
- ✓ Appendrof tubes
- ✓ Spirit burner
- ✓ Tips
- ✓ Sterile cotton
- ✓ Refrigerator
- ✓ Micropipette
- ✓ Incubator
- ✓ Inoculating loop
- ✓ Ethanol

- ✓ Nosemask and Hand gloves
- ✓ Screw cap test tubes

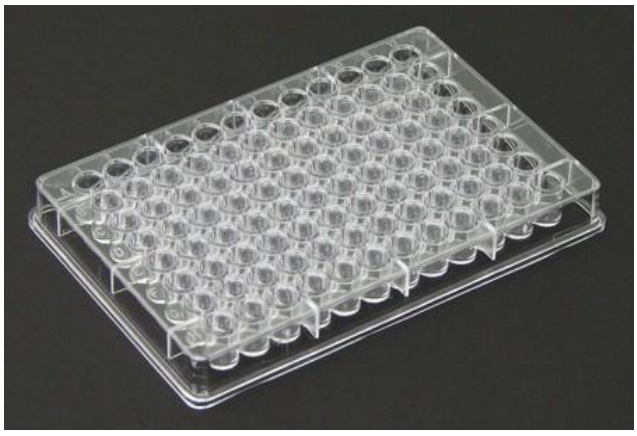
Ingredients	Amount
Bacto beef extract	0.3 gm
Bacto peptone	0.5 gm
Distilled water q.s.	100 ml

3.7 Fig: Table: Composition of nutrient broth medium

Agar and broth medium having this composition was directly brought from the market and the PH = 7.2 + 0.1 at 250C was maintained.

3.8 Procedure:

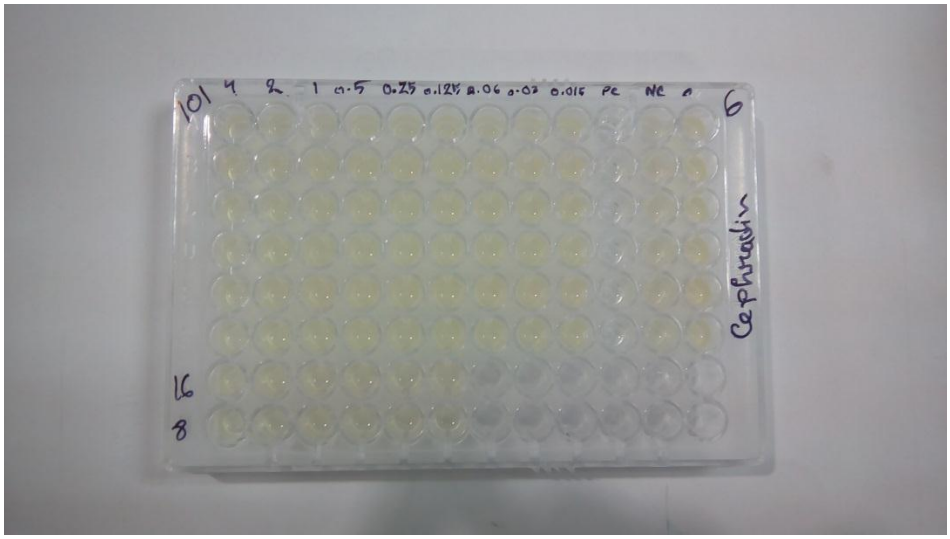
- ✓ Sterile, plastic 96 well containing microtitre plate was used for microdilution where the total plate was cleaned by ethanol to prevent contamination.



3.8.1 Fig: Microtitre plate (96 well)

- ✓ Each microtitre plate contained equal volume of serially diluted drugs along with equal volume of microorganisms from inoculum
- ✓ One column contained positive control (drug solution of highest concentration)
- ✓ One column contained negative control (microorganism from inoculum as growth control)

- ✓ One column contained only broth media to verify the test condition
- ✓ Using micropipette each well was filled with 75 µl of antimicrobial agent along with 75 µl inoculum and mixed by pipetting.



3.8.2 Fig: Microtitre plate with inoculum and drug

- ✓ For each concentration single micro tips has been used
- ✓ After completing addition of microorganisms, plate was labelled and covered by autoclaved foil paper and then incubated for 18-24 hr at (35 ±2)°C (CLSI, 2012).

3.9 Determination of Minimum inhibitory concentration end point:

All plates were taken into micro plate reader to read the optical density of each well at 630 nm. At this point, UV ray can detect the microorganism cells. That's why, as the antimicrobial agents concentration reduced, the growth of organism increased causing increased value of optical density.

MIC value was determined by comparing the growth of microorganisms in antibiotic agent containing well with the growth of well containing no drug at all and this comparison was done on the basis of optical densities at different concentration (CLSI , 2012).

Result & Discussion

Minimum inhibitory concentration (MIC) is defined as lowest concentration of an antimicrobial agent that will inhibit the visible growth of microorganism after overnight incubation (CLSI , 2012).

In case of broth microdilution, growth of microorganism was measured at 630 nm by using microtiter plate reader which provide optical density of each well of microtiter plate. These optical densities were increased as drug concentration has been reduced.
e.g.
Klebsiella-45
Levofloxacin

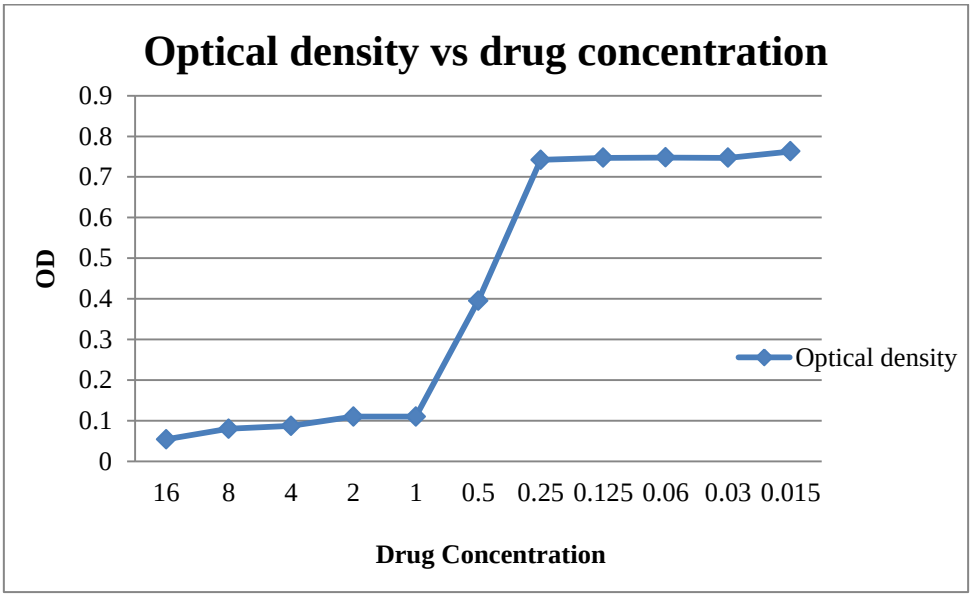


Figure 4.1: Graph of optical density versus drug concentration

These optical densities were used to compare the growth of each well containing antibiotic agents with the growth in growth control well to determine % of inhibition of antimicrobial agents to specify the respective MIC value.
To determine % of inhibition following equation was used-

% of inhibition,

$$= \frac{\text{OD of negative control} - \text{OD at specific drug concentration}}{\text{OD of negative control}} \times 100$$

(CLSI , 2012)

After plotting these values of % of inhibition in graph, gradual fall of % of inhibition according to increased concentration of drug was observed from which MIC value was determined.

The minimum drug concentration at which (up to70 %) microorganism growth was inhibited considered as MIC value for respective antimicrobial agent.
e.g.
Klebsiella-45
Levofloxacin

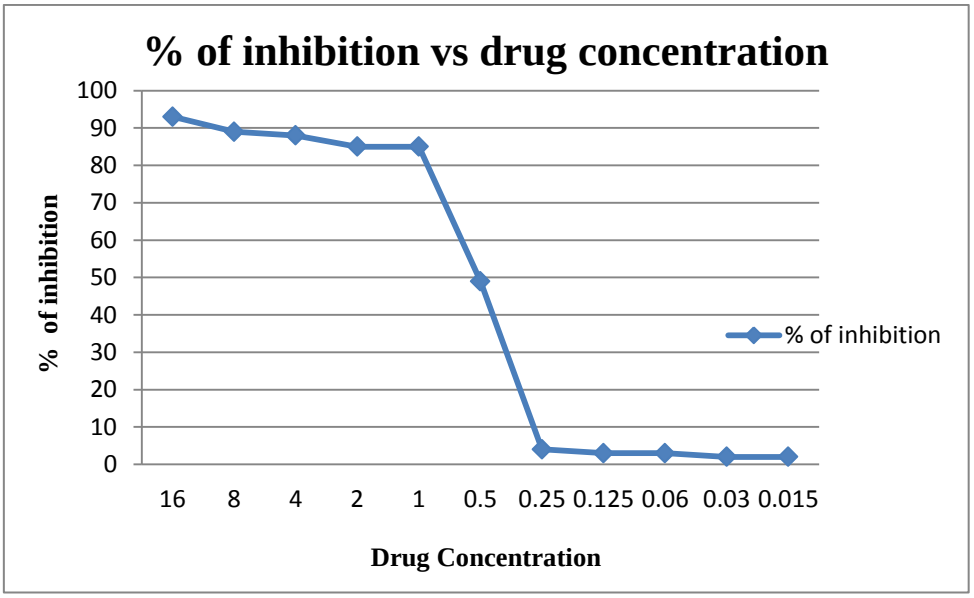


Figure 4.2: Graph of % of inhibition versus drug concentration

In case of this result, value of % of inhibition has been dropped rapidly from 85% to 49%, the lowest drug concentration causing acceptable inhibition is 1µg/ml.
So, MIC value of Levofloxacin against this sample will be 1µg/ml. (Andrews, 2001).

After determining the MIC values of antimicrobial agents, these values were compared with the standard values to state that whether the drug is sensitive or resistant or intermediate.

All results of different antimicrobial agents against microorganisms clinically isolated are mentioned bellow:

4.3.1.1 *Escherichia coli* -28

Ciprofloxacin

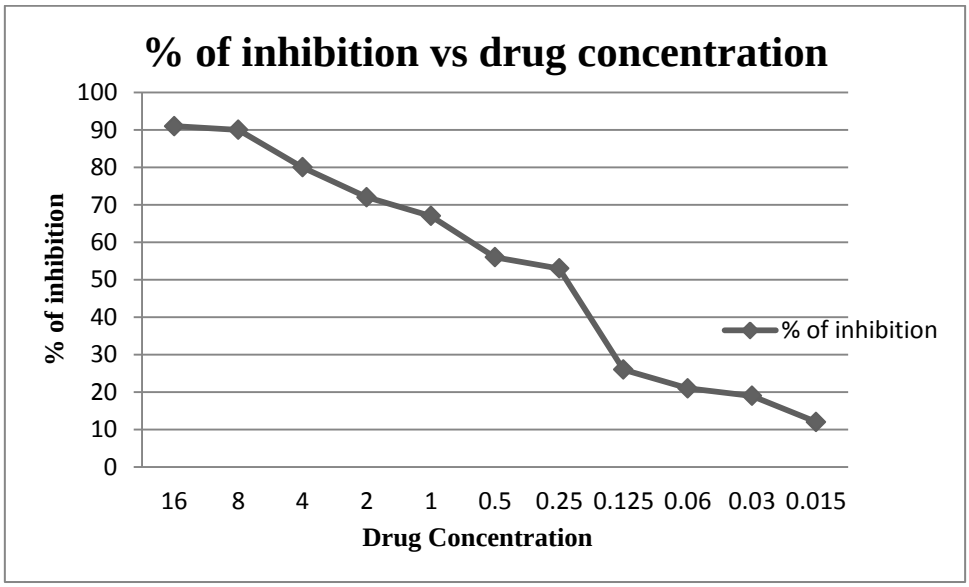


Figure 4.3.1.1: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 80% to 72%, the lowest drug concentration causing acceptable inhibition is 4µg/ml.
So, MIC value of Ciprofloxacin against this sample will be 4µg/ml.

4.3.1.2 *Escherichia coli* -28

Levofloxacin

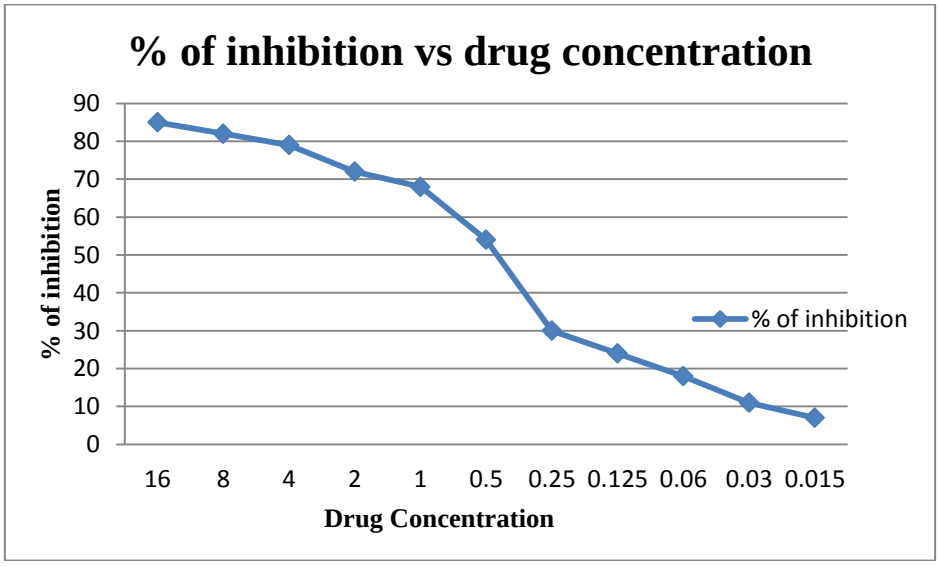


Figure 4.3.1.2: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 79% to 72%, the lowest drug concentration causing acceptable inhibition is 4µg/ml.
So, MIC value of Levofloxacin against this sample will be 4µg/ml.

Table 4.3.1: Determination of MIC status of antimicrobial agent

Antibiotic	MIC value (µg/ml)	Standard MIC range			Status of tested antibiotics
Ciprofloxacin	4	Sensitive ≤	Intermediate	Resistant >	Resistant
		0.5	1	1	
Levofloxacin	4	1	2	2	Rasistant

4.3.2 *Escherichia coli* -34

Vancomycin

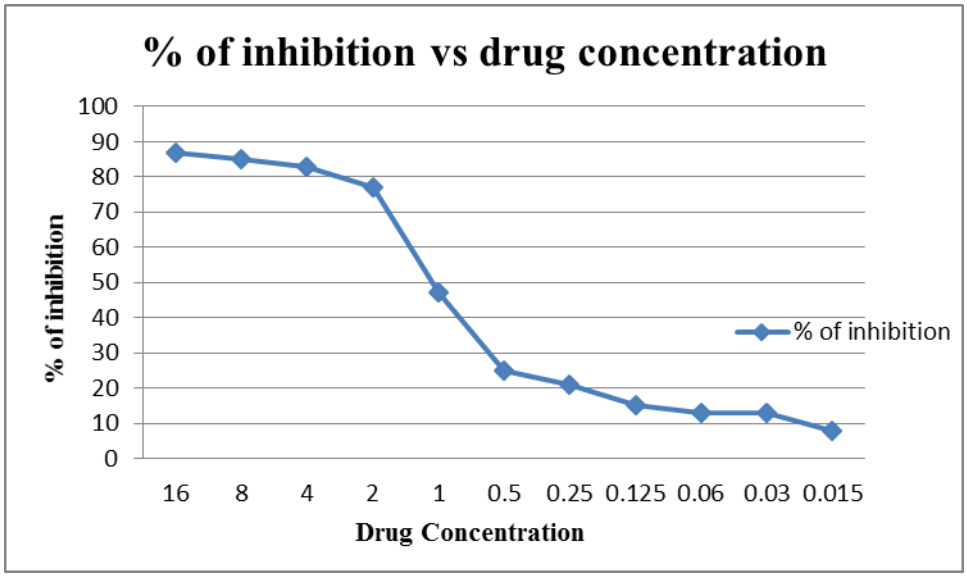


Figure 4.3.2: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 77% to 47%, the lowest drug concentration causing acceptable inhibition is 2µg/ml.
So, MIC value of Vancomycin against this sample will be 2µg/ml.

Table 4.3.2: Determination of MIC status of antimicrobial agent

Antibiotic	MIC value (µg/ml)	Standard MIC range			Status of tested antibiotics
		Sensitive ≤	Intermediate	Resistant >	
Vancomycin	2	2	4	4	Sensitive

4.3.3.1. *Salmonella typhi*-36
Cephradine

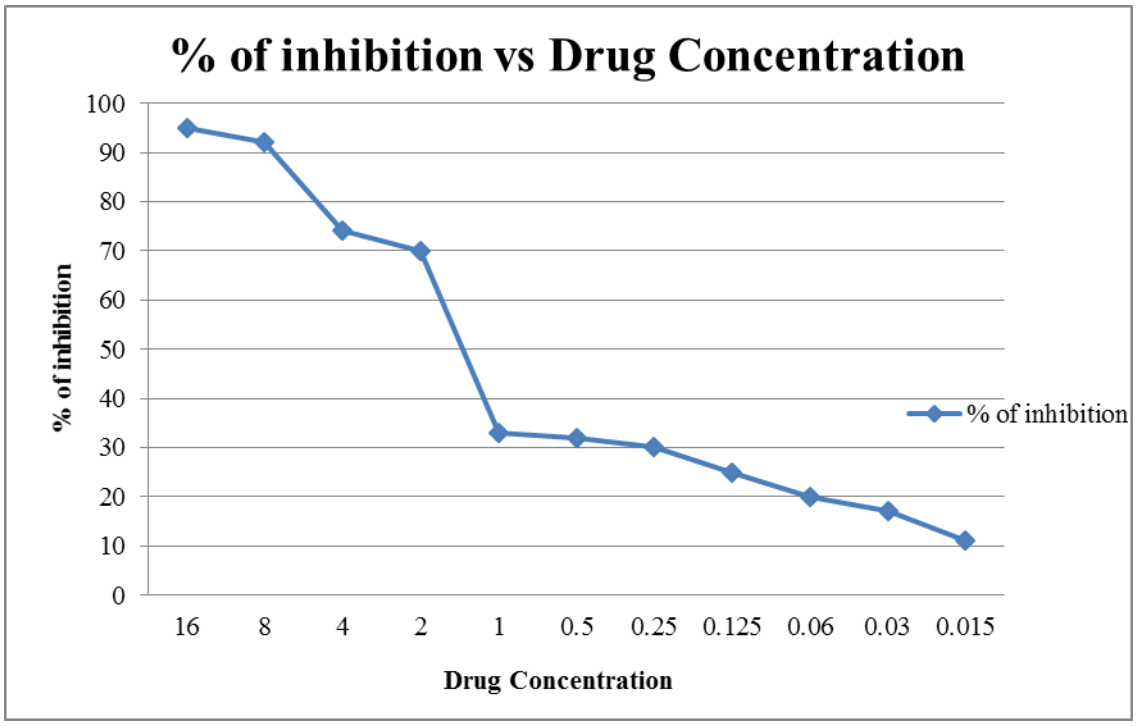


Figure 4.3.3.1: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 70% to 33%, the lowest drug concentration causing acceptable inhibition is 2µg/ml.
So, MIC value of Cephradine against this sample will be 2µg/ml.

4.3.3.2 *Salmonella typhi*-36
Levofloxacin

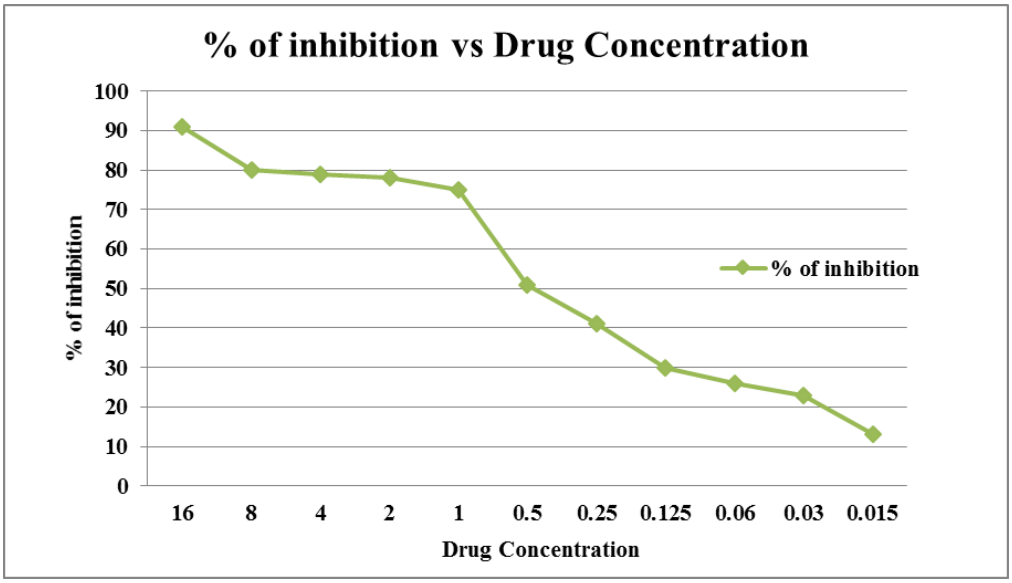


Figure 4.3.3.2: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 75% to 51%, the lowest drug concentration causing acceptable inhibition is 1µg/ml.
So, MIC value of Levofloxacin against this sample will be 1µg/ml.

4.3.3.3 *Salmonella typhi* -36
Azithromycin

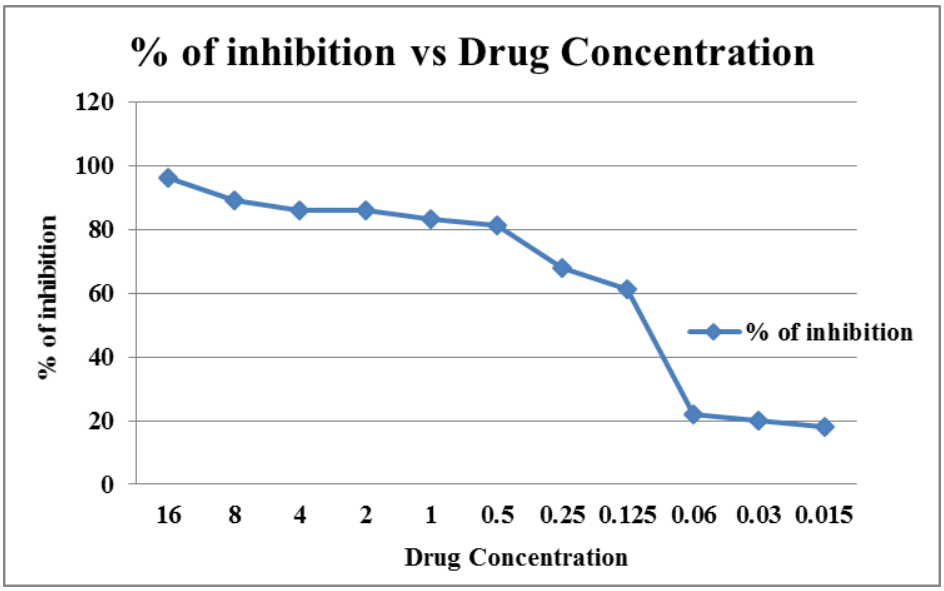


Figure 4.3.3.3: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 81% to 68%, the lowest drug concentration causing acceptable inhibition is 0.5µg/ml.
So, MIC value of Azithromycin against this sample will be 0.5µg/ml.

4.3.3.4 *Salmonella typhi* -36

Ciprofloxacin

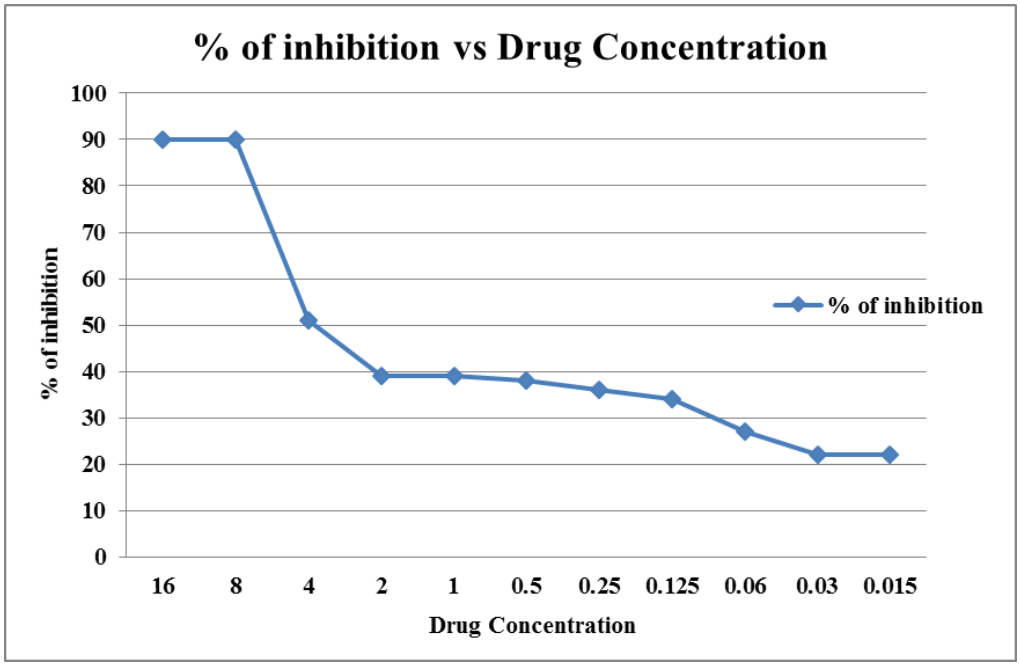


Figure 4.3.3.4: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 90% to 51%, the lowest drug concentration causing acceptable inhibition is 8µg/ml.
So, MIC value of Ciprofloxacin against this sample will be 8µg/ml.

Table 4.3.3: Determination of MIC status of antimicrobial agent

Antibiotic	**MIC value (µg/ml)**	**Standard MIC range**			**Status of tested antibiotics**
Cephradine	2	Sensitive ≤	Intermediate	Resistant >	Intermediate
		1	2	2	
Levofloxacin	1	1	2	2	Sensitive
Azithromycin	0.5	1	2	2	Sensitive
Ciprofloxacin	8	0.5	1	1	Sensitive

4.3.4 *Escherichia coli* -37

Azithromycin

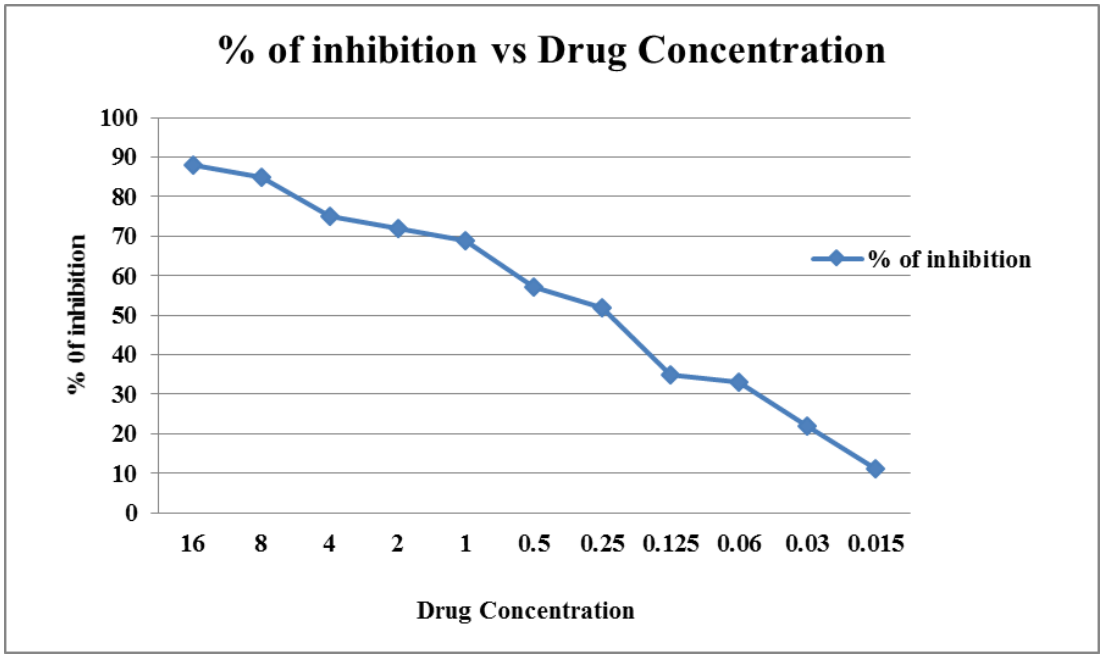


Figure 4.3.4: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 85% to 75%, the lowest drug concentration causing acceptable inhibition is 8µg/ml.
So, MIC value of Azithromycin against this sample will be 8µg/ml.

Table 4.3.4: Determination of MIC status of antimicrobial agent

Antibiotic	**MIC value (µg/ml)**	**Standard MIC range**			**Status of tested antibiotics**
Azithromycin	8	Sensitive ≤	Intermediate	Resistant >	Resistant
		1	1	2	

4.3.5 *Salmonella typhi* -40

Azithromycin

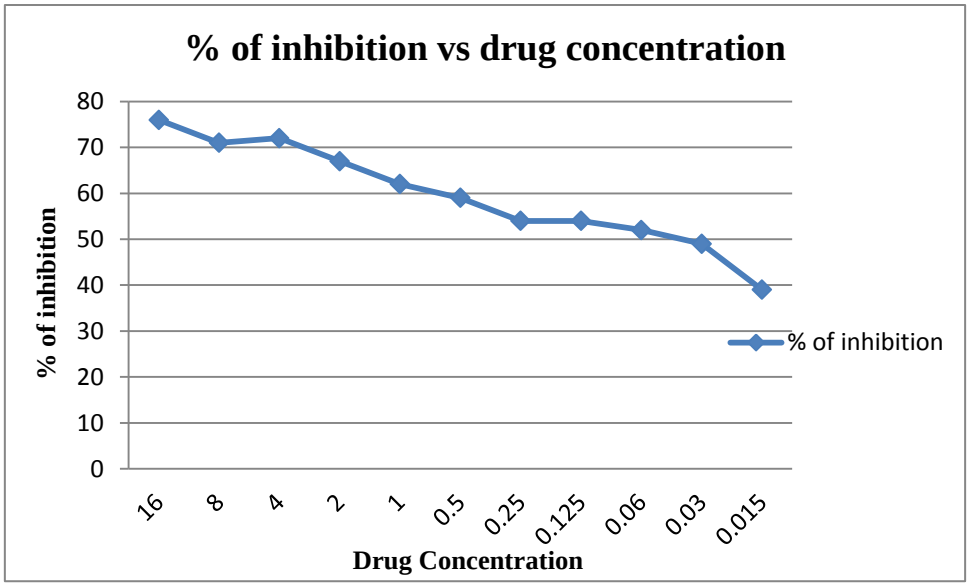


Figure 4.3.5: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 72% to 67%, the lowest drug concentration causing acceptable inhibition is 4µg/ml.
So, MIC value of Azithromycin against this sample will be 4µg/ml.

Table 4.3.5: Determination of MIC status of antimicrobial agent

Antibiotic	MIC value (µg/ml)	Standard MIC range			Status of tested antibiotics
Azithromycin	4	Sensitive ≤	Intermediate	Resistant >	Resistant
		1	2	2	

4.3.6.1 ***Escherichia coli*** **-47**

Levofloxacin

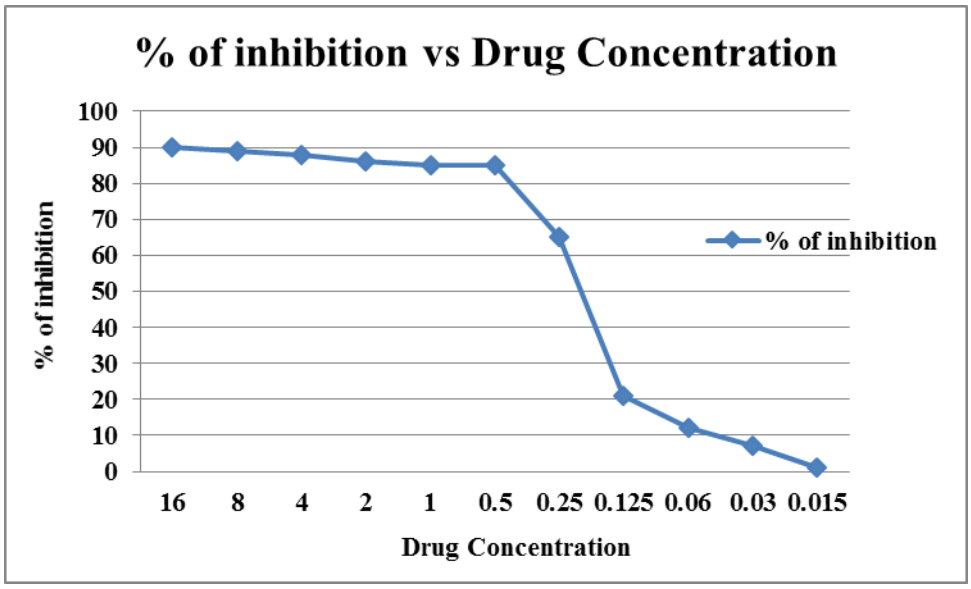


Figure 4.3.6.1: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 85% to 65%, the lowest drug concentration causing acceptable inhibition is 0.5µg/ml.
So, MIC value of Levofloxacin against this sample will be 0.5µg/ml.

4.3.6.2 ***Escherichia coli*** **-47**

Ciprofloxacin

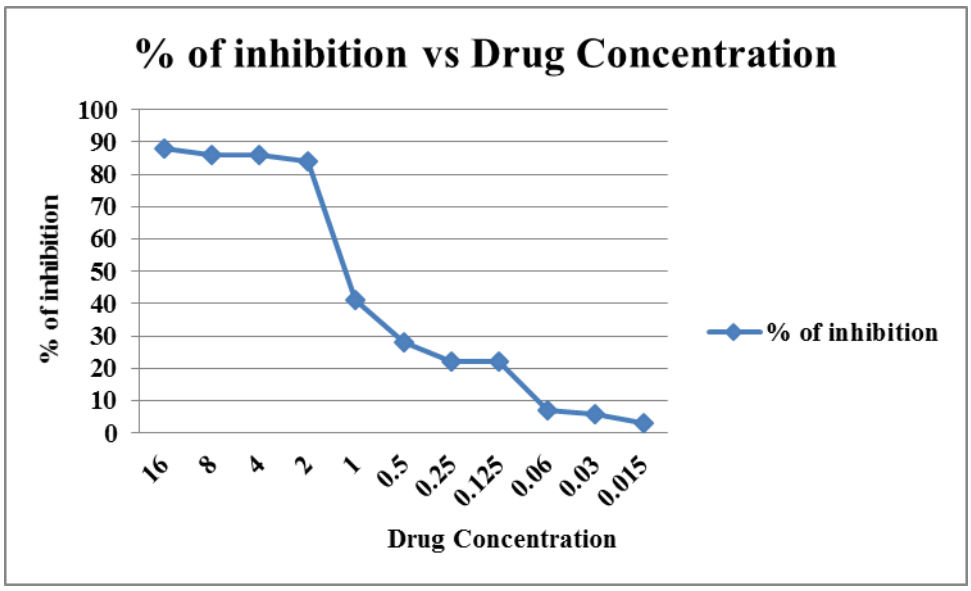


Figure 4.3.6.2: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 84% to 41%, the lowest drug concentration causing acceptable inhibition is 2µg/ml.
So, MIC value of Ciprofloxacin against this sample will be 2µg/ml.

4.3.6.3 *Escherichia coli* -47

Vancomycin

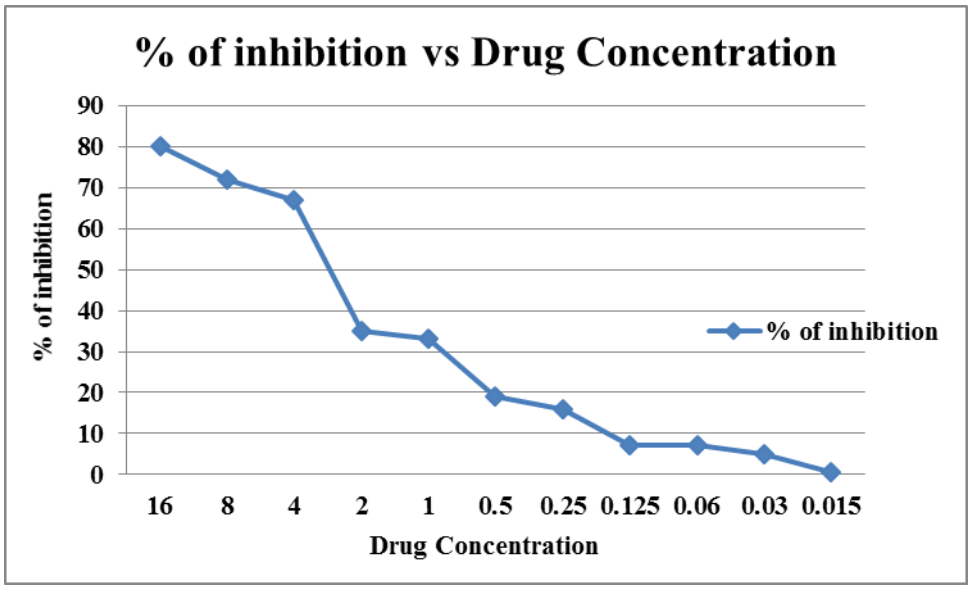


Figure 4.3.6.3: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 80% to 72%, the lowest drug concentration causing acceptable inhibition is 16µg/ml.
So, MIC value of Vancomycin against this sample will be 16µg/ml.

Table 4.3.6: Determination of MIC status of antimicrobial agent

Antibiotic	**MIC value (µg/ml)**	**Standard MIC range**			**Status of tested antibiotics**
Levofloxacin	0.5	Sensitive ≤	Intermediate	Resistant >	Sensitive
		1	1	2	
Vancomycin	16	2	4	4	Resistant
Ciprofloxacin	2	0.5	1	1	Resistant

4.3.7.1 *Klebsiella* -50
Azithromycin

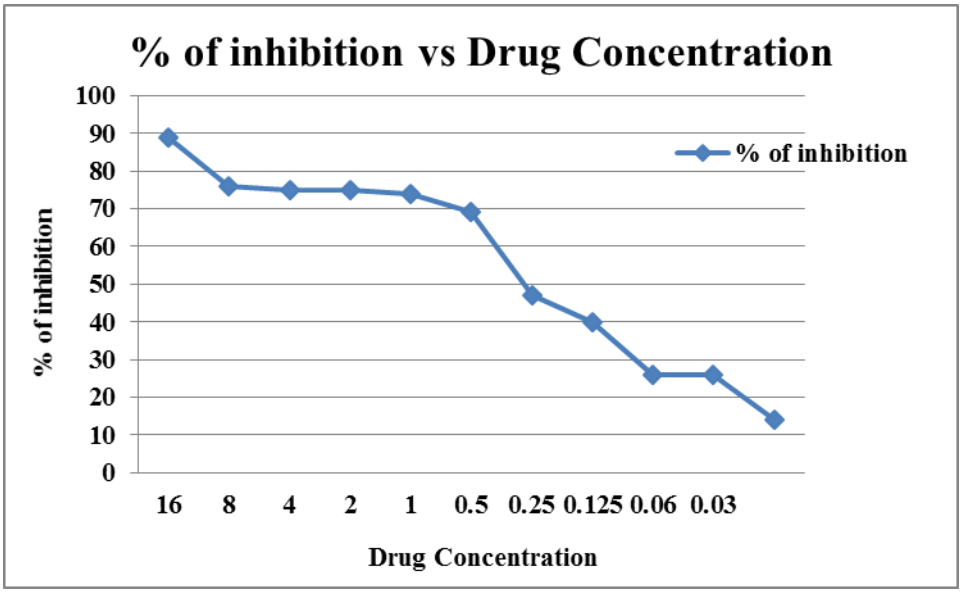


Figure 4.3.7.1: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 74% to 69%, the lowest drug concentration causing acceptable inhibition is 1µg/ml.
So, MIC value of Azithromycin against this sample will be 1µg/ml.

4.3.7.2 *Klebsiella* -50
Ciprofloxacin

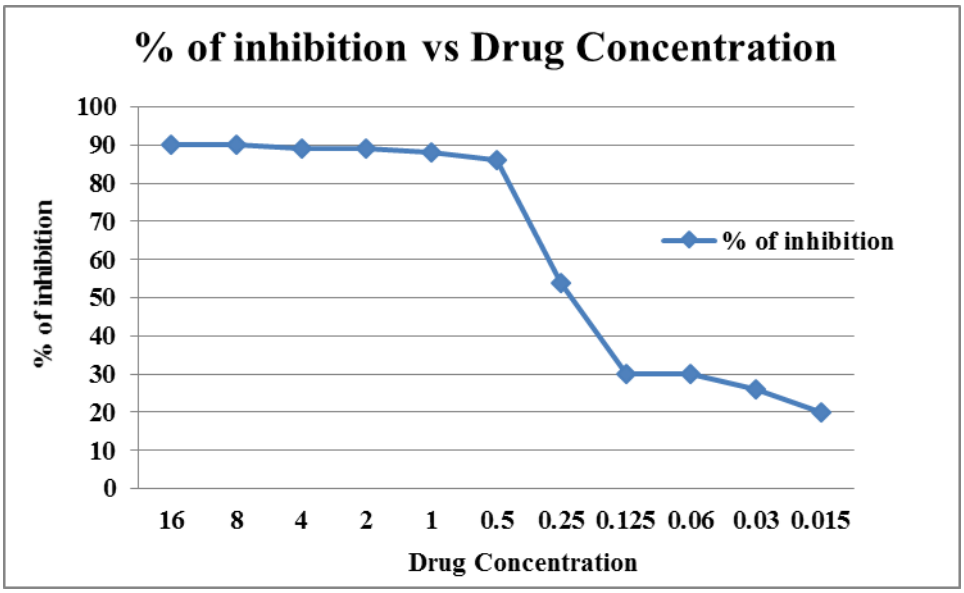


Figure 4.3.7.2: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 86% to 54%, the lowest drug concentration causing acceptable inhibition is 0.5µg/ml.
So, MIC value of Ciprofloxacin against this sample will be 0.5µg/ml.

4.3.7.3 *Klebsiella*-50
Levofloxacin

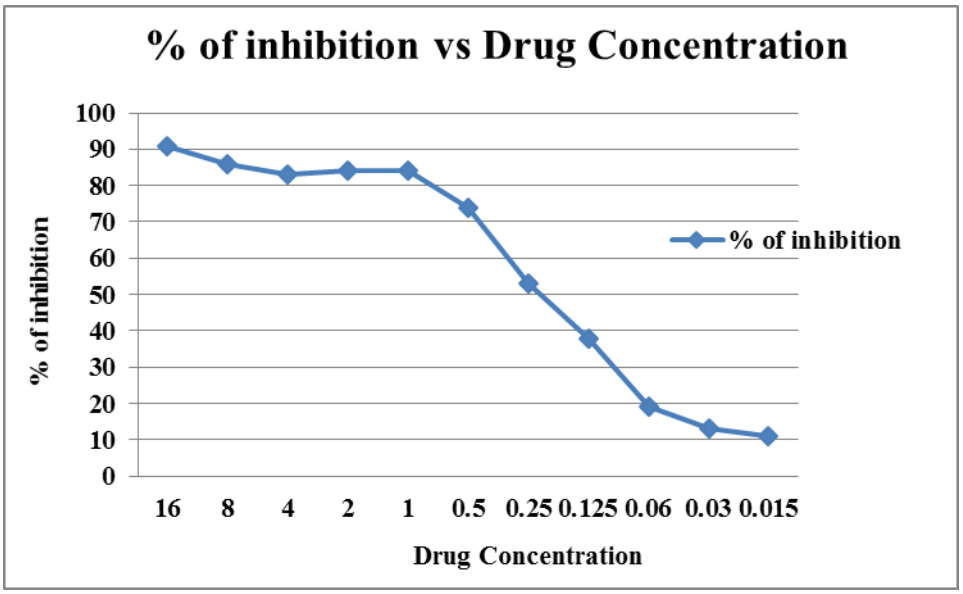


Figure 4.3.7.3: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 74% to 53%, the lowest drug concentration causing acceptable inhibition is 0.5µg/ml.
So, MIC value of Levofloxacin against this sample will be 0.5µg/ml.

Table 4.3.7: Determination of MIC status of antimicrobial agent

Antibiotic	**MIC value (µg/ml)**	**Standard MIC range**			**Status of tested antibiotics**
Azithromycin	1	Sensitive ≤ 1	Intermediate 1	Resistant > 2	Sensitive
Ciprofloxacin	0.5	0.5	1	1	Sensitive
Levofloxacin	0.5	1	2	2	Sensitive

4.3.8.1 *Escherichia coli* -51
Ciprofloxacin

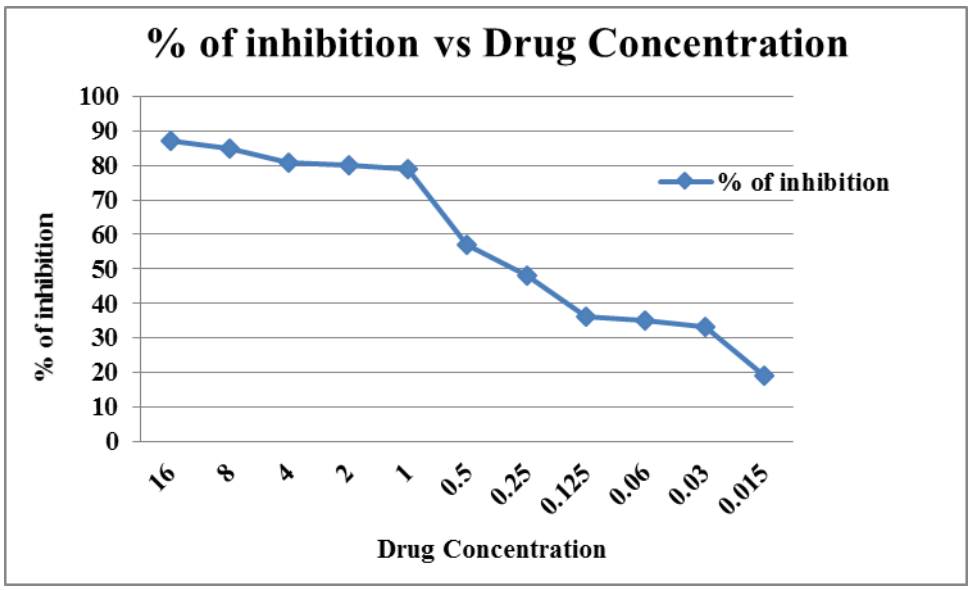


Figure 4.3.8.1: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 79% to 57%, the lowest drug concentration causing acceptable inhibition is 1µg/ml.
So, MIC value of Ciprofloxacin against this sample will be 1µg/ml.

4.3.8.2 *Escherichia coli* -51
Levofloxacin

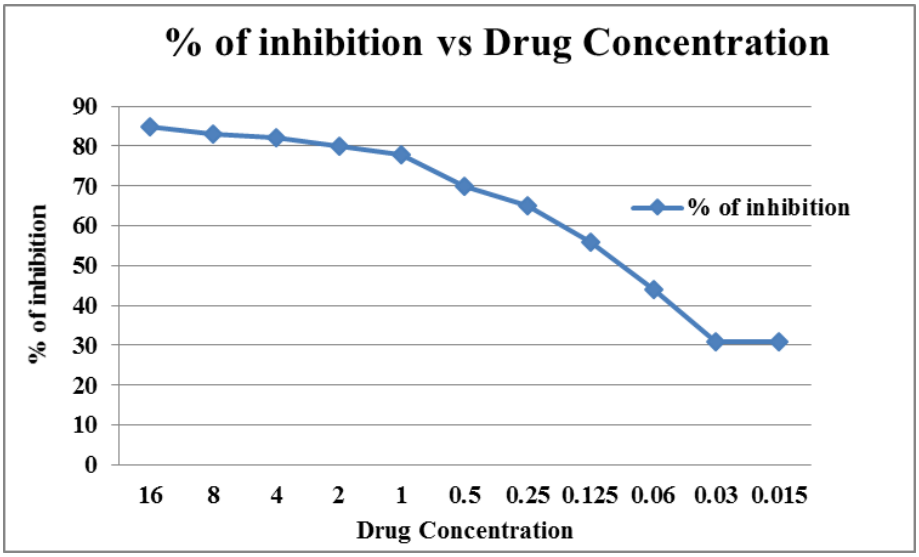


Figure 4.3.8.2: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 78% to 70%, the lowest drug concentration causing acceptable inhibition is 1µg/ml.
So, MIC value of Levofloxacin against this sample will be 1µg/ml.

Table 4.3.8: Determination of MIC status of antimicrobial agent

Antibiotic	MIC value (µg/ml)	Standard MIC range			Status of tested antibiotics
Ciprofloxacin	1	Sensitive ≤	Intermediate	Resistant >	Intermediate
		0.5	1	1	
Levofloxacin	1	1	2	2	Sensitive

4.3.9 *Klebsiella* -54

Ciprofloxacin

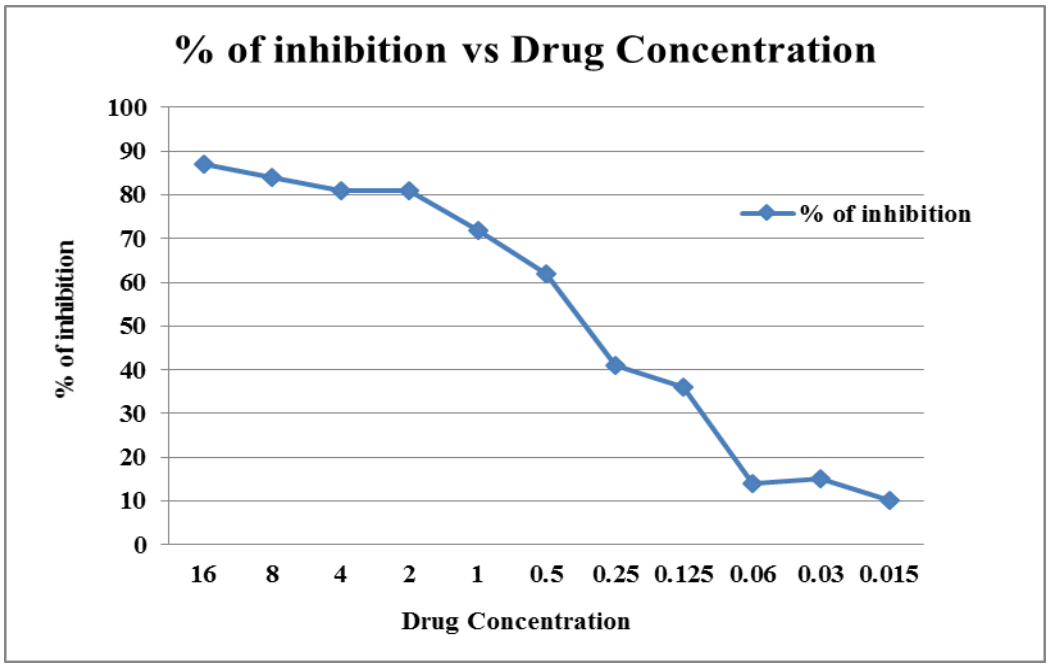


Figure 4.3.9: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 72% to 62%, the lowest drug concentration causing acceptable inhibition is 1µg/ml.
So, MIC value of Ciprofloxacin against this sample will be 1µg/ml.

Table 4.3.9: Determination of MIC status of antimicrobial agent

Antibiotic	MIC value (µg/ml)	Standard MIC range			Status of tested antibiotics
Ciprofloxacin	1	Sensitive ≤	Intermediate	Resistant >	Intermediate
		0.5	1	1	

4.3.10.1 ***Escherichia coli*** **-79**
Azithromycin

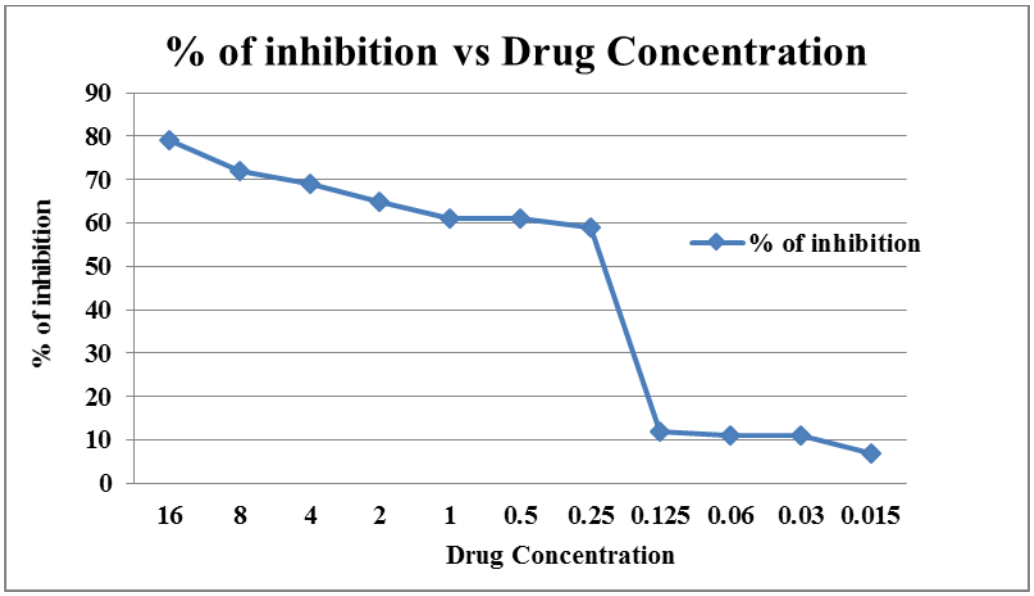


Figure 4.3.10.1: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 79% to 72%, the lowest drug concentration causing acceptable inhibition is 16µg/ml.

So, MIC value of Azithromycin against this sample will be 16µg/ml.

4.3.10.2 *Escherichia coli* -79

Levofloxacin

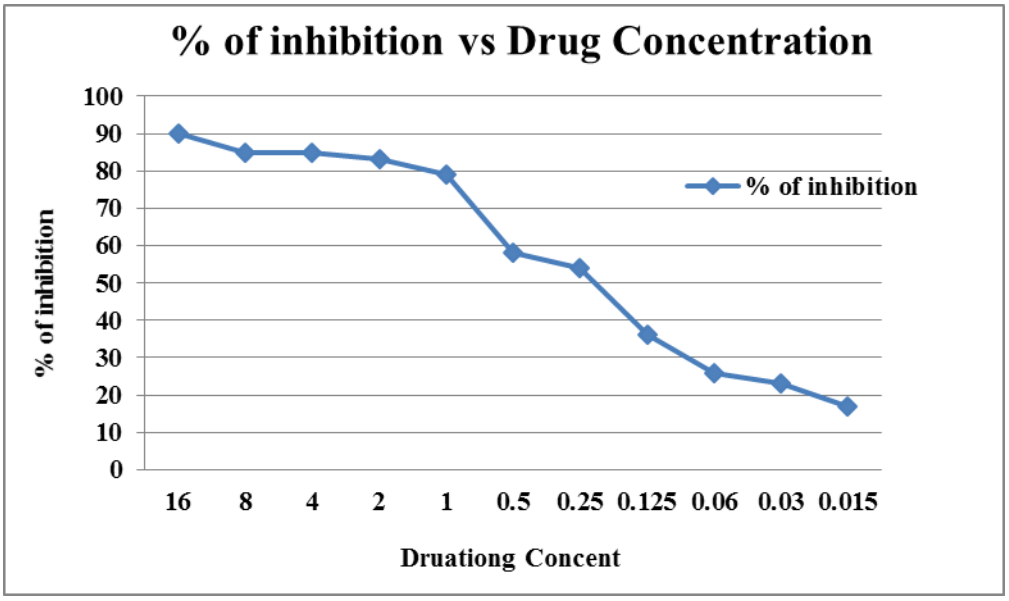


Figure 4.3.10.2: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 79% to 58%, the lowest drug concentration causing acceptable inhibition is 1µg/ml.

So, MIC value of Levofloxacin against this sample will be 1µg/ml.

Table 4.3.10: Determination of MIC status of antimicrobial agent

Antibiotic	**MIC value (µg/ml)**	**Standard MIC range**			**Status of tested antibiotics**
Azithromycin	16	Sensitive ≤ 1	Intermediate 1	Resistant > 2	Resistant
Levofloxacin	1	1	2	2	Sensitive

4.3.11.1 *Klebsiella*-80
Azithromycin

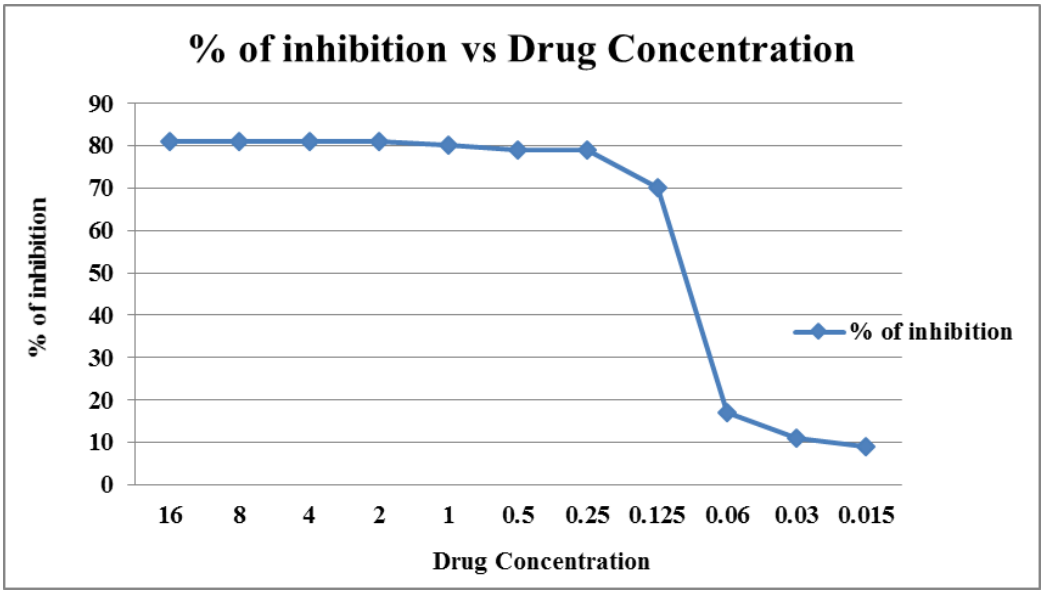


Figure 4.3.11.1: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 75% to 61%, the lowest drug concentration causing acceptable inhibition is 1µg/ml.

So, MIC value of Azithromycin against this sample will be 1µg/ml.

4.3.11.2 *Klebsiella*-80
Levofloxacin

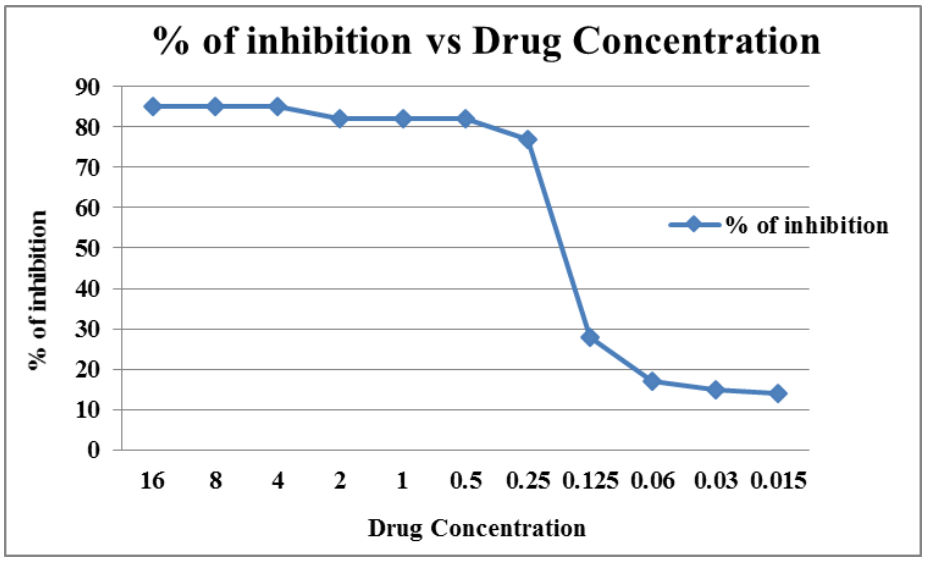


Figure 4.3.11.2: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 82% to 50%, the lowest drug concentration causing acceptable inhibition is 0.5µg/ml.

So, MIC value of Levofloxacin against this sample will be 0.5µg/ml.

Table 4.3.11: Determination of MIC status of antimicrobial agent

Antibiotic	MIC value (µg/ml)	Standard MIC range			Status of tested antibiotics
Azithromycin	1	Sensitive ≤	Intermediate	Resistant >	Sensitive
		1	2	2	
Levofloxacin	0.5	1	2	2	Sensitive

4.3.12. *Pseudomonas* -81

Levofloxacin

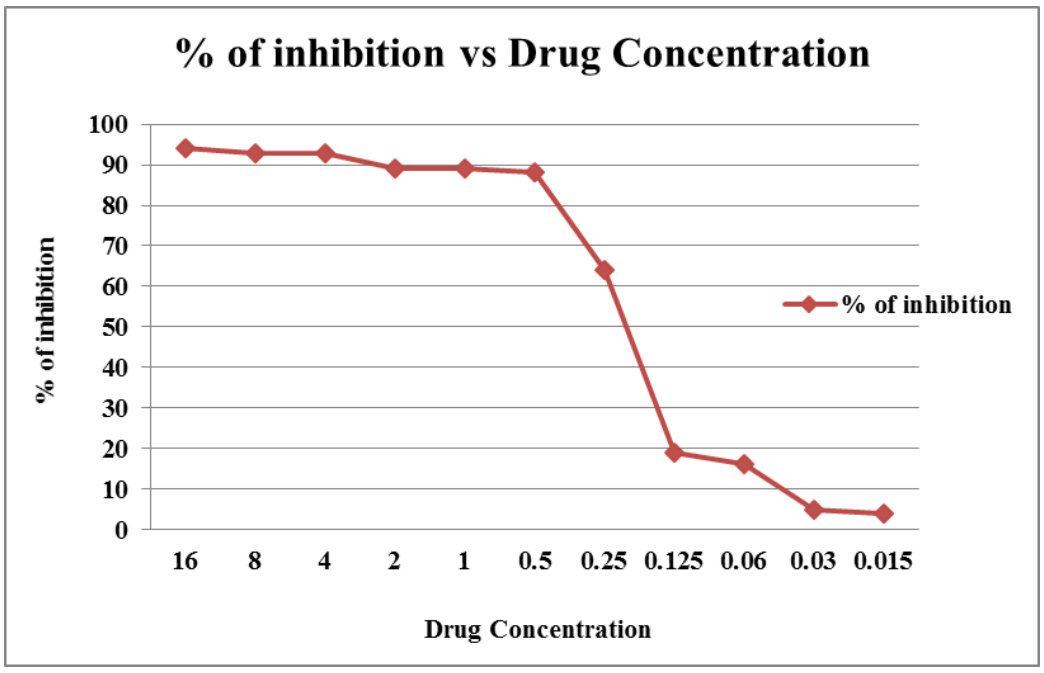


Figure 4.3.12: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 88% to 64%, the lowest drug concentration causing acceptable inhibition is 0.5µg/ml.

So, MIC value of Levofloxacin against this sample will be 0.5µg/ml.

Table 4.3.12: Determination of MIC status of antimicrobial agent

Antibiotic	MIC value (µg/ml)	Standard MIC range			Status of tested antibiotics
Levofloxacin	0.5	Sensitive ≤	Intermediate	Resistant >	Sensitive
		1	2	2	

4.3.13.1 ***Escherichia coli*** **-99**

Levofloxacin

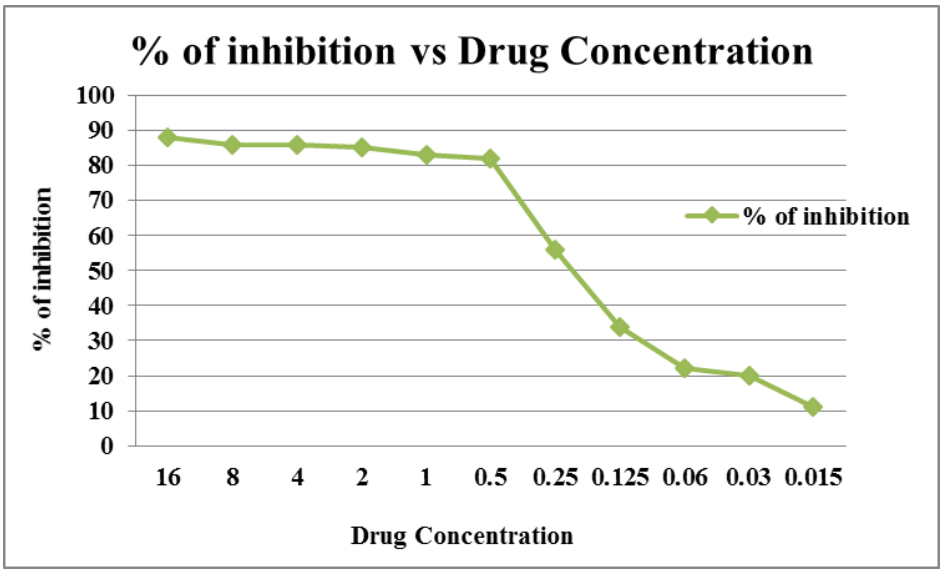


Figure 4.3.13.1: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 82% to 56%, the lowest drug concentration causing acceptable inhibition is 0.5µg/ml.

So, MIC value of Levofloxacin against this sample will be 0.5µg/ml.

4.3.13.2 *Escherichia coli* -99
Cephradine

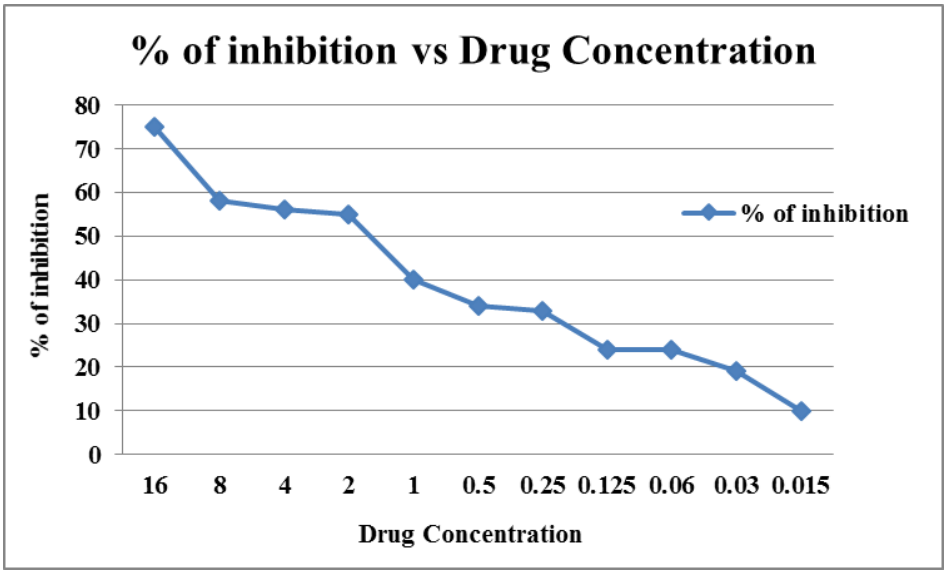


Figure 4.3.13.2: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 75% to 58%, the lowest drug concentration causing acceptable inhibition is 16µg/ml.

So, MIC value of Cephradine against this sample will be 16µg/ml.

4.3.13.3 *Escherichia coli* -99
Azithromycin

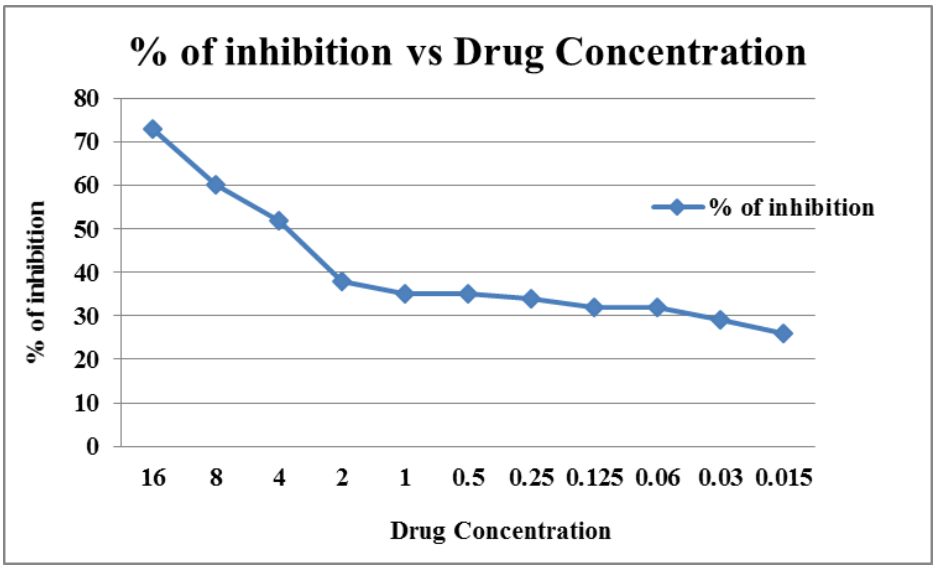


Figure 4.3.13.3: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 73% to 60%, the lowest drug concentration causing acceptable inhibition is 16µg/ml.

So, MIC value of Azithromycin against this sample will be 16µg/ml.

Table 4.3.13: Determination of MIC status of antimicrobial agent

Antibiotic	MIC value (µg/ml)	Standard MIC range			Status of tested antibiotics
Levofloxacin	0.5	Sensitive ≤	Intermediate	Resistant >	Sensitive
		1	1	2	
Cephradine	16	1	2	2	Resistant
Azithromycin	16	1	2	2	Resistant

4.3.14.1 *Klebsiella* -100
Azithromycin

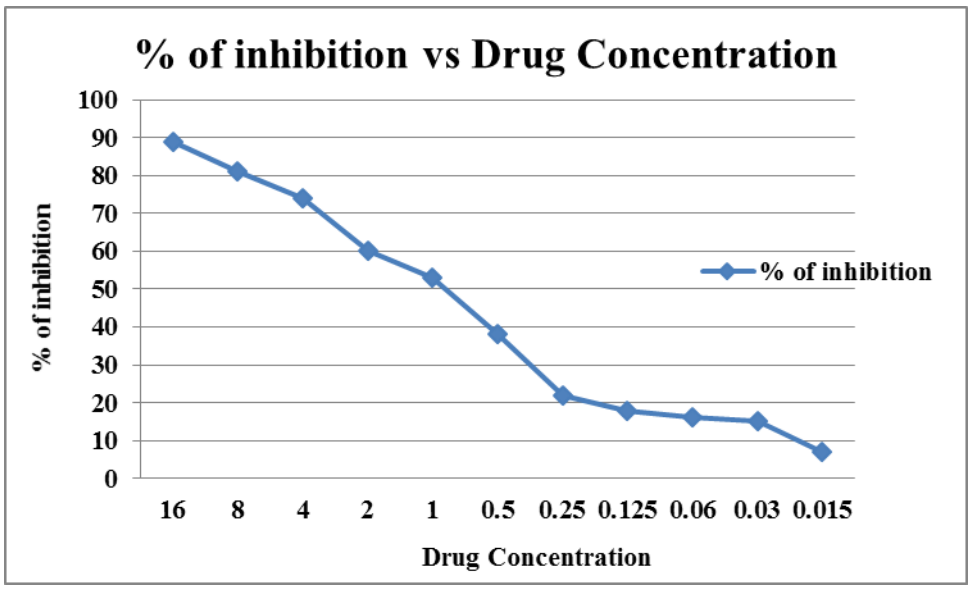


Figure 4.3.14.1: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 74% to 60%, the lowest drug concentration causing acceptable inhibition is 4µg/ml.

So, MIC value of Azithromycin against this sample will be 4µg/ml.

4.3.14.2 *Klebsiella* -100
Levofloxacin

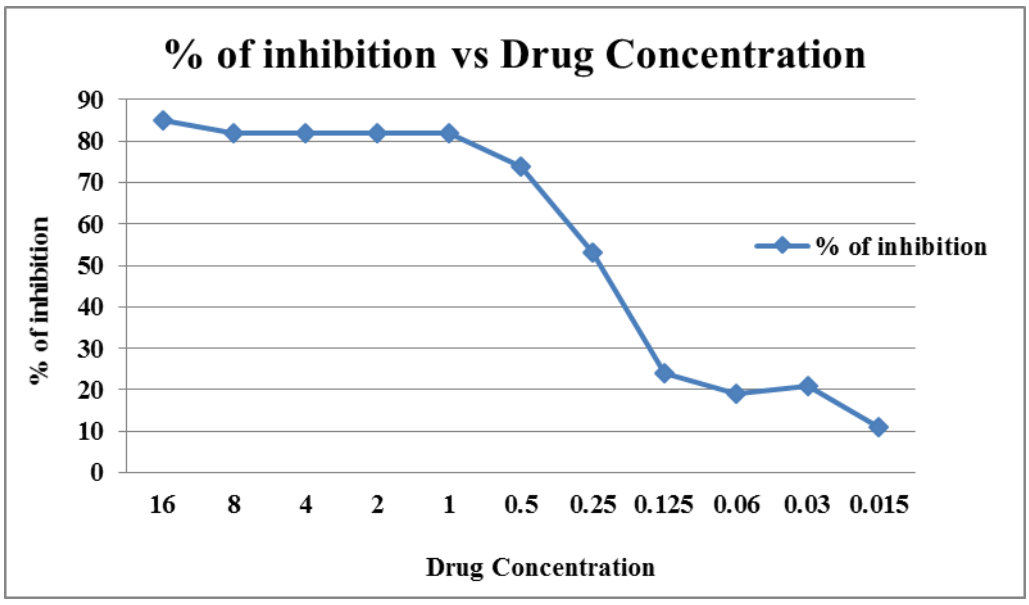


Figure 4.3.14.2: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 74% to 53%, the lowest drug concentration causing acceptable inhibition is 0.5µg/ml.

So, MIC value of Levofloxacin against this sample will be 0.5µg/ml.

4.3.14.3 *Klebsiella* -100
Ciprofloxacin

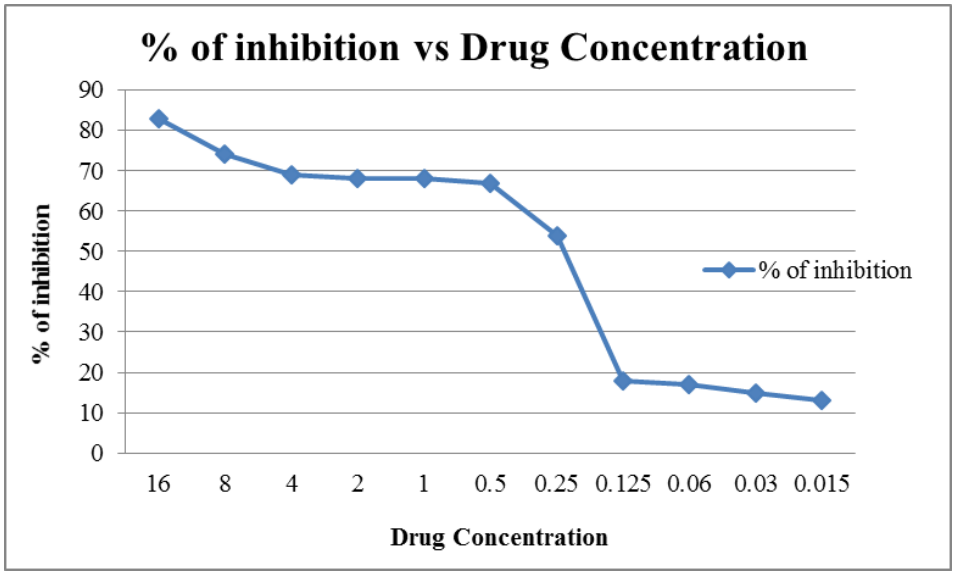


Figure 4.3.14.3: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 67% to 54%, the lowest drug concentration causing acceptable inhibition is 0.5µg/ml.

So, MIC value of Ciprofloxacin against this sample will be 0.5µg/ml.

Table 4.3.14: Determination of MIC status of antimicrobial agent

Antibiotic	MIC value (µg/ml)	Standard MIC range			Status of tested antibiotics
Azithromycin	4	Sensitive ≤	Intermediate	Resistant >	Resistant
		1	1	2	
Levofloxacin	0.5	1	2	2	Sensitive
Ciprofloxacin	0.5	0.5	1	1	Sensitive

4.3.15.1 *Klebsiella* -105
Azithromycin

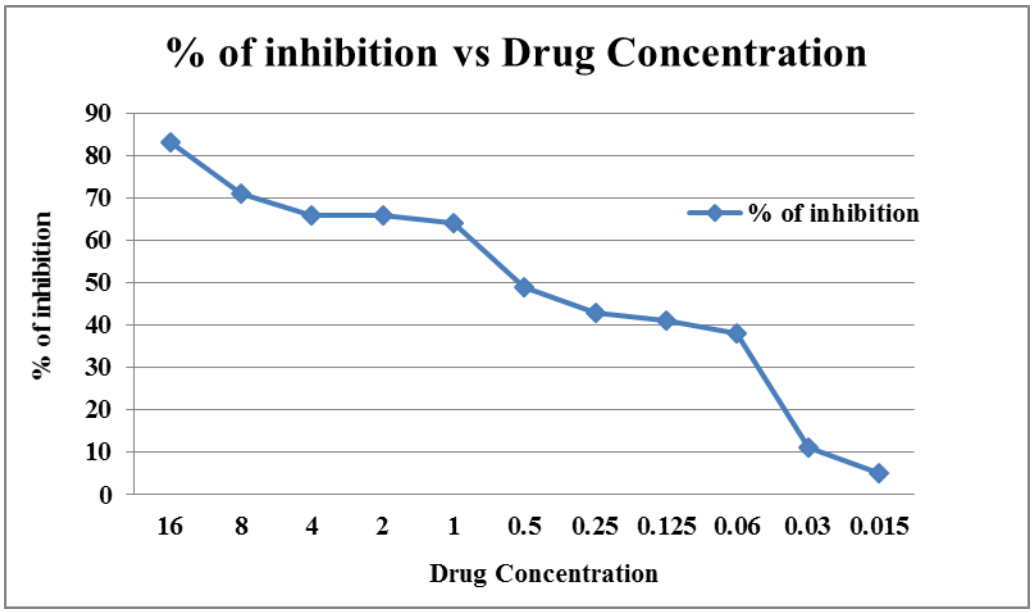


Figure 4.3.15.1: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 83% to 71%, the lowest drug concentration causing acceptable inhibition is 16µg/ml.

So, MIC value of Azithromycin against this sample will be 16µg/ml.

4.3.15.2 *Klebsiella* -105

Levofloxacin

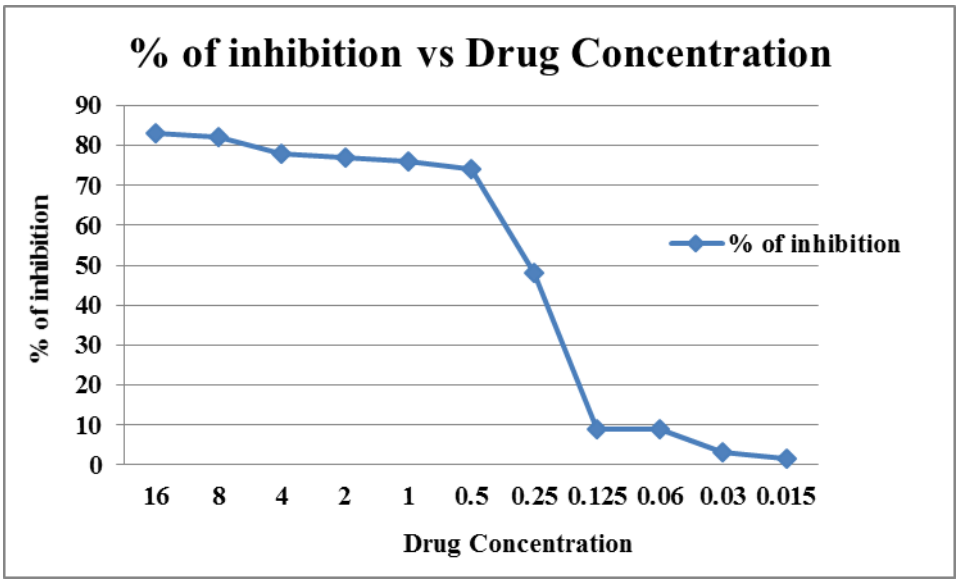


Figure 4.3.15.2: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 74% to 48%, the lowest drug concentration causing acceptable inhibition is 0.5µg/ml.

So, MIC value of Levofloxacin against this sample will be 0.5µg/ml.

Table 4.3.15: Determination of MIC status of antimicrobial agent

Antibiotic	MIC value (µg/ml)	Standard MIC range			Status of tested antibiotics
Azithromycin	16	Sensitive ≤	Intermediate	Resistant >	Resistant
		1	1	2	
Levofloxacin	0.5	1	2	2	Sensitive

4.3.16.1 *Staphoylocccus aureus* -106
Azithromycin

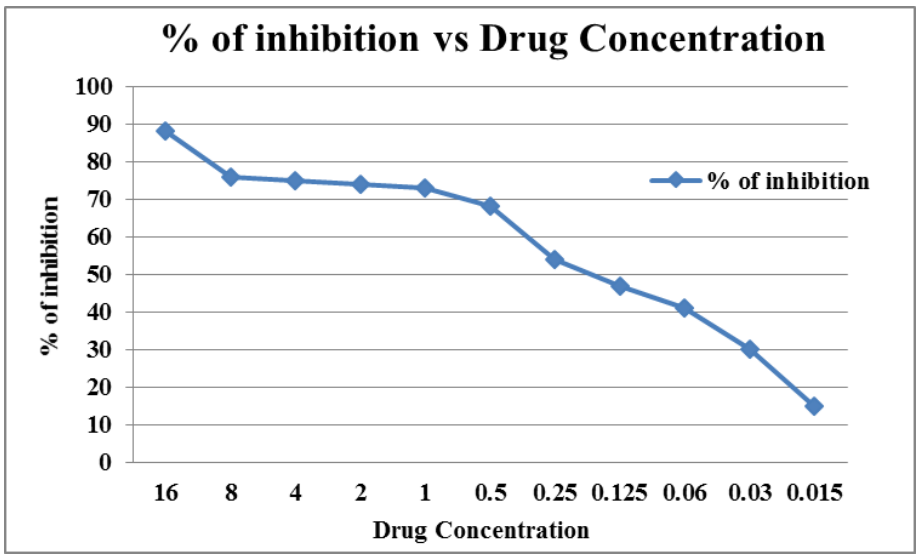


Figure 4.3.16.1: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 73% to 68%, the lowest drug concentration causing acceptable inhibition is 1µg/ml.

So, MIC value of Azithromycin against this sample will be 1µg/ml.

4.3.16.2 *Staphoylocccus aureus* -106
Levofloxacin

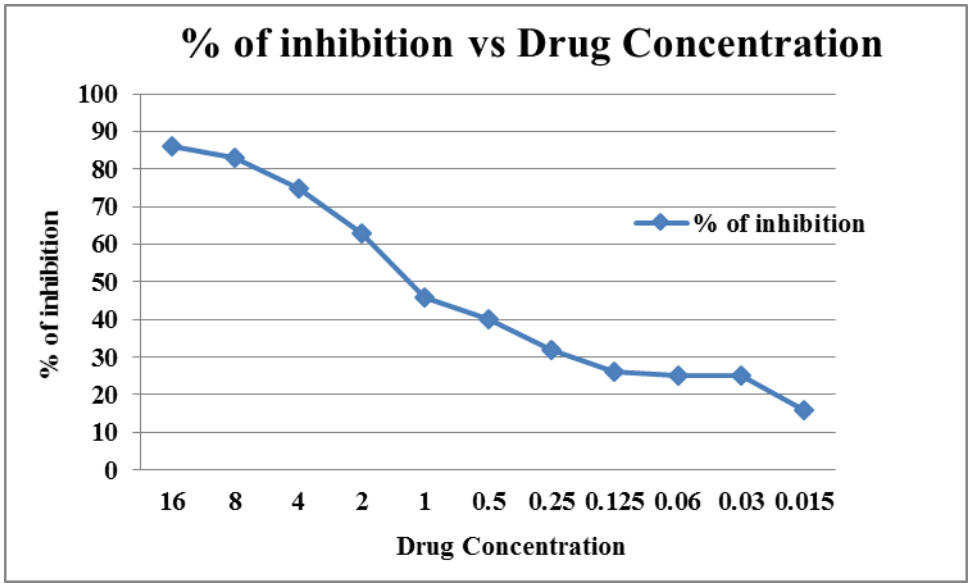


Figure 4.3.16.2: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 75% to 63%, the lowest drug concentration causing acceptable inhibition is 4µg/ml.

So, MIC value of Levofloxacin against this sample will be 4µg/ml.

4.3.16.3 *Staphoylocccus aureus* -106
Ciprofloxacin

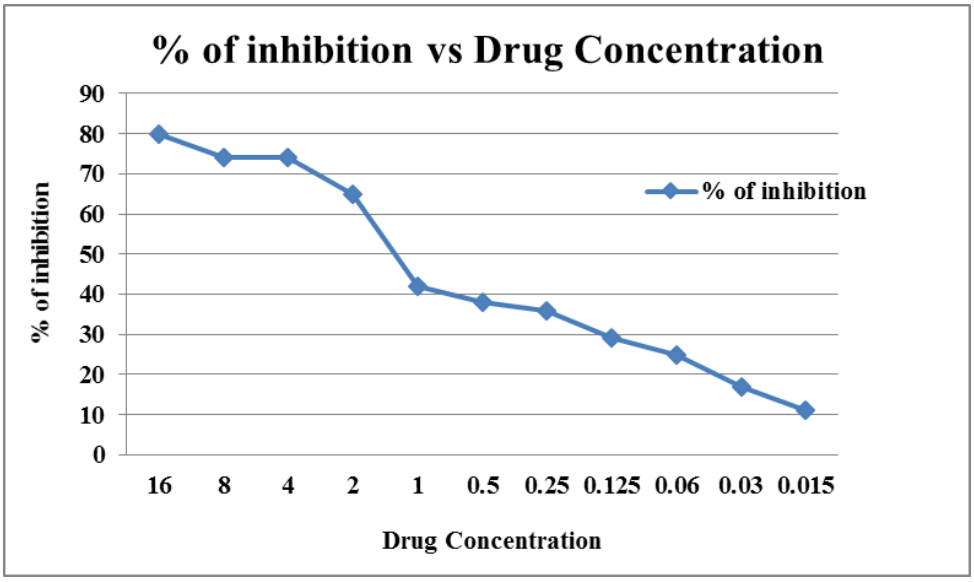


Figure 4.3.16.3: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 74% to 65%, the lowest drug concentration causing acceptable inhibition is 4µg/ml.

So, MIC value of Ciprofloxacin against this sample will be 4µg/ml.

Table 4.3.16: Determination of MIC status of antimicrobial agent

Antibiotic	**MIC value (µg/ml)**	**Standard MIC range**			**Status of tested antibiotics**
Azithromycin	1	Sensitive ≤	Intermediate	Resistant >	Sensitive
		1	1	2	
Levofloxacin	4	1	2	2	Resistant
Ciprofloxacin	4	0.5	1	1	Resistant

4.3.17 *Klebsiella* -110
Levofloxacin

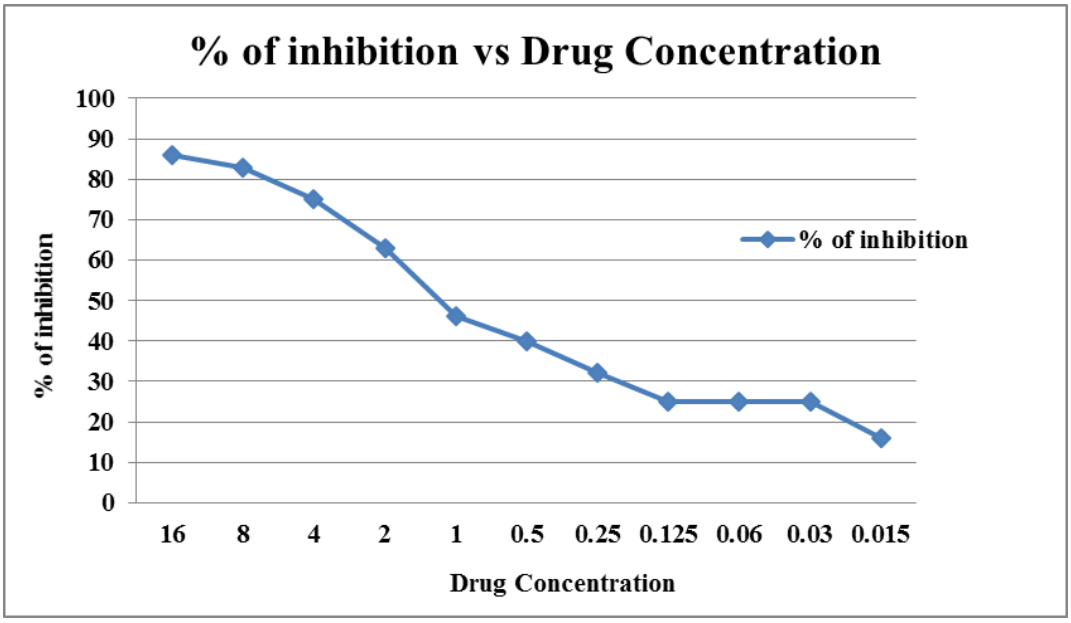


Figure 4.3.17: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 75% to 63%, the lowest drug concentration causing acceptable inhibition is 4µg/ml.

So, MIC value of Levofloxacin against this sample will be 4µg/ml.

Table 4.3.17: Determination of MIC status of antimicrobial agent

Antibiotic	MIC value (µg/ml)	Standard MIC range			Status of tested antibiotics
Levofloxacin	4	Sensitive ≤	Intermediate	Resistant >	Resistant
		1	1	2	

4.3.18 *Klebsiella* -118

Levofloxacin

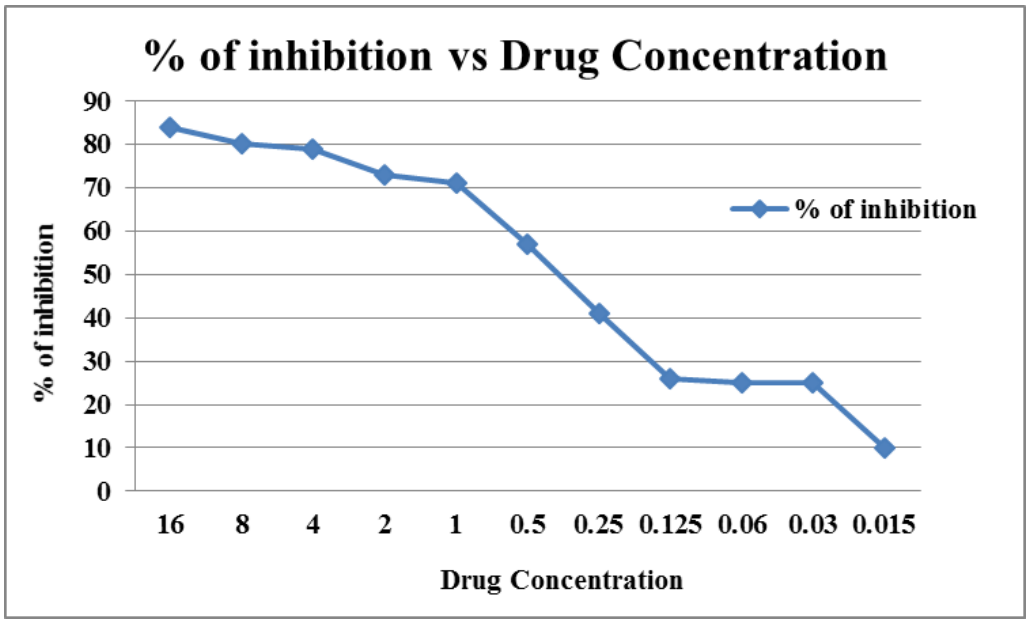


Figure 4.3.18: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 71% to 57%, the lowest drug concentration causing acceptable inhibition is 1µg/ml.

So, MIC value of Levofloxacin against this sample will be 1µg/ml.

Table 4.3.18: Determination of MIC status of antimicrobial agent

Antibiotic	MIC value (µg/ml)	Standard MIC range			Status of tested antibiotics
Levofloxacin	1	Sensitive ≤	Intermediate	Resistant >	Sensitive
		1	1	2	

4.3.19 *Acinetobactor* -119
Levofloxacin

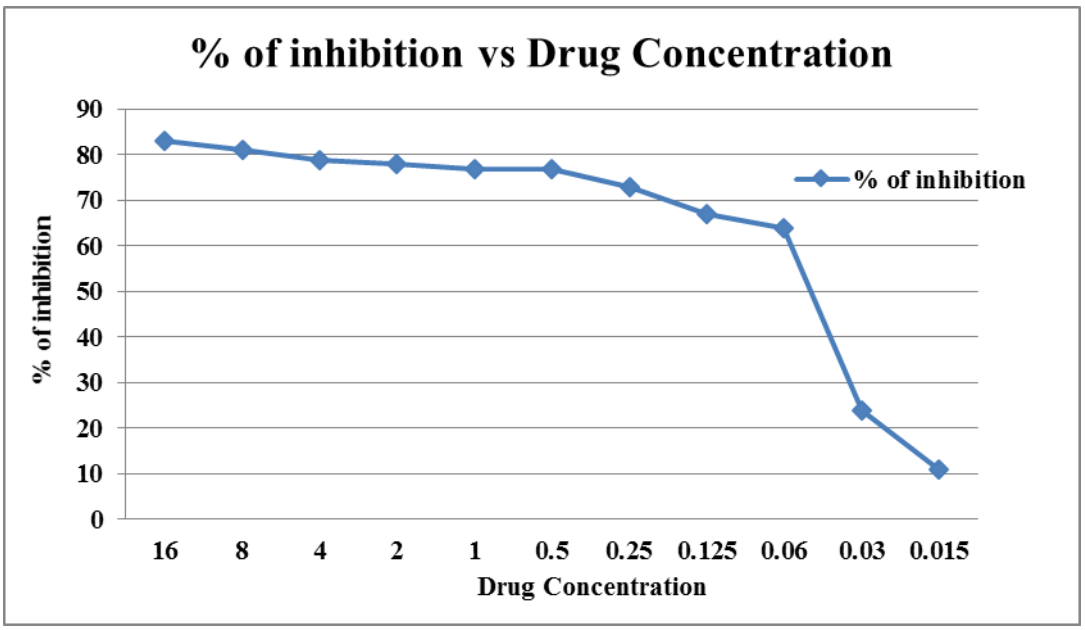


Figure 4.3.19: Graph of % of inhibition versus drug concentration

Value of % of inhibition has been dropped rapidly from 73% to 63%, the lowest drug concentration causing acceptable inhibition is 0.25µg/ml.

So, MIC value of Levofloxacin against this sample will be 0.25µg/ml.

Table 4.3.19: Determination of MIC status of antimicrobial agent

Antibiotic	**MIC value (µg/ml)**	**Standard MIC range**			**Status of tested antibiotics**
Levofloxacin	0.5	Sensitive ≤	Intermediate	Resistant >	Sensitive
		1	1	2	

Discussion

In this study, isolations from clinical cultures covering both gram positive & gram negative organisms have been used. These organisms were seven different isolations of ***Escherichia coli,*** seven different isolations of *Klebsiella*, two different isolations of *Salmonella typhi* and one isolation of *Stahpylococcus aureus, Pseudomonas, Acinetobactor.*

Different isolations of organisms exhibit different pattern of susceptibility against same antimicrobial agents.

(Carmeli Y, 2000)

Table 5.1: Susceptibility pattern of different ***Escherichia coli*** isolation against antibiotics

Antimicrobial agents	Isolation No:28	Isolation No:34	Isolation No:37	Isolation No:47	Isolation No:51	Isolation No:79	Isolation No:99
Levofloxacin	R	-	-	S	S	S	S
Azithromycin	-	-	R	-	-	R	R
Ciprofloxacin	R	-	-	R	S	-	-
Ceftriaxone	-	-	-	-	-	-	-
Cephradine	-	-	-	-	-	-	R
Vancomycin	-	S	-	I	-	-	-

Table 5.2: Susceptibility pattern of different *Klebsiella* isolation against antibiotics

Antimicrobial agents	Isolation No:50	Isolation No:54	Isolation No:80	Isolation No:100	Isolation No:105	Isolation No:110	Isolation No:118
Levofloxacin	S	-	S	S	S	R	S
Azithromycin	S	-	S	R	R	-	-
Ciprofloxacin	S	S	-	S	-	-	-

Table 5.3: Susceptibility pattern of different *Salmonella typhi* isolation against antibiotics

Antimicrobial agents	Isolation No:36	Isolation No:40
Levofloxacin	S	-
Azithromycin	S	R
Ciprofloxacin	R	-
Ceftriaxone	-	-
Cephradine	I	-

Table 5.4: Susceptibility pattern of different *Stahpylococcus aureus* isolation against antibiotics

Antimicrobial agents	Isolation No:106
Levofloxacin	R
Azithromycin	S
Ciprofloxacin	R

Table 5.5: Susceptibility pattern of different *Pseudomonas* isolation against antibiotics

Antimicrobial agents	Isolation No:81
Levofloxacin	S

Table 5.6: Susceptibility pattern of different *Acinetobactor* isolation against antibiotics

Antimicrobial agents	Isolation No:46
Levofloxacin	S

Different changes in susceptibility pattern of similar microorganism species have been found through broth microdilution method against commonly used antimicrobial agents. These changes may occur due to acquired resistance of these clinical isolates.

In this study, significant variations have been found in case of fluoroquinolones because its standard powders showed effective sensitivity towards gram negative isolations whereas resistant activities have been found against gram positive isolation.

On the other hand, macrolides showed resistant activity against different isolations of ***Escherichia coli*** whereas other gram negative isolations were effectively sensitive to macrolides. As macrolides showed this variation against ***Escherichia coli***, it must require further susceptibility tests for these microorganisms in different method.

In order to determine the authentic reasons of this resistance pattern of these clinical isolates genetic sequencing can be carried out.

Conclusion

The principal objective of this study was to determine the susceptibility pattern of different clinically isolated microorganisms for some commonly used antimicrobial agents. To carry out this purpose, most effective method broth microdilution has been used.

Specific deviations in susceptibility pattern of gram positive isolates have been observed as they showed resistance against fluroquinolones. Furthermore, gram negative isolates showed sensitivity towards fluoroquinolones and macrolides.

But ***Escherichia coli*** showed significant difference in their susceptibility pattern as its isolates displayed resistance against macrolides in spite of being gram negative microbes.

References

Andrews J. (2012) *BSAC Methods for Antimicrobial Susceptibility Testing* British society for antimicrobial chemotherapy, [Online] Version 11.1, 25-40 Available form: http://bsac.org.uk/wp-content/uploads/2012/02/Version-11.1-2012-Final-.pdf [Accessed19th Oct. 2015]

Bagg, J. (2005) Voriconazole susceptibility of yeasts isolated from the mouths of patients with advanced cancer. *Journal of Medical Microbiology*, 54(10): 959-964.

Barros, M., Santos, D. and Hamdan, J. (2007) Evaluation of susceptibility of Trichophytonmentagrophytes and Trichophytonrubrum clinical isolates to antifungal drugs using a modified CLSI microdilution method (M38-A). *Journal of Medical Microbiology*, 56(4):514-518.

Brown, B., Wallace, R. and Onyi, G. (1992) Activities of clarithromycin against eight slowly growing species of nontuberculous mycobacteria, determined by using a broth microdilution MIC system. *Antimicrobial Agents and Chemotherapy*, 36(9):1987-1990.

CDC, (2016) *CDC*.gov - *Typhoid Fever: General Information* - *NCZVED*. Available at: http://www.cdc.gov/nczved/divisions/dfbmd/diseases/typhoid_fever/ [Accessed 1 Feb. 2016].

CDC, (2016) CDC.gov *(Escherichia coli)*| *E.coli* |. [online] Available at: http://www.cdc.gov/ecoli/index.html [Accessed 4 Feb. 2016].

CDC, (2016) *CDC*.gov - *Pseudomonas aeruginosa in Healthcare Settings* - *HAI*. [Online] Available at: http://www.cdc.gov/hai/organisms/pseudomonas.html [Accessed 4 Feb. 2016].

CDC, (2016) *CDC*.gov - *Pseudomonas aeruginosa in Healthcare Settings* - *HAI*. [online] Available at: http://www.cdc.gov/hai/organisms/pseudomonas.html [Accessed 4 Feb. 2016].

Chowdhary, A., Randhawa, H., Sundar, G., Kathuria, S., Prakash, A., Khan, Z., Sun, S. and Xu, J. (2011) In vitro antifungal susceptibility profiles and genotypes of 308 clinical and environmental isolates of Cryptococcus neoformans var. grubii and Cryptococcus gattii serotype B from north-western India. *Journal of Medical Microbiology*, 60(7):961-967.

Davies, J., Davies, D. (2010) Origins and Evolution of Antibiotic Resistance. *Microbiology and Molecular Biology Reviews*, 70(3):417-433.

David, W.,Franklin, R. (2012) Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. *Clinical and Laboratory Standards Institute*, 32(2).

Drusano, L., Standiford, C., Plaisance K., Forrest, A., Leslie, J., Caldwell, J. (2014) Absolute oral bioavailability of ciprofloxacin. *Antimicrobial Agents Chemotherapy*, 30(3):444-6.

Flores-Trevino, S., Gutierrez-Ferman, J., Morfin-Otero, R., Rodriguez-Noriega, E., Estrada-Rivadeneyra, D., Rivas-Morales, C., Llaca-Diaz, J., Camacho-Ortiz, A., Mendoza-Olazaran, S. and Garza-Gonzalez, E. (2014) Stenotrophomonasmaltophilia in Mexico: antimicrobial resistance, biofilm formation and clonal diversity. *Journal of Medical Microbiology*, 63(11):1524-1530.

Greyerz S., Bültemann G., Schnyder K., Burkhart C., Lotti B., Hari Y., Pichler W.(2001) Degeneracy and additional alloreactivity of drug-specific human alpha beta(+) T cell clones. *International Immunology*. 13(7): 877-85.

Hachem, C., Clarridge, J., Reddy, R., Flamm, R., Evans, D., Tanaka, S. and Graham, D. (1996) Antimicrobial susceptibility testing of Helicobacter pylori comparison of E-test, broth microdilution, and disk diffusion for ampicillin, clarithromycin, and metronidazole. *Diagnostic Microbiology and Infectious Disease*, 24(1):37-41.

Hannan, P. (1998) Comparative Susceptibilities of Various Aids-Associated and Human Urogenital Tract Mycoplasmas and Strains of Mycoplasma Pneumoniae to 10 Classes of Antimicrobial Agent in Vitro. *Journal of Medical Microbiology*, 47(12):1115-1122.

Harris, G., Foster, S., and Richards, R. (2002) An Introduction to Staphylococcus Aureus, And Techniques for Identifying and Quantifying S. AureusAdhesins in Relation to Adhesion to Biomaterials: Review. .G, Harris *European Cells and Materials*, 4: 39-60.

Held, P. (2003) The Synergy™ HT - A Unique Multi-Detection Microplate Reader for HTS and Drug Discovery. *Journal of the Association for Laboratory Automation*, 8(2): 44-49.

Hugo, W., Russel, A. (1998) Pharmaceutical Microbiology. *Blackwell science Ltd.* p.35, 36,95.

Iatta, R., Figueredo, L., Montagna, M., Otranto, D. and Cafarchia, C. (2014) In vitro antifungal susceptibility of Malassezia furfur from bloodstream infections. *Journal of Medical Microbiology*, 63(11):1467-1473.

Jorgensen, J. and Ferraro, M. (2009) Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. *Clinical Infectious Diseases*, 49(11):1749-1755.

Kaper, J. (2005) Pathogenic Escherichia coli. *International Journal of Medical Microbiology*, 295(6-7):355-356.

Kobayashi, I. (2004) Micro-broth dilution method with air-dried microplate for determining MICs of clarithromycin and amoxycillin for Helicobacter pylori isolates. *Journal of Medical Microbiology*, 53(5):403-406.

Landman, D., Bratu, S. and Quale, J. (2009) Contribution of OmpK36 to carbapenem susceptibility in KPC-producing Klebsiellapneumoniae. *Journal of Medical Microbiology*, 58(10):.1303-1308.

Lavín A., Caviedes R., Carrascosa, F., Mellado, P., Monteagudo, I., Llorca, J., Cobo, M., Campos, R., Ayestarán, B. (2012).] Antimicrobial susceptibility of Helicobacter pylori to six antibiotics currently used in Spain.*The Journal of antimicrobial chemotherapy*, 67: 170-173.

Luber, P., Bartelt, E., Genschow, E., Wagner, J. and Hahn, H. (2003) Comparison of Broth Microdilution, E Test, and Agar Dilution Methods for Antibiotic Susceptibility Testing of Campylobacter jejuni and Campylobacter coli.*Journal of Clinical Microbiology*. 41(3): 1062-8.

Mlinaric-Missoni, E., Hagen, F., Chew, W., Vazic-Babic, V., Boekhout, T. and Begovac, J. (2011) In vitro antifungal susceptibilities and molecular typing of sequentially isolated clinical Cryptococcus neoformans strains from Croatia. *Journal of Medical Microbiology*, 60(10):1487-1495.

Mohammed, M., Marston, C., Popovic, T., Weyant, R. and Tenover, F. (2002) Antimicrobial Susceptibility Testing of Bacillus anthracis: Comparison of Results Obtained by Using the National Committee for Clinical Laboratory Standards Broth Microdilution Reference and Etest Agar Gradient Diffusion Methods. *Journal of Clinical Microbiology*, 40(6): 1902-1907.

Murray, C. and Hospenthal, D. (2004) Determination of Susceptibilities of 26 Leptospira sp. Serovars to 24 Antimicrobial Agents by a Broth Microdilution Technique. *Antimicrobial Agents and Chemotherapy*, 48(10):4002-4005.

Nascente, P., Meinerz, A., Faria, R., Schuch, L., Meireles, M. and Mello, J. (2009) CLSI broth microdilution method for testing susceptibility of Malasseziapachydermatis to thiabendazole. *Brazilian Journal of Microbiology*, 40(2):222-226.

National cancer institute. (2015) Vancomycin. *National cancer institute*. 15(12).

Neiss, E. (1973) Cephradine-a summary of preclinical studies and clinical pharmacology. *Journal of the Irish Medical Association, 1(12).*

Piccolomini, R., Bonaventura, G., Catamo, G., Carbone, F., Neri, N. (1997) Comparative Evaluation of the E Test, Agar Dilution, and Broth Microdilution for Testing Susceptibilities of Helicobacter pylori Strains to 20 Antimicrobial Agents. *Journal of Clinical Microbiology*. 35(7): 1842-1846.

Pirs, T., Avbersek, J., Zdovc, I., Krt, B., Andlovic, A., Lejko-Zupanc, T., Rupnik, M. and Ocepek, M. (2013) Antimicrobial susceptibility of animal and human isolates of Clostridium difficile by broth microdilution. *Journal of Medical Microbiology*, 62(9):1478-1485.

Price, L., Weinstein, R. (2008) Acinetobacter Infection. *New England Journal of Medicine*, 358(26): 2845-2847.

Rhee, J., Choi, J., Choi, M., Song, J., Peck, K. and Ko, K. (2013) Distinct groups and antimicrobial resistance of clinical Stenotrophomonasmaltophilia complex isolates from Korea. *Journal of Medical Microbiology*, 62(5):748-753.

Sader, H., Jones, R., Winokur, P., Pfaller, M., Doern, G. and Barrett, T. (1999) Antimicrobial susceptibility of bacteria causing urinary tract infections in Latin American hospitals: results from the sentry Antimicrobial Surveillance Program (1997). *Clinical Microbiology and Infection*, 5(8):478-487.

Sahm, D., Kissinger, J., Gilmore, M., Murray, P., Mulder, R., Solliday, J. and Clarke, B. (1989) In vitro susceptibility studies of vancomycin-resistant Enterococcus faecalis. *Antimicrobial Agents and Chemotherapy*, 33(9):1588-1591.

Singlas, E. (1995) Clinical pharmacokinetics of azithromycin. *PathologieBiologie.* 43(6): 505-11.

Stock, I., Henrichfreise, B., Wiedemann, B. (2002) Natural antibiotic susceptibility and biochemical profiles of Yersinia enterocolitica-like strains: Y. bercovieri, Y. mollaretii, Y. aldovae and Y. ruckeri. *Journal of Medical Microbiology*. 51: 56-69.

Thornsberry, C., Mcdougal, L. (1983) Successful Use of Broth Microdilution in Susceptibility Testsfor Methicillin-Resistant (Heteroresistant) Staphylococci. *Journal of Clinical Microbiology*, 18(5): 1084-1091.

Tudela, J., Arendrup, M., Barchiesi, F., Bille, J., Chryssanthou, E., Cuenca-Estrella, M., Dannaoui, E., Denning, D., Donnelly, J., Dromer, F., Fegeler, W., Lass-Flörl, C., Moore, C., Richardson, M., Sandven, P., Velegraki, A. and Verweij, P. (2008) EUCAST Definitive Document EDef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts. *Clinical Microbiology and Infection*, 14(4):398-405.

Vipra, A., Desai, S., Junjappa, R., Roy, P., Poonacha, N., Ravinder, P., Sriram, B. and Padmanabhan, S. (2013) Determining the Minimum Inhibitory Concentration of Bacteriophages: Potential Advantages. *Advances in Microbiology*, 03(02):181-190.

Wang, G., Hindler, J., Ward, K. and Bruckner, D. (2006) Increased Vancomycin MICs for Staphylococcus aureus Clinical Isolates from a University Hospital during a 5-Year Period. *Journal of Clinical Microbiology*, 44(11):3883-3886.

Wiegand, I., Hilpert, K. and Hancock, R. (2008) Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, 3(2):163-175.

Yajko, D., Nassos, P. and Hadley, W. (1987) Broth microdilution testing of susceptibilities to 30 antimicrobial agents of Mycobacterium avium strains from patients with acquired immune deficiency syndrome. *Antimicrobial Agents and Chemotherapy*, 31(10):1579-1584.

Yang, Q., Zhang, H., Wang, Y., Xu, Y., Chen, M., Badal, R., Wang, H., Ni, Y., Yu, Y., Hu, B., Sun, Z., Huang, W., Wang, Y., Wu, A., Feng, X., Liao, K., Shen, D., Hu, Z., Chu, Y., Lu, J., Cao, B., Su, J., Gui, B., Duan, Q., Zhang, S., Shao, H., Kong, H., Hu, Y. and Ye, H. (2013) A 10 year surveillance for antimicrobial susceptibility of Escherichia coli and Klebsiellapneumoniae in community- and hospital-associated intra-abdominal infections in China. *Journal of Medical Microbiology*, 62(9):1343-1349.

Zheng, B., Li, A., Jiang, X., Hu, X., Yao, J., Zhao, L., Ji, J., Ye, M., Xiao, Y. and Li, L. (2014) Genome sequencing and genomic characterization of a tigecycline-resistant Klebsiellapneumoniae strain isolated from the bile samples of a cholangiocarcinoma patient. *Gut Pathogens*, 6(1).