In Vitro Antimicrobial Susceptibility Test of Ciprofloxacin hydrochloride- 500mg (Ciprocin® and Ciprofloxacin®)

A Thesis Paper is submitted to the Department of Pharmacy, East West University in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy

Submitted By

Prakrety Chakraborty
ID- 2008-1-70-024

July, 2012

DEPARTMENT OF PHARMACY
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In the name of God
The most Gracious
The most Merciful

CERTIFICATE

This is to certify that the thesis submitted to the Department of Pharmacy, East West University, Aftabnagar, Dhaka in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy was carried out by Prakrety Chakraborty, ID- 2008-1-70-024.

Sufia Islam

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Ms. Sufia Islam, Ph.D.
Chairperson and Associate Professor
Department of Pharmacy
East West University

CERTIFICATE

This is to certify that the thesis submitted to the Department of Pharmacy, East West University, Aftabnagar, Dhaka in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy was carried out by Prakrety Chakraborty ID-2008-1-70-024 under my guidance and supervision and that no part of the thesis has been submitted for any other degree. I further certify that all the sources of information, laboratory facilities availed of this connection is fully acknowledged.

Farhana Rizwan

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Supervisor
Senior Lecturer
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I would like to convey deepest love and obedience to my caring parents for their support and guiding me all through my life until today, which keeps me strong and honest to do the things I needed to do.

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Abstract

Ciprofloxacin is a fluroquinolone antibiotic act against a broad spectrum of bacteria including gram positive and negative. It is effective against urinary tract infection, acute prostatitis, sinusitis, lower respiratory tract infection etc. To get therapeutic effect in maximum level the quality of the drug need to control. In a pharmaceutical sense, quality means checking and directing the degree or grade of excellence of processes and products. Antimicrobial susceptibility test is one of the quality control tests to estimate the possible effectiveness of antibiotics. It is performed by using disk diffusion test. The objective of the study is to measure the sensitivity of the antibiotic by comparing with the standard. It is significant because of the rapid occurrence of antibiotic resistance due to use of substandard or poor quality drugs or due to the irrational use of drug. The test was performed using Ciprocin® and Ciprofloxacin® 500mg Tablet in concentration of 5μg/disc. Different species of bacteria such as three different strains of *E. coli* and two strains of *Pseudomonas aeruginosa, Salmonella typhi* were subcultured in agar plate which was isolated from the urine, stool and blood sample of the pediatric patients. Different concentrations of sample were applied by disc diffusion method and incubated overnight. Inhibitory activity of the antibiotic was measured as zone of inhibition in mm. The zone of inhibition of the sample was compared with the zone of inhibition of the standard. Results show that both Ciprocin ® and Ciprofloxacin® 500mg possibly contain the sufficient amount of active ingredient which is required to achieve desired therapeutic activity. It was the possible indication of drug effectiveness which was almost similar with the standard. Further studies need to confirmation of results.

Chapter- 1

Introduction

1.1. Overview

Man is the best creation on earth. Although for many developing countries of the world, including Bangladesh, this sentence seems to be only a paradox. In our country, for example, it is man who makes the most environmental pollution to make his own life miserable. He spits here and there, urinates and defecates at all possible places, and throws all unwanted spoilage around the household and street corners. In addition, black smoke of vehicles contains lead and dangerous gases which directly affect human health. Children are the worst victims. Morbidity and mortality rates are therefore alarmingly high in such communities.

In Bangladesh, more than 90% of the slum children are malnourished and 25% of the families live below the line of hard core poverty. Acute respiratory tract infection (ARI), Diarrheas and Typhoid are the most common among the diseases which causes morbidity and mortality among infants and children in developing countries. The International Centre for Diarrheal Disease Research, Bangladesh (ICDDRB), is a major center for research into diarrheal diseases. The center treats more than 100,000 patients a year (Albert *et al*, 1999).

Pathogens involve to produce these disease are the different strains of the *E.coli*, *Sallmonela* and *Pseudomonas* species in most of the cases. There are many antimicrobial agents active against these species.

Fluroquinolone antibiotic Ciprofloxacin is one of them. It acts against various types of bacteria. It has the ability to injure or kill invading microorganisms without harming the cells of the host. It should be noted that in instances, the selective toxicity is relative rather than absolute which requires that the concentration of the drug be carefully controlled to attack the microorganism while still being tolerated by the host. Moreover, there are also risks of developing antibiotic resistance by the microorganisms which will ultimately makes the drugs inactive to treat infections. Many strains of the microorganism become resistance against many antibiotics due to irrational use of drug. The risk of drug resistance is an alarming condition and various necessary steps and precautions should be taken by the respective authority to handle these problems.

The purpose of the study is to determine the sensitivity of different brands of Ciprofloxacin against different bacteria by using disc diffusion method.

1.2 Microorganism

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 A, 2008, p.1).

1.2.1 Classification of microorganism

1.2.1.1 According to shape

According to shape bacteria can be classified as two types, Bacilli and Cocci. Bacilli are rod shape, in example; *Cornybacterium, Mycobacterium* and cocci are round shape, in example; *Stephylococcus, Streptococcus* (Pelczar *et al*, 2009, p.74)

1.2.1.2 According to Gram stain

Bacteria can be classified as two types, Gram positive; in example, *Clostridium* and Gram negative; in example, *Pseudomonas*. The thickness of cell walls of Gram negative bacteria is generally thinner than those of Gram positive bacteria (Pelczar *et al*, 2009, p.86,87)

1.2.1.3 According to gaseous requirement

According to gaseous requirement bacteria can be classified as two types, Aerobic; in example, *Neisseria* and Anaerobic; in example, *Bordetella*. Bacteria which requires oxygen as their living condition is called aerobic. Bactria do not requires oxygen, even low level of oxygen can be toxic for them is called (Pelczar *et al*, 2009, p.109).

1.2.2 Some pathogenic bacteria

1.2.2.1 *Salmonella*

Salmonella typhi is the causal organism of typhoid fever, *Sal. paratyphi* causes paratyphoid fever, whilst *Sal. typhimurium, Sal. enteritidis* and very many other closely related organisms are

a cause of bacterial food poisoning (Hugo and Russel, 1998, p.35).

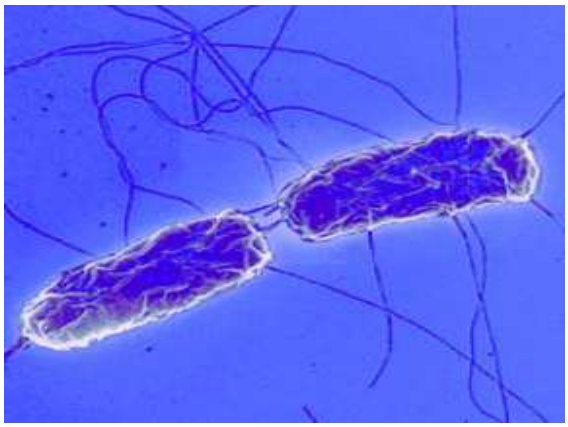

Figure 1.1 *Salmonella typhi* (Harvey R.A *et al*, 2006).

1.2.2.2 *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, p. 35).

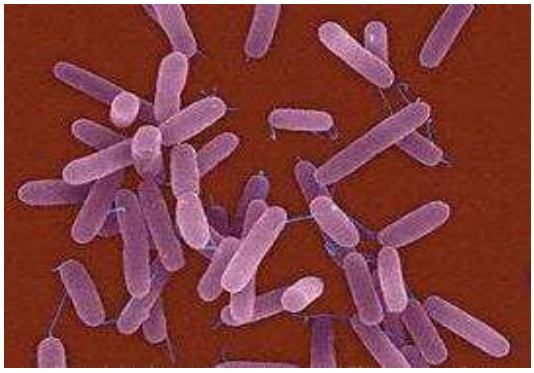

Figure 1.2 *Pseudomonas* (Harvey R.A *et al*,2006).

1.2.2.3 *Escherichia*

Escherichia coli are members of a group of microorganisms known as the enterobacteria, so called because they inhabit the intestines of humans and animals. Many selective and diagnostic media anddifferential biochemical reactions are available to isolate and distinguish members of this group, as they are of great significance in public health. *Escherichia coli* is a cause of enteritis in young infants and the young of farm animals, where it can cause diarrhoea and fatal dehydration. It is a common infectant of the urinary tract and bladder in humans, and is a cause of pyelitis, pyelonephritis and cystitis (Hugo and Russel, 1998, p. 35).

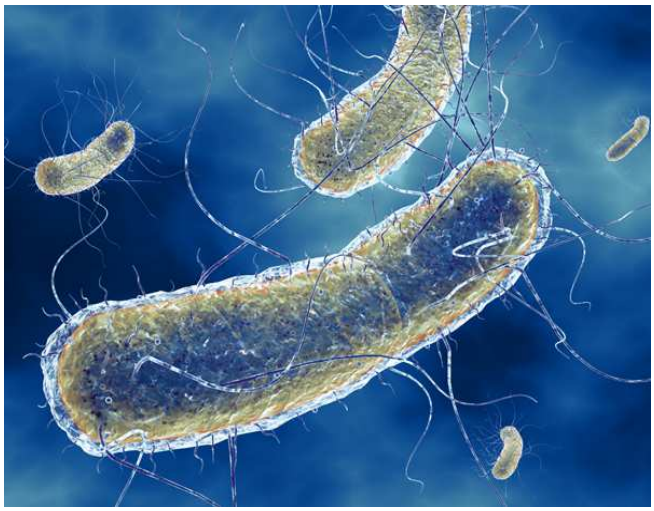

Figure 1.3 *Escherichia* (Harvey R.A *et al*, 2006).

1.2.3 Isolation of microorganism

1.2.3.1 Culture

The cultural techniques is used in microbiology to demonstrate the presence of organisms which may be causing disease, and when indicated, to test the susceptibility of pathogens to antimicrobial agents. Different types of culture media are used in this technique. In example, Basic, Enriched, Selective, Indicator, Transport and Identification media. Basic media can be again classified as three types, solid; semisolid and liquid. Solid media is Media are solidified by incorporating a gelling agent such as agar. Example of liquids media is Broth. Liquid media are

most commonly used as enrichment where organisms are likely to be few e.g. blood culture. The pH of most culture media is near neutral. An exception is alkaline peptone water. Culture media can be sterilized by steaming, autoclaving and filtration (Cheesbrough, 2006. p.45,46,47).

1.2.3.2 Subculture

To obtain as pure a culture as possible it is necessary to reduce the number of commensals inoculated. Inoculation technique is used to reduce commensal numbers. Blood agar and MacConkey agar is used for Gram positive bacteria; Chocolate agar is used for Gram negative bacteria inoculation (Cheesbrough, 2006, p.74).

1.2.3.3 Biochemical tests

Table 1.1: Biochemical tests to identify the bacteria.

Tests	Purpose
Beta-glucuronidase	To identify *E. coli*
Bile solubility	To differentiate *S. pneumoniae* from other alpha-haemolytic streptococci
Citrate utilization	To differentiate enterobacteria
Coagulase	To identify *S. aureus*
DNA-ase	To help identify *S. aureus*

Indole	To differentiate Gram negative rods, particularly *E. coli*
Litmus milk	To help identify decolorization *Enterococcus* and some clostridia
Lysine decarboxylase	To assist in the identification ofsalmonellae and shigellae
Oxidase	To help identify *Neisseria,Pasteurella, Vibrio, Pseudomonas*
Urease	To help identify *Proteus,Morganella, Y.enterocolitica,H. pylori*

(Cheesbrough, 2006, p.62, 63).

1.2.4 Antimicrobial susceptibility test

To treat and control the infectious diseases, especially when caused by pathogens that are often drug resistant, susceptibility (sensitivity) testing is used to select effective antimicrobial drugs. Susceptibility testing is not usually indicated when the susceptibility reactions of a pathogen can be predicted, for example; *Proteus* species are generally resistant to nitrofurantoin and tetracyclines; *S. pyogenes* is usually susceptible to penicillin, Anaerobes are susceptible to metronidazole. Susceptibility tests must never be performed on commensal organisms or contaminants because this could result in the patient receiving ineffective and unnecessary antimicrobial therapy, causing possible side effects and resistance to other potentially pathogenic organisms. Laboratory antimicrobial susceptibility testing can be performed using dilution technique and disc diffusion technique (Cheesbrough, 2006, p.135).

1.2.4.1 Disk diffusion test:

The disk diffusion susceptibility method is simple and practical and has been well-standardized. The test is performed by applying a bacterial inoculum of approximately $1–2\times10^{8}$CFU/mL to the

surface of a large (150 mm diameter) Mueller-Hinton agar plate. Up to 12 commercially-prepared, fixed concentration, paper antibiotic disks are placed on the inoculated agar surface.

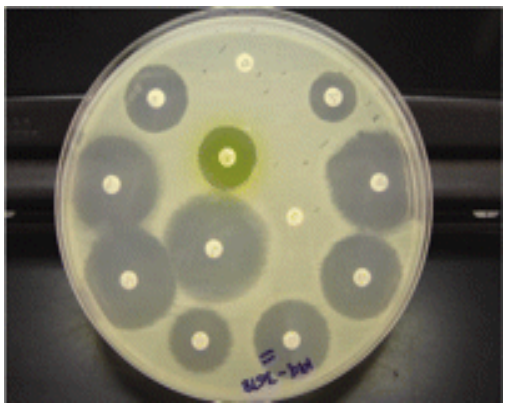

Figure 1.4 A disk diffusion test with an isolate of *Escherichia coli* from a urine culture (Reller LB *et al*, 2003).

Plates are incubated for 16–24 h at 35°C prior to determination of results. The zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each drug are interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI). The results of the disk diffusion test are "qualitative," in that a category of susceptibility (ie, susceptible, intermediate, or resistant) is derived from the test rather than an MIC (Reller LB *et al*,). Mueller-Hinton agar is considered to be the best for routine susceptibility testing of non fastidious bacteria due to acceptable batch to batch reproducibility, provide satisfactory growth of most non fastidious pathogen and it is low in sluphonamides,tetracycline, trimethoprim inhibitors (Lalitha M.K)..

1.2.4.2 Dilution susceptibility tests:

Dilution susceptibility tests are performed both manually or semi-automatically in Microbiology Reference Laboratories for epidemiological purposes or when a patient does not respond to treatment thought to be adequate, relapses while being treated, or when there is immunosuppression. Dilution techniques measure the minimum inhibitory concentration (MIC).

They can also be used to measure the minimum bactericidal concentration (MBC) which is the lowest concentration of antimicrobial required to kill bacteria. A dilution test is carried out by adding dilutions of an antimicrobial to a broth or agar medium. A standardized inoculum of the test organism is then added. After overnight incubation, the MIC is reported as the lowest concentration of antimicrobial required to prevent visible growth. By comparing the MIC value with known concentrations of the drugobtainable in serum or other body fluids, the likely clinical response can be assessed (Cheesbrough, 2006, p. 136). Dilution susceptibility test is again two types; Broth dilution and Agar dilution (Lalitha M.K).

1.2.4.3 Factors affecting antimicrobial susceptibility tests

Moisture and pH of the medium should be checked. The agar medium should have a pH between 7.2 and 7.4 at room temperature after gelling. Too low pH cause certain drugs to lose the potency such as aminoglycoslides, quinolones and macrolides, while other agents may appear to have excessive activity such as tetracycline. To high pH also hampers the drug potency by showing opposite effects. To reduce excess moisture plates should be placed in incubator at 35°C or laminar flow hood at room temperatute until excess surface moisture is lost by evaporation. Usually the time is 10 to 30 minutes (Lalitha M.K).

1.2.4.4 Limitations of antimicrobial susceptibility tests

Susceptibility tests measure antimicrobial activity against bacteria under laboratory conditions (*in vitro* activity), not in the patient (*in vivo* activity). It cannot be assumed therefore, that an antimicrobial which kills or prevents an organism from growing *in vitro* will be a successful treatment. Selecting appropriate antimicrobial treatment also involves considering the patient's clinical condition, any underlying condition (e.g. liver or kidney disease), the type and site of the infection, any history of drug hypersensitivity, age of patient and whether a patient is pregnant. It is also necessary to know the activity of the different drugs including their rates of absorption, diffusion in the tissues, metabolism, excretion and also possible toxicity and effects on the patient's normal microbial flora. The cost and availability of a drug will also need to be considered (Cheesbrough, 2006, p.136).

1.3 Antimicrobial agent

The terminology ‘antibiotic’ etymologically evidently signifies anything against life. (Kar A, 2008, p.12). Antibiotics are antibacterial substances produced by various species of microorganisms (bacteria, fungi, and actinomycetes) that suppress the growth of other microorganisms. Common usage often extends the term antibiotics to include synthetic antimicrobial agents, such as sulfonamides and quinolones (Goodman *et al*, 2006, p.718.)

1.3.1 Classification of antibiotics

Antibiotics differ markedly in physical, chemical, and pharmacological properties, in antimicrobial spectra, and in mechanisms of action. Knowledge of molecular mechanisms of bacterial replication has greatly facilitated rational development of compounds that can interfere with their replication.Antibiotics can be classified as the spectra of bacteria particularly in which these are effective.

1.3.1.1 Narrow-spectrum antibiotics

Chemotherapeutic agents acting only on a single or a limited group of microorganisms are said to have a narrow spectrum. For example, isoniazid is active only against mycobacteria (Finkel *et al*, 2009, p.353).

1.3.1.2 Extended-spectrum antibiotics

Extended spectrum is the term applied to antibiotics that are effective against gram-positive organisms and also against a significant number of gram-negative bacteria. For example, ampicillin is considered to have an extended spectrum, because it acts against gram-positive and some gram-negative bacteria (Finkel *et al*, 2009, p.353).

1.3.1.3 Broad-spectrum antibiotics

Drugs such as tetracycline and chloramphenicol affect a wide variety of microbial species and are referred to as broad-spectrum antibiotics (Finkel et al, 2009, p.353).

Antibiotics can be again classified according to their chemical composition. There are six main

classes of antibiotics acts against different spectra of bacteria. These include Aminoglycosides, Fluroquinolones, β-lactam antibiotics, Macrolides, tetracyclines and Chloramphenicol (Kar A, 2003, p.46).

Table 1.2: Classification of antibiotics

Class	Designated Antibiotics
Aminoglycosides	Amikasin, Gentamycin , Kanamycin ; Neomycin ; Netilmicin ; Streptomycin ; and Tobramycin
Ansamycins	Maytansine and Rifampicin ;
Beta-lactam antibiotic	Amoxycillin ; Ampicillin ; Cephalosporin ; Clavulanic acid
Cyclic polypeptides	Gramicidin ; and Polymixins A, B, C, D and E
Fluoroquinolones	Ciprofloxacin ; Enoxacin ; Norfloxacin ; and Ofloxacin
Macrolides	Azithromycin ; Bacitracin ; Clarithromycin
Polyenes	Amphotericin B ; Griseofulvin ; and Nystatin
Tetracyclines	Aureomycin ; Doxycycline ; Tetracycline ;
Miscellaneous	Adriamycin ; Chloramphenicol;Clindamycin ; Cycloserine ; and Mitomycins

(Kar A, 2003, p. 47).

1.3.2 Mechanism of action of antibiotics

All antimicrobials are not completely non-toxic, at the concentration required to be effective to the human cells. Most, however, show sufficient selective toxicity to be of value in the treatment of microbial diseases. Antibacterial agents can be grouped by their mode of action, i.e. their ability to inhibit the synthesis of the cell wall, cell membrane, proteins, and nucleic acids of bacteria (Cheesbrough,2006,p.132). The many modes of antibiotic action are shown schematically in the diagram below.

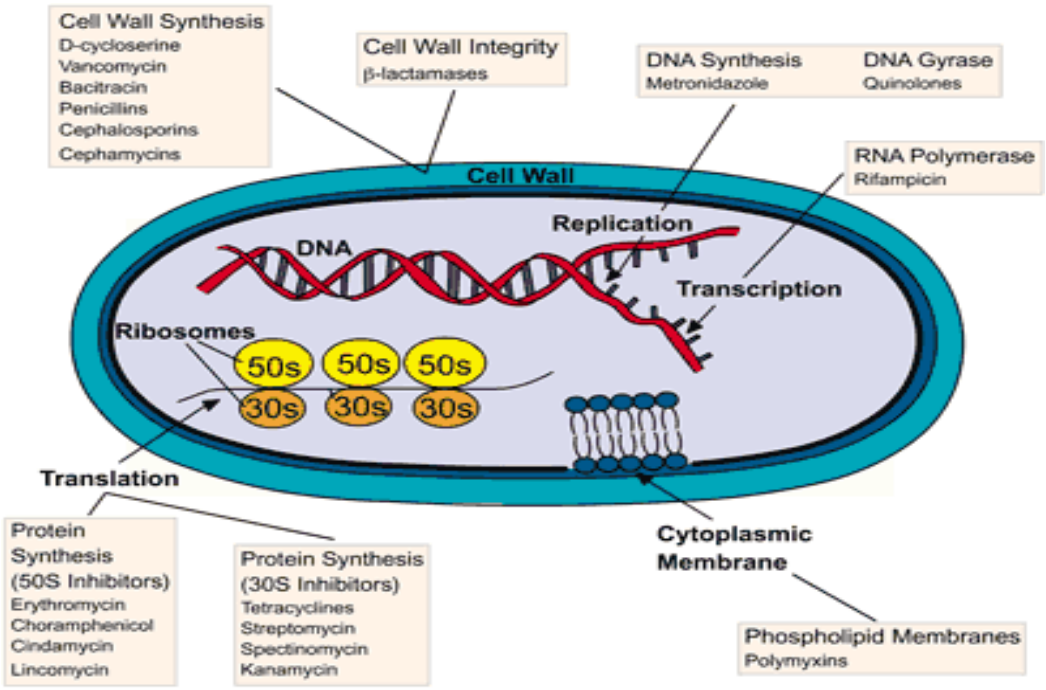

Figure 1.5 Mechanism of action of different antibiotics on bacterium (Finkel *et al*, 2009, p.357).

1.3.3 Antibiotic resistance

Due to the extensive use and misuse of antimicrobial drugs most of the antimicrobial resistance is occurring which is now making it difficult to treat some infectious diseases. Drug-resistant strains are common among staphylococci, gonococci, meningococci, pneumococci, enterococci, Gram negative bacteria (e.g. *Salmonella*, *Shigella*, *Klebsiella*, *Pseudomonas*) and *M. tuberculosis*. Bacteria become resistant to antimicrobial agents by a number of mechanisms, the commonest being: production of enzymes which inactivate or modify antibiotics, changes in the

bacterial cell membrane, preventing the uptake of an antimicrobial, modification of the target so that it no longer interacts with the antimicrobial, development of metabolic pathways by bacteria which enable the site of antimicrobial action to be bypassed. To acquire these new properties bacteria must undergo a genetic change. Such a genetic change may occur by mutation or by the acquisition of new genetic material. New genetic material is acquired by the transfer of resistance genes, (located on plasmids and transposons) from one bacterium to another. Some plasmids encode for resistance to several antibiotics and can be transferred between bacterial species, e.g. from *Escherichia coli* to *Shigella dysenteria* (Cheesbrough, 2006, p.134,135).

1.4 Ciprofloxacin

Ciprofloxacin is the most commonly used fluoroquinolone and a broad-spectrum antibiotic effective against both Gram-positive and Gram-negative organisms (Rang H.P *et al*, 2007, p.673).

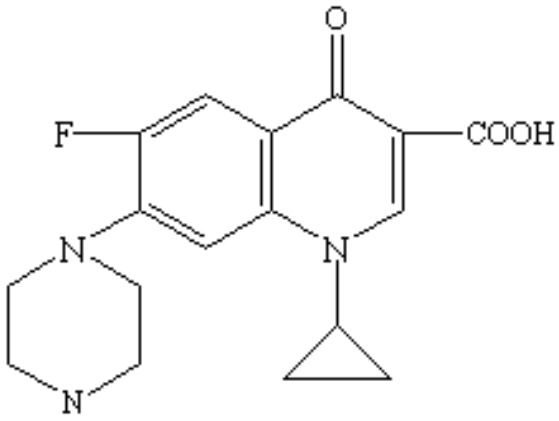

Figure1.6: Ciprofloxacin (Rang H.P *et al*, 2007, p.672).

Chemical formula $C_{17}H_{18}FN_3O_3$

Molecular mass 336.346 (Kar A, 2003, p.57).

1.4.1 Mechanism of action

The caiprofloxacin selectively inhibit DNA gyrase (topoisomerase II) and , topoisomerase IV

which is not found in mammalian cells. In gram positive bacteria topoisomerase IV is the primary activity inhibited by the quinolones. In gram negative bacteria DNA gyrase is the primary quinolone target. To permit DNA replication or transcription individual strands of double helix DNA must be separated. Anything that separates the DNA strands results in "overwinding" or excessive positive supercoiling of the DNA in front of the point of separation.To overcome this mechanical obstacle,the bacterial enzyme DNA gyrase is responsible for the continuous introduction of negative super coils into DNA via an ATP dependent reaction requiring that both strands of the DNA be cut to permit passage of a segment of DNA through the break; the break then is resulted (Sanders,1987, p.518).

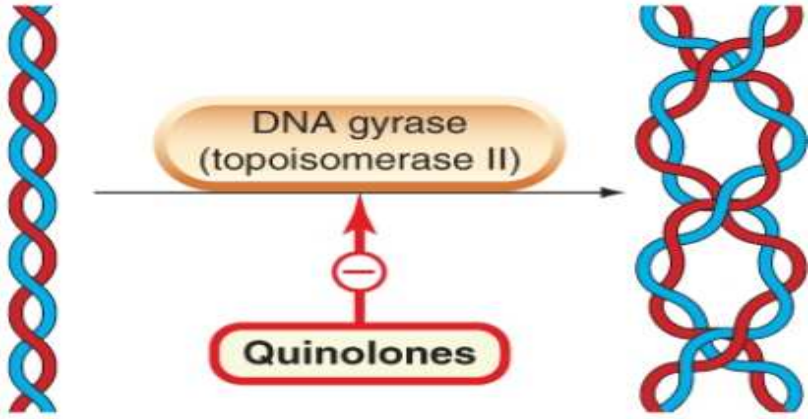

Figure 1.7 Mechanism of action of Ciprofloxacin (Rang H.P *et al*, 2007, p.672).

1.4.2 Pharmacokinetic

1.4.2.1 Absorption

After oral administration the ciprofloxacin are well absorbed and widely distributed in body tissues except to the brain. Ciprofloxacin should not be given with food (Goodman *et al*, 2011, p. 1473).

1.4.2.2 Distribution

Ciprofloxacin is widely distributed in body tissues. Peak serum levels of the drug are obtained

within 1-3 hours of an oral dose of 400mg.Food may delay the time to peak serum concentration. Bioavailability of the fluroquinolones are >50% for all agents and 95% for several. The volume of distribution of Ciprofloxacin is high, with concentration of quinolones in urine, kidney, lung and prostate tissue, stool, bile and macrophage and neutrophils higher than serum levels. Ciprofloxacin concentration in cerebrospinal fluid, bone and prostatic fluid are lower than the serum and levels has been detected in human breast milk (Goodman *et al*, 2011, p.1473).

1.4.2.3 Excretion

Mostly Ciprofloxacin is cleared predominantly by the kidney and dosage must be adjusted for renal failure. None of the fluroquinolones is removed efficiently by peritoneal dialysis or hemodialysis (Goodman *et al*, 2011, p.1473).

1.4.2.4 Dosage

Ciprofloxacin hydrochloride (tablet, oral suspension, and pellets for suspension,powder for intravenous injection) should be given 1 hour before or 2 hours after meals when administered orally. The dose of Ciprofloxacin are Oral: 250, 500, 750 mg tablets; 50, 100 mg/mL suspension, Parenteral: 2, 10 mg/mL for IV infusion, Ophthalmic: 3 mg/mL solution; 3.3 mg/g ointment. Ciprofloxacin 500 mg, two times daily is indicated mostly in case of urinary tract infection. (Katzung *et al*, 2003, p.1085, 1088).

1.4.3 Indication

Ciprofloxacin is effective in both uncomplicated and complicated urinary tract infections, but is best reserved for complicated, hospital-acquired or recurrent infections. Ciprofloxacin is effective in complicated and severe lower respiratory tract infections, including those in patients with infective exacerbations of chronic bronchitis and cystic fibrosis, and pseudomonal infections. Ciprofloxacin is effective in the treatment of serious, non-self-limiting intra-abdominal infections, peritonitis in CAPD, pelvic inflammatory disease, endometritis and gall-bladder infections. Ciprofloxacin is effective in the treatment of a range of serious, non-self-limiting gastrointestinal infections (e.g. Salmonella and typhoid fever). Ciprofloxacin is effective in the empirical treatment of febrile neutropenic episodes, and prophylaxis of gram-negative

bacteraemia in neutropenia and bone marrow transplantation. Ciprofloxacin is also effective in a range of other indications (e.g. eye infections, skin and soft tissue infections, bone and joint infections,andgonorrhoea) (Finch RG *et al,* 2000).

1.4.3.1 Urinary tract infection

Urinary tract infection caused by *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Serratia marcescens, Proteus mirabilis, Providencia rettgeri, Morganella morganii, Citrobacter diversus, Citrobacter freundii, Pseudomonas aeruginosa,* methicillin-susceptible*Staphylococcus epidermidis, Staphylococcus saprophyticus,* or *Enterococcus faecalis* (Goodman *et al,*2011, p.1472). .

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1.4.3.2 Acute Uncomplicated Cystitis in females

Acute uncomplicated cystitis in female caused by *Escherichia coli* or *Staphylococcus saprophyticus* (Goodman *et al,* 2011, p.1472). .

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1.4.3.3 Chronic Bacterial Prostatitis

Chronic bacterial prostaitis caused by *Escherichia coli* or *Proteus mirabilis* (Goodman *et al,*2011, p.1472). .

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1.4.3.4 Lower Respiratory Tract Infections

Lower respiratory tract infection caused by *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Proteus mirabilis, Pseudomonas aeruginosa, Haemophilus influenzae, Haemophilus parainfluenzae,* orpenicillin-susceptible *Streptococcus pneumonia* (Goodman *et al,*2011, p.1472). .

1.4.3.5 Acute Sinusitis

Acute sinusitis caused by *Haemophilus influenzae,* penicillin-susceptible *Streptococcus pneumoniae,* or *Moraxella catarrhalis* (Goodman *et al,* 2011, p.1473).

1.4.3.6 Skin and Skin Structure Infections

Skin and skin structure infection caused by *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Proteus mirabilis, Proteus vulgaris, Providencia stuartii, Morganella morganii, Citrobacter freundii, Pseudomonas aeruginosa,* methicillin-susceptible*Staphylococcus aureus,*methicillin-susceptible *Staphylococcus epidermidis,* or *Streptococcus pyogenes*(Goodman *et al,*2011, p.1473).

1.4.3.7 Bone and Joint Infections

Bone and joint infections caused by *Enterobacter cloacae, Serratia marcescens,* or*Pseudomonas aeruginosa* (Goodman *et al,* 2011, p.1473)..

1.4.3.8 Complicated Intra-Abdominal Infections

Complicated intra abdominal infection (ciprofloxacin used in combination with metronidazole) caused by *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae,* or *Bacteroides fragilis* (Goodman *et al,*2011, p.1473)..

1.4.3.9 Infectious Diarrhea

Infectious Diarrhea caused by *Escherichia coli* (enterotoxigenic strains), *Campylobacter jejuni, Shigella boydii* **,** *Shigella dysenteriae, Shigella flexneri* or *Shigella sonnei* when antibacterial therapy is indicated (Goodman *et al,*2011, p.1473).

1.4.3.10 Typhoid Fever

Typhoid fever caused by *Salmonella typhi.* The efficacy of ciprofloxacin in the eradication of the chronic typhoid carrier state has not been demonstrated. Uncomplicated cervical and urethral gonorrhea due to *Neisseria gonorrhoeae* (Goodman *et al,* 2011, p.1473).

1.4.4 Side Effects

Table 1.3: Side Effects of Ciprofloxacin

Nausea	Phototoxicity
Vomiting	Dizziness
Diarrhea	Headache
Central nervous system problem	Nephrotoxicity
Insomnia	Skin rash

(R.Finkel *et al*, 2009, p.391).

1.4.5 Ciprofloxacin resistance

Close structural relationship of the fluorinated 4-quinolones ciprofloxacin to the parent compound nalidixic acid results the mechanism of resistance is often perceived to be the same. A number of mutations in the chromosome of *Escherichia coli* have been identified and these are described. These mutations affect the interaction of quinolones with their presumed targets (DNA gyrases) and the transport of quinolones through the cell membrane. A small number of other mutations giving rise to low-level resistance to nalidixic acid, which affect neither gyrase nor membrane function, are also recognized. Despite the recognition of these mutations, the practical application of this information is hampered by lack of knowledge of the normal uptake mechanisms, the processes involved in selection and maintenance of resistance, the relationship of resistance to pathogenicity and the frequency of occurrence of these mutations (Crumplin G.C, 1990).

Chapter-2

Significance and Aims of the study

Significance of the Study

Antimicrobial susceptibility testing of significant bacterial isolates is a very important function of microbiology laboratory. The goals of testing are to detect possible drug resistance in common pathogens, to measure the probable susceptibility to drugs of choice for particular infections and also compare the possible drug activity to the standard. There are many methods to conduct the test. Each method has strengths and weaknesses, including organisms that may be accurately tested by the method. Some methods provide quantitative results (e.g. minimum inhibitory concentration), and all provide qualitative assessments using the categories susceptible, intermediate, or resistant. In general, current testing methods provide accurate detection of common antimicrobial resistance mechanisms. However, newer or emerging mechanisms of resistance require constant vigilance regarding the ability of each test method to accurately detect resistance (James *et al*, 2011).

Antibiotic resistance occurs when an antibiotic has lost its ability to effectively control or kill bacterial growth. The bacteria targeted adapt by natural selection to become 'resistant' and continue to multiply despite the presence of the antibiotic. Controlling the deadliest infectious diseases in the world such as diarrheal diseases, respiratory tract infections, sexually transmitted infections, meningitis, pneumonia, and hospital acquired infections, is more difficult today because of the emergence of antimicrobial drug resistance. Resistance has emerged for most bacterial infections, which causes a significant proportion of the burden of disease in developing countries (Ramanan *et al*, 2006).

In 1990 it was estimated that 78% of world's population lived in developing countries and of 39.5 million deaths in the developing world, 9.2 million were estimated to have been caused by infectious and parasitic disease. Infections of the lower respiratory tract were the third most common cause of death worldwide (Murray *et al*, 1997). Ninety eight per cent of deaths in children occur in the developing world, mostly as a result of infections (C A Hart *et al*, 1998).

Bacterial resistance to different antibiotics is more severe in developing countries. Inappropriate, excessive use of antibiotics, insufficient control on drug prescribing, inadequate compliance with treatment regimens, prescribing inappropriate doses and irrational use of antibiotic provides favorable conditions for resistant microorganisms to emerge and spread. For example, when patients do not take the full course of a prescribed antimicrobial or when poor quality

antimicrobials are used, resistant microorganisms can emerge and spread (Ramanan *et al*, 2006).

Poor quality medicine is medicine that does not meet official standards for strength, quality, purity, packaging, and/or labeling. They may be legally registered innovator or generic products, or they could be counterfeits—deliberately mislabeled for identity, strength, or source. Whether counterfeit or unintentionally substandard, poor quality drugs result in serious health implications including treatment failure, adverse effects, increased morbidity, mortality, development of drug resistance, and wasted resources. Recent reports indicate the availability of substandard and counterfeit drugs has reached a disturbing proportion in many low-income countries (US Pharmacopoeia 30, NF-25, p.2756).

Ciprofloxacin is chosen for the study because it can treat a broad spectrum of bacterial infections and it is the first line choice of drug for urinary tract infection. This fluorinated quinolones offer greater potency, a broader spectrum of antimicrobial activity, greater in vitro efficacy against resistant organisms, and in some cases, a better safety profile than older quinolones and other antibiotics (R.Finkel *et al*, 2009).

Aim of the Study

The main goals of antimicrobial susceptibility testing are-

- Possible determination of susceptibility of various micro-organisms against Ciprofloxacin hydrochloride.
- Comparison of the probable activity of drugs available in the market with the standard

Chapter- 3

Materials and Method

2.1 Study design

2.1.1 Sample collection

Drug samples for the study are collected from different drug stores of the Mohakhali, Dhaka with their Manufacturer name, Manufacturing date (MFG. date), Batch number, Expiry date (EXP. Date), Manufacturing license number, prices etc.

Table 2.1: Details of drug sample collection.

Brand name	Manufacturer name	MFG. License no.	Batch no.	MFG. date	EXP. date
Ciprocin	Square pharmaceuticals	235 & 460	202012	February 2012	January 2016
Ciprofloxacin	Albion pharmaceuticals	109 & 191	02 C 12	March 2012	March 2015

Clinical isolate of blood, stool and urine sample is collected from Pathology Department, Institute of Child Health & Shishu Sasthya Foundation Hospital (ICH), Mirpur-2, Dhaka.

Table 2.2: Details of the collected clinical isolates

Sample	Clinical isolates
Urine	*E.coli* strain 1
Blood	*E.coli* strain 2
Stool	*E.coli* strain 3
Blood	*Pseudomonas aeruginosa*
Blood	*Salmonella typhi*

2.1.2 Place and Period of the study

Study is conducted in microbiology lab of the East West University, Mohakhali campus. The perid of the study was six months.

3.1 Materials

3.1.1 Apparatus

Table 3.1: Name & sources of materials required for sensitivity test.

Materials	Sources
Micro pipette	Eppendrof, Germany
Hot air oven	YCO-N01, Gemmy industrial crop, Taiwan
Electronic balance	ELB 3000, Shimadzu, Japan.
Laminar air flow	EQU/03-EHC, ESCO, USA
Autoclave	Hydroclave MC8, Barnsted International
Incubator	BK 4266
Peridishes,Wireloops, Forceps, Calipter or ruler	
Vortex machine and Bunsen burners, Whatman filter paper	
1000ml bottles, Eppendrof tube, 100ml conical flask, cotton bud	

3.1.2 Reagents

Nutrient agar, source is Himedia laboratories, India. Agar powder source is BDH laboratory, England, Normal Saline(0.9% NaCl) **,** Distilled Water**.**

3.2 Method

3.2.1 Sample preparation

• A 500mg two different brans of Ciprofloxacin hydrochloride Tablets were weighed and recorded in the Record Book.

• The tablets were crushed gently by mortar and pestle then 5mg equivalent tablet powder was weighed and each of them kept in different tubes.

• Distilled water was added with Ciprofloxacin hydrochloride powder kept in each tube to make 10ml solution.

• The solutions were mixed by shaking carefully where Ciprofloxacin hydrochloride readily soluble with the distilled water.

• The solutions were filtered by using filter papers. The filtrate solutions were used for antimicrobial test.

3.2.2 Standard Preparation

•5mg of standard Ciprofloxacin hydrochloride powder was weighed and kept in tubes.

•Then distilled water was added to the tube to make 10ml of solution.

•The solutions were mixed by shaking the tubes carefully where standards of Ciprofloxacin hydrochloride powder readily soluble with the distilled water.

3.2.3 Preparation of dried filter paper discs

Whatman filter paper no. 1 is used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized in hot air oven. The loop is used for delivering the antibiotics solution is made.

3.2.4 Media preparation

•11.2 mg of nutrient agar and 3mg of agar powder were weighed and mixed with 400ml of distilled water.

•Then the solution was mixed vigorously to create a homogenous mixture.

•The mixture was kept into an autoclave for a certain period of time under specific conditions of sterilization.

3.2.5 Procedure

• Nutrient agar plates with appropriate turbidity were prepared by pour plate method.
• Bacterial suspensions were prepared by taking sample from bacterial growth containing plate with a wire loop and soak it to the eppendrof tube containing 1ml normal saline. Vortex machine was used to make the suspension uniform.

• A sterile cotton bud swapped in the bacterial suspension and the cotton bud was streaked in at least three directions over the surface of the nutrient agar for obtaining uniform growth.

• Then the plates are allowed to dry at least for five minutes in laminar air flow.

• Disks containing the test antibiotic were placed on the surface of the agar, using autoclave-sterile forceps to dispense each antibiotic disk one at a time.

• The disks was placed on the surface of the agar such a way that the distribution of the disks

should apart from each other and not close to the edges of the plate.

• Then the plates were incubated within 15 minutes after applying the disks.

• The temperature range of $35^0 \pm 2^0$C is normally required for incubation and the incubation time was 24 which were considered as standard for this test.

Chapter- 4

Results

4.1 Data of zone of inhibition for 5µg/disc

Table 4.1 Data of zone of inhibition for standard ciprofloxacin in 5µg/disc concentration for *E.coli* from urine sample

Clinical isolate	Zone of inhibition (mm)	
	Standard	Blank
Escherichia coli	21	0

From the table 4.1 it is observed that the standard of Ciprofloxacin showed the 21 mm zone of inhibition in the concentration of 5µg/disc against the *E.coli*, where the blank result was zero.

Table 4.2 Data of zone of inhibition for standard ciprofloxacin in 5µg/disc concentration for *E.coli* from blood sample

Clinical isolate	Zone of inhibition (mm)	
	Standard	Blank
Escherichia coli	12	0

From the table 4.2 it is observed that the standard of Ciprofloxacin showed the 12 mm zone of inhibition in the concentration of 5µg/disc against the *E.coli*, where the blank result was zero.

Table 4.3 Data of zone of inhibition for standard ciprofloxacin in 5µg/disc concentration for *E.coli* from stool sample

Clinical isolate	Zone of inhibition (mm)	
	Standard	Blank
Escherichia coli	19	0

From the table 4.3 it is observed that the standard of Ciprofloxacin showed the 19 mm zone of inhibition in the concentration of 5µg/disc against the *E.coli*, where the blank result was zero.

Table 4.4 Data of zone of inhibition for Standard in 5µg/disc concentration for *Pseudomonas aeruginosa*

Clinical isolate	Zone of inhibition (mm)	
	Standard	Blank
Pseudomonas aeruginosa	41	0

From the table 4.4 it is observed that the standard of Ciprofloxacin showed the 41 mm zone of inhibition in the concentration of 5µg/disc against the *Pseudomonas aeruginosa*, where the blank result was zero.

Table 4.5 Data of zone of inhibition for Standard in 5µg/disc concentration for *Salmonella typhi*

Clinical isolate	Zone of inhibition (mm)	
	Standard	Blank
Salmonella typhi	41	0

From the table 4.5 it is observed that the standard of Ciprofloxacin showed the 41 mm zone of inhibition in the concentration of 5µg/disc against the *Salmonella typhi* where the blank result was zero.

Table 4.6 Data of zone of inhibition for Ciprocin in 5µg/disc concentration for *E.coli* from urine sample

Clinical isolate	Zone of inhibition (mm)	
	Ciprocin	Blank
Escherichia coli	20	0

From the table 4.6 it is observed that one of two brands Ciprocin showed the 20 mm zone of inhibition in the concentration of 5µg/disc against the *E.coli*, where the blank result was zero.

Table 4.7 Data of zone of inhibition for Ciprocin in 5µg/disc concentration for *E.coli* from blood sample

Clinical isolate	Zone of inhibition (mm)	
	Ciprocin	Blank
Escherichia coli	12	0

From the table 4.3 it is observed that one of two brands Ciprocin showed the 12 mm zone of inhibition in the concentration of 5µg/disc against the *E.coli*, where the blank result was zero.

Table 4.8 Data of zone of inhibition for Ciprocin in 5µg/disc concentration for *E.coli* from stool sample

Clinical isolate	Zone of inhibition (mm)	
	Ciprocin	Blank
Escherichia coli	18	0

From the table 4.3 it is observed that one of two brands Ciprocin showed the 18 mm zone of inhibition in the concentration of 5µg/disc against the *E.coli*, where the blank result was zero.

Table 4.9 Data of zone of inhibition for Ciprocin in 5µg/disc concentration for *Pseudomonas aeruginosa*

Clinical isolate	Zone of inhibition (mm)	
	Ciprocin	Blank
Pseudomonas aeruginosa	40	0

From the table 4.9 it is observed that one of two brands Ciprocin showed the 40mm zone of inhibition in the concentration of 5µg/disc against the *Pseudomonas aeruginosa* , where the blank result was zero.

Table 4.10 Data of zone of inhibition for Ciprocin in 5µg/disc concentration for *Salmonella typhi*

Clinical isolate	Zone of inhibition (mm)	
	Ciprocin	Blank
Salmonella typhi	41	0

From the table 4.10 it is observed that one of two brands Ciprocin showed the 41mm zone of inhibition in the concentration of 5µg/disc against the *Salmonella typhi*, where the blank result was zero.

Table 4.11 Data of zone of inhibition for Ciprofloxacin in 5µg/disc concentration for *E.coli* from urine sample

Clinical isolate	Zone of inhibition (mm)	
	Ciprofloxacin	Blank
Escherichia coli	20	0

From the table 4.11 it is observed that one of two brands Ciprofloxacin showed the 20mm zone of inhibition in the concentration of 5µg/disc against the *E.coli*, where the blank result was zero.

Table 4.12 Data of zone of inhibition for Ciprofloxacin in 5µg/disc concentration for *E.coli* from blood sample

Clinical isolate	Zone of inhibition (mm)	
	Ciprofloxacin	Blank
Escherichia coli	11	0

From the table 4.12 it is observed that one of two brands Ciprofloxacin showed the 11mm zone of inhibition in the concentration of 5µg/disc against the *E.coli*, where the blank result was zero.

Table 4.13 Data of zone of inhibition for Ciprofloxacin in 5µg/disc concentration for *E.coli* from stool sample

Clinical isolate	Zone of inhibition (mm)	
	Ciprofloxacin	Blank
Escherichia coli	16	0

From the table 4.13 it is observed that one of two brands Ciprofloxacin showed the 16mm zone of inhibition in the concentration of 5µg/disc against the *E.coli*, where the blank result was zero.

Table 4.14 Data of zone of inhibition for Ciprofloxacin in 5µg/disc concentration for *Pseudomonas aeruginosa*

Clinical isolate	Zone of inhibition (mm)	
	Ciprofloxacin	Blank
Pseudomonas aeruginosa	39	0

From the table 4.14 it is observed that one of two brands Ciprofloxacin showed the 39mm zone of inhibition in the concentration of 5µg/disc against the *Pseudomonas aeruginosa* , where the blank result was zero.

Table 4.15 Data of zone of inhibition for Ciprofloxacin in 5µg/disc concentration for *Salmonella typhi*

Clinical isolate	Zone of inhibition (mm)	
	Ciprofloxacin	Blank
Salmonella typhi	40	0

From the table 4.15 it is observed that one of two brands Ciprofloxacin showed the 40mm zone of inhibition in the concentration of 5µg/disc against the *Salmonella typhi*, where the blank result was zero.

4.2 Comparison of zone of inhibition of Ciprocin and Ciprofloxacin with the standard

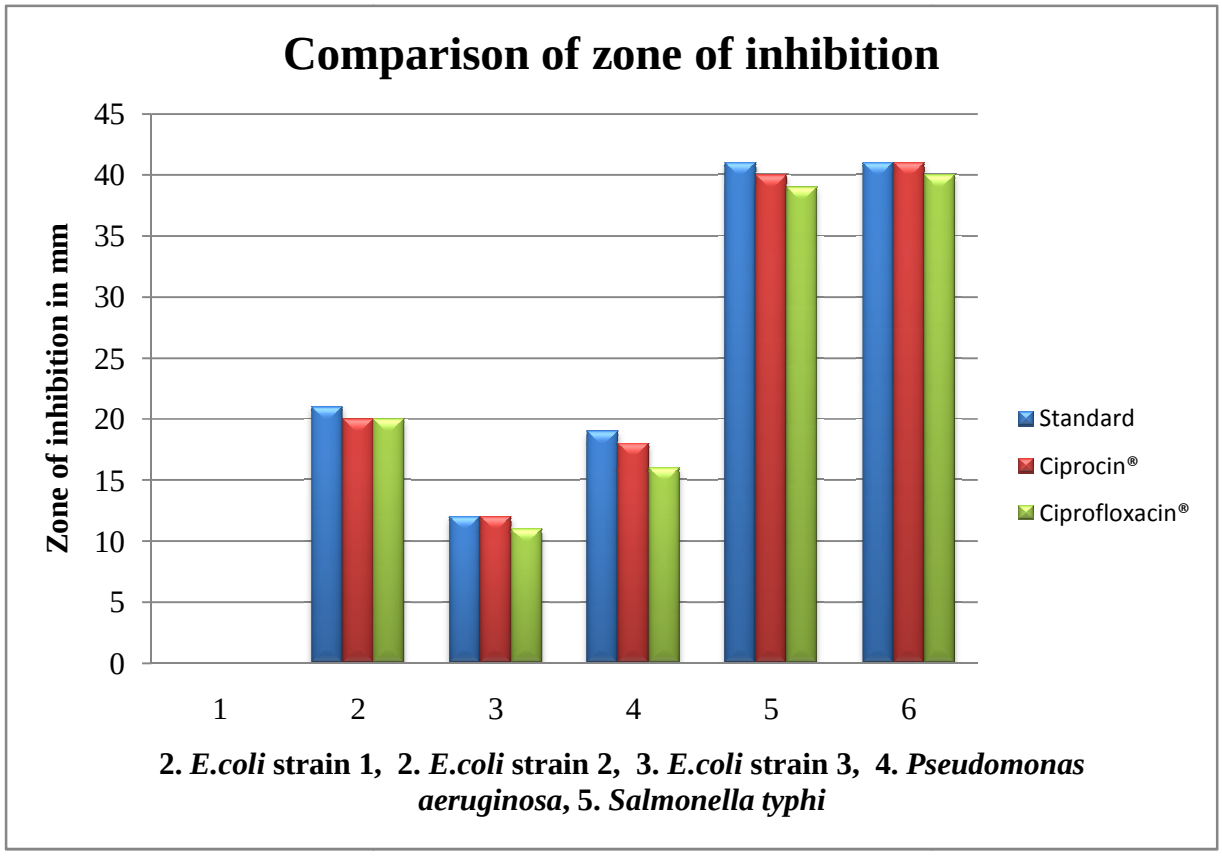

Figure 1.8 Comparison of zone of inhibition of two brands of Ciprofloxacin hydrochloride with the standard against different clinical isolates.

Above figure shows that the probable effectiveness of two brands are almost similar with the standard. Although diameter of the zone of inhibition showed that the drug is resistance against that microorganism. Two brands and the standard show the zone of inhibition 11mm, 12mm and 12mm respectively against the *E.coli* strain 3. From this data it can be said that the drug may become resistance against that strain of *E.coli*. Data of zone of inhibition of the drug against the *Pseudomonas aeruginosa* and *Salmonella typhi* showed that the drug is probably effective against these microorganisms. The diameter of zone of inhibition were almost similar with the standard.

Chapter -5

Discussion

Discussion

Disc diffusion method is one of the effective methods for antimicrobial susceptibility test (Cheesbrough, 2006, p.135).

Ciprofloxacin is the most effective drug of choice to treat the urinary tract infection caused by *E.coli* (Goodman et al, 2006, p.749).

There are many serotypes of Salmonella typhi which can be treated mostly by using the quinolones, specially by the ciprofloxacin (Harvey R.A et al, 2006, p.123).

The increasing recognition that *S.* Typhi isolates with reduced susceptibility to ciprofloxacin may lead to treatment failure has led to calls for a revision of their breakpoints. A study shows that by using disk sensitivity testing, isolates with reduced susceptibility were detected by a Ciprofloxacin (5-μg) disk inhibition zone diameter of ≤30 mm with a sensitivity of 94.0% and specificity of 94.2% (Christofer M.P *et al*, 2010).

Where this study showed that the zone of inhibition 41mm for the brand Ciprocin® and 40 mm for the brand Ciprofloxacin® at the same concentration. Another study shows that by using disk diffusion method zone of inhibition for *Pseudomonas aeruginosa* was 17mm by using the same concentration of Ciprofloxacin per disc (Idu F.K, 2009).

Where this study result showed zone of inbition 41mm which is not similar with the above mentioned study. Another part of the study mentioned above shows 31mm zone of inhibition for the *Salmonella ty*phi by using same concentration of Ciprofloxacin. Where this study showed 41mm of zone of inhibition with the same concentration. Which is not also similar. It may be due to different strains of the microorganism or may be due to mechanical error during the test process. Mean value of the zone of inbition should be taken to confirm the result (Cheesbrough, 2006). But due to time limitation performance the test repeatedly was not possible. These can be the major cause of lack of confirmation about the antibiotics effectiveness against the clinical isolates.

Chapter -6

Conclusion

Conclusion

Drug sector is one of the growing sectors of Bangladesh. Although as a developing country we have to fight several realities like lacking of education, monitoring of drug regulatory authorities, low standard of life, poor hygiene, irrational and substandard drug use etc. Local company meets 95% of drug demand of the country now a day. Not only that, drug export is the growing business of the country. So, we have to be more concern about the quality of drug produced locally.

The study results showed that the two brands Ciprocin® and Ciprofloxacin® of Ciprofloxacin hydrochloride 500mg possibly contain the sufficient amount of active ingredient required for antimicrobial activity and *in vivo* use of these agents can be as effective as the standard which indicates that these are may be the quality products. Further studies need to confirm the effectiveness of the drugs in comparison with the standard. There are many analytical methods and computerized method by which purity as well as various other parameters of a quality drug can be determined more precisely, efficiently and rapidly.

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