Evaluation of In-Vitro Antimicrobial Activity of Five different Brands of Ciprofloxacin

Submitted by:

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ID - 2006-3-70-015

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

DEPARTMENT OF PHARMACY
EAST WEST UNIVERSITY

CERTIFICATE

This is to certify that the research report is submitted to the Department of Pharmacy, East West University, Mohakhali, Dhaka in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy was carried out by Kazi Imtiaz Ibne Mahtab (ID-2006-3-70-015)

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CERTIFICATE

This is to certify that the Research Project of Evaluation of the Antimicrobial Activity of Five different Brands of Ciprofloxacin in Bangladesh, 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 Kazi Imtiaz Ibne Mahtab (ID - 2006-3-70-015), under my guidance and supervision and that no part of the project has been submitted for any other degree. I further certify that all the sources of information, laboratory facilities availed for this connection is dully acknowledged.

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Abstract

Ciprofloxacin is considered to be one of the most widely used drug in Antibiotic Chemotherapy. In the developed world its use has been minimized with the upcoming of newer generations of Quinolone drugs. But in the developing countries like Bangladesh, Ciprofloxacin is still the most commonly used antibiotic. Therefore in this study we tried to put forward a comparative anti-microbial study of different available brands of Ciprofloxacin drugs. The aim was to evaluate the antibiotic activity of these drugs against available strains of bacteria common in Bangladesh.

Five common Ciprofloxacin brands were selected - DFX 500 Tablet (Delta Pharma Limited), Neofloxin 500 Tablet (Beximco Pharmaceuticals Ltd.), Flontin 500 Tablet (Renata Ltd.), Beuflox 500 Tablet (Insepta Pharmaceuticals Ltd.) and Ciprocin 500 Tablet (Square Pharmaceuticals Ltd.)

Three concentrations of the drugs were prepared 500 µg/ml, 750 µg/ml and 1000 µg/ml respectively. The different concentrations were used to measure the antimicrobial activity by using disc diffusion method against a blank and a positive control. The positive control is the crude ciprofloxacin supplied by Delta Pharmaceuticals Ltd. The zone of inhibition for each concentration, blank and positive control was measured and compared with other concentrations of all the brands.

The zone of inhibitions of each concentrations were compared and it was observed that all the drugs show almost similar zone of inhibitions in each concentrations compared to the standard against most the strains of bacteria.

So after carrying out extensive comparison between the different brands it was concluded that all the five brands show activity in the acceptable range. Under antimicrobial susceptibility test, all the five brands have passed the test parameter in comparison with the standard against most of the bacterial strains.

Key words : Ciprofloxacin, reference standard, zone of inhibition, antimicrobial sensitivity

1.1 Ciprofloxacin

Systematic (IUPAC) name :

1-cyclopropyl- 6-fluoro- 4-oxo- 7-piperazin- 1-yl- quinoline- 3-carboxylic acid

Ciprofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class. It is a second-generation fluoroquinolone antibacterial. It kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops synthesis of DNA and of protein.

Ciprofloxacin is marketed worldwide with over three hundred different brand names. In the United States, Canada, and the UK, it is marketed as Baycip, Ciloxan, Ciflox, Cipro, Cipro XR, Cipro XL, Ciproxin, Prociflor, and most recently, Proquin. In addition, ciprofloxacin is available as a generic drug under a variety of different brand names and is also available for limited use in veterinary medicine.

Ciprofloxacin was first patented in 1983 by Bayer A.G. and subsequently approved by the United States Food and Drug Administration (FDA) in 1987. Ciprofloxacin has 12 FDA-approved human uses and other veterinary uses, but it is often used for unapproved uses (off-label). Ciprofloxacin interacts with other drugs, herbal and natural supplements, and thyroid medications.

1.2 Antimicrobial Activity of Ciprofloxacin

Ciprofloxacin is highly active in vitro against a wide range of gram-negative and gram-positive organisms. Ciprofloxacin has been shown to be active against most strains of the following microorganisms in vitro and in clinical infections:

Aerobic gram-negative microorganisms

- *Campylobacter jejuni (C jejuni)*
- *Citrobacter diversus (C diversus)*
- *Citrobacter freundii (C freundii)*
- *Enterobacter cloacae (E cloacae)*
- *E coli*

- *H influenzae*
- *Haemophilus parainfluenzae (H parainfluenzae)*
- *Klebsiella pneumoniae (K pneumoniae)*
- *M catarrhalis*
- *Morganella morganii (M morganii)*
- *Neisseria gonorrhoeae (N gonorrhoeae)*
- *P mirabilis*
- *Proteus vulgaris (P vulgaris)*
- *Providencia rettgeri (P rettgeri)*
- *Providencia stuartii (P stuartii)*
- *P aeruginosa*
- *Salmonella typhi (S typhi)*
- *Serratia marcescens (S marcescens)*
- *Shigella boydii (S boydii)*
- *Shigella dysenteriae (S dysenteriae)*
- *Shigella flexneri (S flexneri)*
- *Shigella sonnei (S sonnei)*

Against all indicated gram-negative organisms, MIC90 values range from 0.016 µg/mL to 1.0 µg/mL.

Aerobic gram-positive microorganisms.

- *Enterococcus faecalis (E faecalis)*
- *Staphylococcus aureus (S aureus) (methicillin susceptible)*
- *Staphylococcus epidermidis (S epidermidis)*
- *Staphylococcus saprophyticus (S saprophyticus)*
- *Streptococcus pneuomoniae (S pneumoniae)*
- *Streptococcus pyogenes (S pyogenes)*

The MIC90 values for indicated gram-positive organisms range between 0.5 µg/mL and 2 µg/mL.

(Danesil.R,Lupetti.A.,Barbari.C.,Ghelardi.E.,Chella.A, 2003),

1.3 History of Ciprofloxacin

The patent history for ciprofloxacin makes reference to a 1982 European Patent (patent number 0049355), as well a German patent dated 21 January 1986. Bayer introduced ciprofloxacin in 1987 and was later approved by the U.S. FDA on 22 October 1987 for use in the United States to treat specific bacterial infections. In 1991, the intravenous formulation was introduced. The current United States patent appears to be held by Bayer, being the assignee. The United States patent was applied for in January 1987, but was not approved until 1996 according to the patent history.

In 2004, ciprofloxacin and levofloxacin together commanded 65% ($3.3 billion) of the global sales of the fluoroquinolone class. The first nine months of 2008 sales for ciprofloxacin were $242 million, as compared to $324 million for Bayer aspirin. Ciprofloxacin has proven to be a blockbuster drug for Bayer A. G., generating billions of dollars in additional revenue. "In 1999, Cipro was the eleventh most prescribed drug in the United States based on new prescriptions, and ranked twentieth in total United States sales. In 1999, Bayer's gross sales of Cipro in the United States were approximately $1.04 billion."

The sale of ciprofloxacin increased dramatically following the anthrax scare of 2001. On 24 October 2002, the Bush Administration (2001–2009) announced a deal between the government and Bayer Pharmaceuticals to purchase 100 million tablets of ciprofloxacin at a reduced price of $0.95 per pill. A full course of ciprofloxacin for postexposure prophylaxis (60 days) resulting from this arrangement costs the government $204 per person treated, compared with $12 per person treated with doxycycline, the drug normally used to treat anthrax, a difference of $192.Generic equivalents.

On 24 October 2001, The Prescription Access Litigation (PAL), filed suit to dissolve an agreement between Bayer and three of its competitors which produced generic versions of drugs (Barr Laboratories, Rugby Laboratories, and Hoechst-Marion-Roussel) that it claimed was blocking access to adequate supplies and cheaper, generic versions of ciprofloxacin. The plaintiffs charged that Bayer Corporation, a unit of Bayer AG, had unlawfully paid the three competing companies a total of $200 million to prevent cheaper, generic versions of ciprofloxacin from being brought to the market, as well as manipulating the price and supply of ciprofloxacin.

Numerous other consumer advocacy groups joined the lawsuit. On 15 October 2008, five years after Bayer's patent had expired, the United States District Court for the Eastern District of New York granted Bayer's and the other defendants' motion for summary judgment, holding that any anticompetitive effects caused by the settlement agreements between Bayer and its codefendants were within the exclusionary zone of the patent and thus could not be redressed by federal antitrust law, in effect upholding Bayer's agreement with its competitors.

1.4 Chemistry of Ciprofloxacin

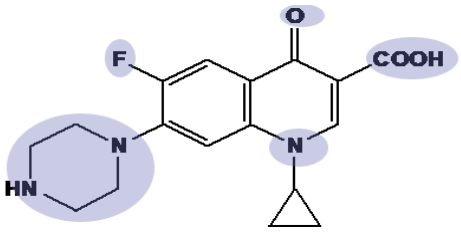

Fig - 1.4 Structure of Ciprofloxacin

Ciprofloxacin is a fluorinated quinolone structurally related to nalidixic acid, the prototype 4-quinolone antibiotic. Ciprofloxacin has a fluorine atom at the 6-position, a piperazine moiety at the 7-position, and a cyclopropyl ring at the 1-position. Enhanced gram-negative in vitro activity is associated with the presence of the piperazine group at the 7-position; presence of this group may enhance penetration of the quinolones into bacteria and may also be responsible for increased activity against *P. aeruginosa.* A fluorine attachment at the 6-position is thought to broaden the spectrum and increase antimicrobial activity up to 30 times. A 2-carbon group at the 1-position is essential for antimicrobial activity. Larger or smaller groups generally lead to reduced activity. Ciprofloxacin has a cyclopropyl attachment, which may be spatially equivalent to a 2-carbon group and is thought to enhance overall antibacterial activity.

Ciprofloxacin hydrochloride is a faintly yellowish to light yellow crystalline substance with a molecular weight of 385.8. Ciprofloxacin is a faintly yellowish to light yellow crystalline substance with a molecular weight of 331.4. It is soluble in dilute (0.1 N) hydrochloride acid and is practically insoluble in water and ethanol. The suspension is composed of ciprofloxacin microcapsules and diluent, which are mixed prior to dispensing. Ciprofloxacin IV is available as a clear, colorless to slightly yellowish solution. (Lode.H., Borner.K., Koeppe.P.and Schaberg.T.,1996)

1.5 Mechanism of action of Ciprofloxacin

The primary mechanism of action of ciprofloxacin is inhibition of the activity of the A subunit of DNA gyrase. Inhibition of bacterial gyrase causes relaxation of the supercoiled DNA. These changes lead to termination of chromosomal replication and to interference with cell division and gene expression. A secondary mechanism, inhibition of the activity of topoisomerase IV, leads to separation of 2 united DNA molecules and subsequent interference with cellular replication.

Ciprofloxacin has been shown to kill bacteria in both growth and stationary phases. This may provide an advantage over other classes of antibiotics such as b-lactams, which are not bactericidal when bacteria are in the stationary phase of growth or growing slowly.

(Lode.H.,*et al*.,1996)

1.6 Clinical and Microbiological Efficacy of Ciprofloxacin

The bactericidal action of ciprofloxacin results from interference with the enzyme DNA gyrase, which is needed for the synthesis of bacterial DNA. Ciprofloxacin does not cross-react with other antimicrobial agents such as b-lactams or aminoglycosides; therefore, organisms resistant to these drugs may be susceptible to ciprofloxacin. Ciprofloxacin is indicated for the treatment of infections caused by susceptible strains of the designated microorganisms in the conditions listed below.

- Acute sinusitis caused by *H influenzae, S pneumoniae, or M catarrhalis.*
- Lower respiratory tract infections caused *by E coli, K pneumoniae, E cloacae, P mirabilis, P aeruginosa, H influenzae, H parainfluenzae, or S pneumoniae.* Also *M catarrhalis* for the treatment of acute exacerbations of chronic bronchitis.
- UTIs caused *by E coli, K pneumoniae, E cloacae, S marcescens, P mirabilis, P rettgeri, M morganii, C diversus, C freundii, P aeruginosa, S epidermidis, S saprophyticus, or E faecalis.*
- Acute uncomplicated cystitis in females caused by *E coli or S saprophyticus.*
- Chronic bacterial prostatitis caused by *E coli or P mirabilis*.

- Complicated intra-abdominal infections (used in combination with metronidazole) caused by *E coli, P aeruginosa, P mirabilis, K pneumoniae, or B fragilis*.
- Skin and skin structure infections caused by *E coli, K pneumoniae, E cloacae, P mirabilis, P vulgaris, P stuartii, M morganii, C freundii, P aeruginosa, S aureus (methicillin susceptible), S epidermidis, or S pyogenes.*
- Bone and joint infections caused by *E cloacae, S marcescens, or P aeruginosa*.
- Infectious diarrhea caused *by E coli (enterotoxigenic strains), C jejuni, S boydii, S dysenteriae, S flexneri, or S sonnei* when antibacterial therapy is indicated.
- Typhoid fever caused by *S typhi.*
- Uncomplicated cervical and urethral gonorrhea caused by *N gonorrhoeae*

(Noedl. H., Krudsood.S., Chalermratana. K., 2006).

1.7 Dosage Information of Ciprofloxacin

Table 1.7 - It shows the type of infection and its recomended dose

(as suggested in Cipro® literature, 2011

Infection	Type / Sensitivity	Dose and Duration
Acute uncomplicated cystitis	Mild/Moderate	250 mg bid for 3 days
Urinary tract infection	Mild/Moderate	250 mg bid for 7-14 days
	Severe/Complicated	500 mg bid for 7-14 days
Chronic bacterial prostatitis	Mild/Moderate	500 mg bid for 28 days
Urethral and cervical gonococcal infection	Uncomplicated	250 mg single dose
Lower respiratory tract	Mild/Moderate	500 mg bid for 7-14 days
	Severe/Complicated	750 mg bid for 7-14 days
Acute sinusitis	Mild/Moderate	500 mg bid for 10 days
Skin and skin structure	Mild/Moderate	500 mg bid for 7-14 days
	Severe/Complicated	750 mg bid for 7-14 days
Bone and Joint	Mild/Moderate	500 mg bid for 4-6 weeks
	Severe/Complicated	750 mg bid for 4-6 weeks
Inhalation anthrax	Adult	500 mg bid for 60 days

(post exposure)	Pediatric	15 mg/kg/dose not to exceed 500 mg dose bid for 60 days
Intra-abdominal (in combination with metronidazole)	Complicated	500 mg bid for 7-14 days
Infectious diarrhea	Mild/Moderate/Severe	500 mg bid for 5-7 days
Typhoid fever	Mild/Moderate	500 mg bid for 10 days

1.8 Adverse effects of Ciprofloxacin

Serious adverse events occur more commonly with fluoroquinolones than with any other antibiotic drug classes. In most, adverse reactions are mild to moderate; however, occasionally serious adverse effects occur. There have been a number of regulatory actions taken as a result of such adverse reactions, which included published warnings, additional warnings and safety information added to the package inserts together with the issuance of "Dear Doctor Letters concerning the recent addition of Black Box Warnings. In 2004, the U.S. FDA requested new warning labels to be added to all of the fluoroquinolones, including ciprofloxacin, regarding peripheral neuropathy (irreversible nerve damage), tendon damage, heart problems (prolonged QT Interval / torsades de pointes), pseudomembranous colitis, rhabdomyolysis (muscle breakdown), Stevens-Johnson syndrome, as well as concurrent usage of NSAIDs contributing to the severity of these reactions.

The serious adverse effects that may occur as a result of ciprofloxacin therapy include irreversible peripheral neuropathy, spontaneous tendon rupture and tendonitis, acute liver failure or serious liver injury (hepatitis), QTc prolongation/torsades de pointes, toxic epidermal necrolysis (TEN) and Stevens-Johnson syndrome, severe central nervous system disorders (CNS) and Clostridium difficile associated disease (CDAD: pseudomembranous colitis), as well as photosensitivity/phototoxicity reactions.

Psychotic reactions and confusional states, acute pancreatitis, bone marrow depression, interstitial nephritis and hemolytic anemia may also occur during ciprofloxacin therapy. Additional serious adverse reactions include temporary, as well as permanent, loss of vision, irreversible double vision, drug induced psychosis and chorea (involuntary muscle movements), impaired color vision, exanthema, abdominal pain, malaise, drug fever,

dysaesthesia and eosinophilia.Pseudotumor cerebri, commonly known as idiopathic intracranial hypertension (IIH), (also referred to as increased intracranial pressure), has been reported to occur as a serious adverse reaction to ciprofloxacin.

Children and the elderly are at a much greater risk of experiencing such adverse reactions.Such reactions may manifest during fluoroquinolone therapy, and long after it had been discontinued.

Serious visual complications have also been reported to occur with ophthalmic fluoroquinolone therapy, which may also occur with ciprofloxacin eye drops, especially corneal perforation, but also evisceration and enucleation. This increased incidents of corneal perforation may be due to fluoroquinolones causing alterations in stromal collagen, leading to a reduction in tectonic strength. As noted previously permanent double vision (diplopia) has also been reported. An unusual case of seizures has also been reported with ciprofloxacin ear drops in an elderly patient.

Some groups refer to these adverse events as "fluoroquinolone toxicity". These groups of people claim to have suffered serious long term harm to their health from using fluoroquinolones. This has led to a class action lawsuit by people harmed by the use of fluoroquinolones, as well as legal action by the consumer advocate group Public Citizen. Partly as a result of the efforts of the State of Illinois and Public Citizen, the FDA ordered black box warnings on all fluoroquinolones advising consumers of the possible toxic effects of fluoroquinolones on tendons.

(Fry.A.M.,Jha.H.C.,Leitman.T.M.,Chaudhary.J.S.P.,Bhatta.R.C.,Elliott.J.,Hyde.T.,Schuch at.A.,Gaynor.B. and Dowell.S.F., 1998)

1.9 Side effects of Ciprofloxacin

Get emergency medical help if you have any of these signs of an allergic reaction to ciprofloxacin: hives; difficult breathing; swelling of your face, lips, tongue, or throat.

❖ **Serious ciprofloxacin side effects may include :**

- severe dizziness, fainting, fast or pounding heartbeats;

- sudden pain, snapping or popping sound, bruising, swelling, tenderness, stiffness, or loss of movement in any of your joints;
- diarrhea that is watery or bloody;
- confusion, hallucinations, depression, unusual thoughts or behavior;
- seizure (convulsions);
- severe headache, ringing in your ears, dizziness, nausea, vision problems, pain behind your eyes;
- pale or yellowed skin, dark colored urine, fever, weakness;
- urinating less than usual or not at all;
- easy bruising or bleeding;
- numbness, tingling, or unusual pain anywhere in your body;
- the first sign of any skin rash, no matter how mild; or
- severe skin reaction -- fever, sore throat, swelling in your face or tongue, burning in your eyes, skin pain, followed by a red or purple skin rash that spreads (especially in the face or upper body) and causes blistering and peeling.

❖ **Less serious ciprofloxacin side effects may include:**

- nausea, vomiting;
- dizziness or drowsiness;
- blurred vision;
- feeling nervous, anxious, or agitated; or
- sleep problems (insomnia or nightmares).

(Fry.A.M., *et al.*, 1998)

1.10 Contraindications and cautions

❖ **Ciprofloxacin is contraindicate for use in:**

- Pseudomembranous colitis
- Deficiency of glucose-6-phosphate dehydrogenase
- Pregnancy
- Lactation (breastfeeding);

- Children and adolescents under 18 years of age;
- Hypersensitivity to ciprofloxacin or another fluoroquinolon

❖ **Ciprofloxacin should be used with caution in:**

- Cerebral atherosclerosis
- Stroke
- Mental diseases
- Epilepsy
- Seizures
- Severe hepatic or renal impairment
- Elderly patients

❖ **Ciprofloxacin Pregnancy Warnings**

Ciprofloxacin has been assigned to pregnancy category C by the FDA. Animal studies failed to reveal embryotoxicity or teratogenicity, although maternal toxicity in some animal studies resulted in increased incidence of abortion. There are no controlled data in human pregnancy. However, ciprofloxacin has been shown to distribute into amniotic fluid. Concentrations reported were 57% (at 2 to 4 hours post dose) to 1000% (at 10 to 12 hours post dose) of that found in maternal serum. A review by the Teratogen Information System concluded that a substantial risk is unlikely; however, there were insufficient data to state that there is no risk. Cartilage damage and arthropathies associated with ciprofloxacin have been reported in immature animals of various species, giving rise to concern over its possible toxic effects on human fetal bone formation. Because safer alternatives are generally available, some experts consider ciprofloxacin contraindicated during pregnancy, especially during the first trimester. The manufacturer recommends use of ciprofloxacin during pregnancy only when benefit outweighs risk.

❖ **Ciprofloxacin Breastfeeding Warnings**

Ciprofloxacin is excreted into human milk. Concentrations found in breast milk have ranged from 85% (at 24 hours post dose) to 214% (at 4 hours post dose) of maternal serum concentration. In one case report, a 2-month-old girl developed perforated

pseudomembranous colitis following ingestion of ciprofloxacin via the mother's milk and subsequently required a bowel resection. In addition, quinolone-induced cartilage erosion and arthropathies that have been observed in juvenile animals render some concern over its possible toxic effects on the developing joints of nursing infants. Ciprofloxacin is considered compatible with breast-feeding by the American Academy of Pediatrics. Because of the potential for serious adverse effects in nursing infants, a decision should be made to discontinue nursing or discontinue administration of ciprofloxacin, taking into account the importance of the drug to the mother

(Noedl H.,*et al.*,2006)

1.11 Drug-Drug interaction of Ciprofloxacin

- When Ciprofloxacin is applied simultaneously with didanosine the absorption of ciprofloxacin is reduced due to the formation of complexes with ciprofloxacin contained in didanosine salts of magnesium and aluminum.
- Due to decreased activity of microsomal oxidation processes in hepatocytes ciprofloxacin increases the concentration of theophylline iother xanthine.
- Ciprofloxacin decreases prothrombin index when is used in combination with hypoglycemic agents
- Ciprofloxacin increases the risk of seizures when used with antidiabetic drugs.
- Simultaneous treatment with antacids, as well as products containing aluminum ions, zinc, iron and magnesium, may decrease the absorption of ciprofloxacin, therefore, the interval between the appointment of these drugs should not be less than 4 hours
- With the simultaneous use of ciprofloxacin and cyclosporin nephrotoxicity is enhanced.
- Metoclopramide increases the absorption of ciprofloxacin, which reduces the time required to reach its maximum concentration in plasma.
- Uricosuric drugs may cause slower excretion (50%) and increased plasma concentrations of ciprofloxacin.
- Ciprofloxacin increases the effects of other antibiotics.

(Nahata.M., 1996)

1.12 Drug-Food Interactions Ciprofloxacin

1.12.1 Ciprofloxacin and Caffeine :

Coadministration with certain quinolones may increase the plasma concentrations and pharmacologic effects of caffeine due to inhibition of the CYP450 1A2 metabolism of caffeine. Quinolones that may inhibit CYP450 1A2 include ciprofloxacin, enoxacin, grepafloxacin, nalidixic acid, norfloxacin, pipemidic acid, and pefloxacin (not all commercially available). In healthy volunteers, enoxacin (100 to 400 mg twice daily) increased systemic exposure (AUC) of caffeine by 2- to 5-fold and reduced its clearance by approximately 80%. Pipemidic acid (400 to 800 mg twice daily) increased AUC of caffeine by 2- to 3-fold and reduced its clearance by approximately 60%. Ciprofloxacin (250 to 750 mg twice daily) increased AUC and elimination half-life of caffeine by 50% to over 100%, and reduced its clearance by 30% to 50%. Norfloxacin 400 mg twice daily increased caffeine AUC by 16%, while 800 mg twice daily increased caffeine AUC by 52% and reduced its clearance by 35%. Pefloxacin (400 mg twice daily) has been shown to reduce caffeine clearance by 47%.

1.12.2 Ciprofloxacin and multivitamins with minerals :

Oral preparations that contain magnesium, aluminum, or calcium may significantly decrease the gastrointestinal absorption of quinolone antibiotics. Absorption may also be reduced by sucralfate, which contains aluminum, as well as other polyvalent cations such as iron and zinc. The mechanism is chelation of quinolones by polyvalent cations, forming a complex that is poorly absorbed from the gastrointestinal tract. The bioavailability of ciprofloxacin has been reported to decrease by as much as 90% when administered with antacids.

When co-administration cannot be avoided, quinolone antibiotics should be dosed either 2 to 4 hours before or 4 to 6 hours after polyvalent cation-containing products to minimize the potential for interaction.

1.12.3 Moderate Food Interaction :

Concurrent ingestion of dairy products (milk, yogurt) or calcium-fortified foods (i.e., cereal, orange juice) may decrease the activity of certain oral fluoroquinolone antibiotics. The mechanism is chelation of calcium and the quinolone, resulting in decreased bioavailability. In the case of orange juice, inhibition of intestinal transport mechanisms (P-glycoprotein or organic anion-transporting polypeptides) by flavones may also be involved. One study reported an average 41% decrease in maximum plasma concentrations and a 38% decrease in AUC when ciprofloxacin was given with calcium-fortified orange juice instead of water.

(Nahata.M.,1996)

1.13 Drug-Disease Interactions of Ciprofloxacin

1.13.1 Antibiotics (Includes Ciprofloxacin) ↔ Colitis :

Pseudomembranous colitis has been reported with most antibacterial agents and may range in severity from mild to life-threatening, with an onset of up to several weeks following cessation of therapy. Antibiotic therapy can alter the normal flora of the colon and permit overgrowth of Clostridium difficile, whose toxin is believed to be a primary cause of antibiotic-associated colitis. The colitis is usually characterized by severe, persistent diarrhea and severe abdominal cramps, and may be associated with the passage of blood and mucus. The most common culprits are clindamycin, lincomycin, the aminopenicillins (amoxicillin, ampicillin), and the cephalosporins. Therapy with broad-spectrum antibiotics and other agents with significant antibacterial activity should be administered cautiously in patients with a history of gastrointestinal diseases, particularly colitis. There is some evidence that pseudomembranous colitis, if it occurs, may run a more severe course in these patients and that it may be associated with flares in their underlying disease activity. The offending antibiotic(s) should be discontinued if significant diarrhea occurs during therapy. Stool cultures for Clostridium difficile and stool assay for C. difficile toxin may be helpful diagnostically. A large bowel endoscopy may be considered to establish a definitive diagnosis in cases of severe diarrhea.

1.13.2 Quinolones (Includes Ciprofloxacin) ↔ CNS Disorders :

Quinolones may cause CNS stimulation manifested as tremors, agitation, restlessness, anxiety, confusion, hallucinations, paranoia, insomnia, toxic psychosis, and/or seizures. Benign intracranial hypertension has also been reported. Therapy with quinolones should be administered cautiously in patients with or predisposed to seizures or other CNS abnormalities. In addition, these patients should be advised to avoid the consumption of caffeine-containing products during therapy with some quinolones, most notably ciprofloxacin, enoxacin, and cinoxacin, since these agents can substantially reduce the clearance of caffeine and other methylxanthines, potentially resulting in severe CNS reactions.

1.13.4 Quinolones (Includes Ciprofloxacin) ↔ Crystalluria :

Crystalluria has been reported rarely during quinolone therapy. Although it is not expected to occur under normal circumstances with usual recommended dosages, patients who are dehydrated (e.g., due to severe diarrhea or vomiting) may be at increased risk and should be encouraged to consume additional amounts of liquid or given intravenous fluid to ensure an adequate urinary output. Alkalinity of the urine should be avoided, since it may also increase the risk of crystalluria. Renal function tests should be performed periodically during prolonged therapy (> 2 weeks).

1.13.5 Quinolones (Includes Ciprofloxacin) ↔ Diabetes :

The use of certain quinolones such as clinafloxacin, gatifloxacin, temafloxacin, levofloxacin, and moxifloxacin has been associated with disturbances in blood glucose homeostasis possibly stemming from effects on pancreatic beta cell ATP-sensitive potassium channels that regulate insulin secretion. Hypoglycemia and, less frequently, hyperglycemia have been reported, although the latter may also occur due to infection alone. Hypoglycemia has usually occurred in patients with diabetes receiving concomitant oral hypoglycemic agents and/or insulin. Administration of ciprofloxacin, levofloxacin, norfloxacin, and especially gatifloxacin in patients treated with sulfonylureas or other oral hypoglycemic agents has resulted in severe, refractory hypoglycemia and hypoglycemic coma. Elderly patients and patients with reduced renal function are particularly susceptible. Blood glucose should be monitored more closely whenever quinolones are prescribed to patients with diabetes.

Gatifloxacin has been known to cause hypoglycemic episodes generally within the first 3 days of therapy and sometimes even after the first dose, while hyperglycemia usually occurs 4 to 10 days after initiation of therapy. Patients should be counseled to recognize symptoms of hypoglycemia such as headache, dizziness, drowsiness, nausea, tremor, weakness, hunger, excessive perspiration, and palpitations. If hypo- or hyperglycemia occur during quinolone therapy, patients should initiate appropriate remedial therapy immediately, discontinue the antibiotic, and contact their physician.

1.13.6 Quinolones (Includes Ciprofloxacin) ↔ Hemodialysis :

The following quinolones are known to be partially removed by hemodialysis and should be administered after dialysis: ciprofloxacin, enoxacin, gatifloxacin, levofloxacin, lomefloxacin, and ofloxacin.

1.13.7 Quinolones (Includes Ciprofloxacin) ↔ Renal Dysfunction:

Quinolones (except trovafloxacin, moxifloxacin, and nalidixic acid) and their metabolites are eliminated by the kidney. Patients with renal impairment may be at greater risk for adverse effects from quinolones, including nephrotoxicity, due to decreased drug clearance. Dosage adjustments may be necessary and modifications should be based on the degree of renal impairment and severity of infection in accordance with the individual product package labeling. Renal function tests should be performed periodically during therapy. (Nahata.M.,1996)

1.14 Pharmacokinetics of Ciprofloxacin

Absorption: Ciprofloxacin given as an oral tablet is rapidly and well absorbed from the gastrointestinal tract after oral administrations.1 The absolute bioavailability of ciprofloxacin tablets and oral suspension is approximately 70% with no substantial loss by first pass metabolism. The pharmacokinetic parameters for ciprofloxacin tablets and IV are shown in Table 1.14

Table 1.14 - it shows the pharmacokinetic parameters of Ciprofloxacin in oral and IV form (as in Cipro® literature, 2011)

	Oral			IV	
Parameters	250 mg	500 mg	750 mg	200 mg	400 mg
C_{max}	1.2	2.4	4.3	2.1	4.6
$T_{1/2}$	4	4	4	5-6	5-6
AUC	4.8	11.6	20.2	5.7	11.3
Cl renal	18	18	18	22	22
Dose in Urine	40-50	40-50	40-50	50-70	50-70
	C_{max} - maximum plasma concentration, $T_{1/2}$ - half life, AUC - area under the curve, Cl renal - renal clearance				

Distribution: Ciprofloxacin is distributed throughout the extravascular space and has been detected in most body tissues and fluids.22-25 Ciprofloxacin achieves high concentrations (up to 6 times serum levels) in urine and bile and also kidney, gallbladder, and lung.16,25,26 Other sites where serum concentration levels are exceeded include muscle tissue, skin-blister fluid, bone, and prostatic tissue.27-29 Extensive distribution of ciprofloxacin throughout the extravascular space and relatively low protein binding (30%) may contribute to its high volume of distribution.

Metabolism: 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. ciprofloxacin is an inhibitor of human cytochrome P450 1A2 mediated metabolism. Co-administration of ciprofloxacin with other drugs promarily metabolized by CYP1A2 results in increase plasma concentrations of these drugs and could lead to clinically significant adverse events of the co-administered drug.

Elimination: Ciprofloxacin undergoes predominantly renal and some extrarenal excretion. About 20% to 35% of tablets and oral suspension and 15% of IV dose is recovered in the feces within 5 days after dosing. The serum elimination half-life in subjects with normal renal function is approximately 4 hours. Approximately 40% to 50% of an orally administered dose

is excreted in the urine as unchanged drug. After a 250-mg oral dose, urine concentrations of ciprofloxacin usually exceed 200 µg/mL during the first 2 hours and are approximately 30 µg/mL at 8 to 12 hours after dosing. The urinary excretion of ciprofloxacin is virtually complete within 24 hours after dosing. The renal clearance of ciprofloxacin, which is approximately 300 mL/minute, exceeds the normal glomerular filtration rate of 120 mL/minute. Thus, active tubular secretion would seem to play a significant role in its elimination.

When ciprofloxacin tablets are given concomitantly with food, there is a delay in the absorption of the drug, resulting in peak concentrations that occur closer to 2 hours postdose rather than 1 hour; however, there is no delay observed when ciprofloxacin suspension is given with food. Concurrent administration of antacids containing magnesium hydroxide or aluminum hydroxide may reduce the bioavailability of ciprofloxacin by as much as 90%.

(Lucchi.M.,Damle.B.,Fang.A., De Caprariis.P.J.,Mussi.A.,Sanchez.S.P.,Pasqualetti.G., and Tacca.M.D., 2008)

1.15 Overprescribing and Bacterial Resistance of Ciprofloxacin

Ciprofloxacin is commonly used for urinary tract and intestinal infections (traveler's diarrhea) and was once considered a powerful antibiotic of last resort, used to treat especially tenacious infections. Not all physicians agreed with this assessment, as evidenced by its wide spread use to treat minor infections as well as non-approved uses. As a result in recent years many bacteria have developed resistance to this drug, leaving it significantly less effective than it would have been otherwise.

Resistance to ciprofloxacin and other fluoroquinolones may evolve rapidly, even during a course of treatment. Numerous pathogens, including Staphylococcus aureus, enterococci, and Streptococcus pyogenes now exhibit resistance worldwide. Widespread veterinary usage of the fluoroquinolones, particularly in Europe, has been implicated.

Fluoroquinolones had become the most commonly prescribed class of antibiotics to adults in 2002. Nearly half (42%) of these prescriptions were for conditions not approved by the FDA, such as acute bronchitis, otitis media, and acute upper respiratory tract infection, according to a study that was supported in part by the Agency for Healthcare Research and Quality.

Additionally they are commonly prescribed for medical conditions that are not even bacterial to begin with, such as viral infections, or those to which no proven benefit exist.

(Fry.A.M., *et al.*, 1998)

1.16 Availability of Ciprofloxacin

Ciprofloxacin tablets are available in 250-mg, 500-mg, and 750-mg strengths. Ciprofloxacin oral suspension is available in 5% (5 g ciprofloxacin in 100 mL) and 10% (10 g ciprofloxacin in 100 mL) strengths. Ciprofloxacin IV is available in 200-mg and 400-mg strengths

1.17 Quality Control and Assurance

Antibiotic sensitivity is a term used to describe the susceptibility of bacteria to antibiotics. Antibiotic susceptibility testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection *in vivo*. Testing for antibiotic sensitivity is often done by the Kirby-Bauer method. Small wafers containing antibiotics are placed onto a plate upon which bacteria are growing. If the bacteria are sensitive to the antibiotic, a clear ring, or zone of inhibition, is seen around the wafer indicating poor growth. Ideal antibiotic therapy is based on determination of the aetiological agent and its relevant antibiotic sensitivity. Empiric treatment is often started before laboratory microbiological reports are available when treatment should not be delayed due to the seriousness of the disease. The effectiveness of individual antibiotics varies with the location of the infection, the ability of the antibiotic to reach the site of infection, and the ability of the bacteria to resist or inactivate the antibiotic. Some antibiotics actually kill the bacteria (bactericidal), whereas others merely prevent the bacteria from multiplying (bacteriostatic) so that the host's immune system can overcome them.

Standardized susceptibility test procedures require the use of quality control microorganisms to control the technical aspects of the test procedures. Quality control microorganisms are specific strains of organisms with intrinsic biological properties. QC strains are very stable strains which will give a standard and repeatable susceptibility pattern. The specific strains used for microbiological quality control are not clinically significant.QC is performed to check the quality of medium, the potency of the antibiotic, to check manual errors. Quality

control strains should be included daily with the test. Not more than 1 in 20 results should be outside accuracy limits. No zone should be more than 4 standard deviations away from midpoint between the stated limits.

If, for reasons of expense or manpower constraints, it is not possible to include all strains on a daily basis, then the following guidelines should be followed.

The frequency can be decreased to once weekly if proficiency has been demonstrated by

1. Performing QC daily for 30 days with less than 10% inaccuracy for each drug
2. Proficiency testing is repeated for each new drug included in the testing
3. All documentation is maintained indefinitely
4. Proficiency testing is repeated for each new batch of media or reagents

All tests must be within accuracy limits if QC is done once weekly

(Lalitha.M.K., 2004).

Significance of the study

- A laboratory test which determines how effective antibiotic therapy is against a bacterial infections.
- Antibiotic sensitivity testing will control the use of Antibiotics in clinical practice
- Testing will assist the clinicians in the choice of drugs for the treatment of infections.
- The identification of relevant pathogens in exudates and body fluids collected from patients
- Sensitivity tests done to determine the degree of sensitivity or resistance of pathogens isolated from patient to an appropriate range of antimicrobial drugs
- Assay of the concentration of an administered drug in the blood or body fluid of patient required to control the schedule of dosage.
- Helps to guide the Physician in choosing Antibiotics
- The accumulated results on different pathogens their sensitivity will guide the physician in choosing empirical treatment in serious patients before the individual's laboratory results are analyzed in the Microbiology laboratory.
- Reveals the changing trends in the local isolates.
- Helps the local pattern of antibiotic prescribing.
- Bacteria have the ability to develop resistance following repeated or subclinical (insufficient) doses, so more advanced antibiotics and synthetic antimicrobials are continually required to overcome them.
- The in vitro testing of bacterial cultures with antibiotics to determine susceptibility of bacteria to antibiotic therapy. Bauer-Kirby test.

Aim of the study

The major objective of this study was to find out the effectiveness or efficacy of different brands of Ciprofloxacin available in Bangladesh.

2. Kirby-Bauer disc Diffusion Susceptibility Test Procedure

2.1 Principle of Disc Diffusion Method

Solutions of known concentration (μg/ml) of the test samples are made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) are then impregnated with known amounts of the test substances using micropipette. Discs containing the test material are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs (impregnated with solvents) are used as positive and negative control. These plates are then kept at low temperature (4 ^{0}C) for 24 hours to allow maximum diffusion. During this time dried discs absorb water from the surrounding media and then the test materials are dissolved and diffused out of the sample disc. The diffusion occurs according to the physical law that controls the diffusion of molecules through agar gel. As a result there is a gradual change of test materials concentration in the media surrounding the discs.

The plates are then incubated at 37 ^{0}C for 24 hours to allow maximum growth of the organisms. If the test materials have any antimicrobial activity, it will inhibit the growth of the microorganisms and a clear, distinct zone of inhibition will be visualized surrounding the medium. The antimicrobial activity of the test agent is determined by measuring the diameter of zone of inhibition expressed in millimeter.

2.2 Apparatus and reagents

1. Petri dish
2. Autoclave
3. Laminar Air Flow
4. Hot Air Oven
5. 1000ml Bottle
6. Nutrient Agar(Media)
7. Normal Saline(0.9% NaCl)
8. Distilled Water
9. Forceps
10. Bunsen Burner
11. Inoculating Loop
12. Electronoc Balance
13. Calipter or Ruler
14. Disc (Prepared by filter paper)

15. Cotton Buds

16. Micropipette

17. Centrifuge Tube

18. 100ml Volumetric Flask

19. Beaker

2.3 Sterilization procedures

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glassware's were sterilized by autoclaving at a temperature of 121 ^{0}C and a pressure of 15-lbs./sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

2.4 Sample Preparation

1. Three 500mg Ciprofloxacin Tablet were weighed and recorded in the Record Book.
2. Crushed the tablet gently by mortar and pestle then 1000mg equivalent powder
1. weighed and that kept in tube.
2. Added water to tube containing Ciprofloxacin powder.
3. Mixed the solution by shaking carefully.
4. Ciprofloxacin is partially soluble in water at room temperature, so the solution was subject to heating with a water bath, with temperature set to 65 Degree Celsius for 1 hour.
5. After heating, the solution was filtered out and stored in a screw cap test tube.

2.5 Preparation of medium

To prepare required volume of this medium, calculated amount of the constituent was taken in a conical flask and distilled water was added to it to make the required volume. The contents were heated in a water bath to make a clear solution. The P^{H} (at 25 ^{0}C) was adjusted

at 7.2 – 7.6 using NaOH or HCl. 10 ml and 5 ml of the medium was then transferred in screw cap test tubes to prepare plates. The test tubes were then capped and sterilized by autoclaving at 15-lbs. pressure/sq. inch at 121 ^{0}C for 20 minutes.

2.6 Preparation of subculture

In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the broth slants (prepared prior with sterilization) with the help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24 hours at 37 ^{0}C for their optimum growth. These fresh cultures were used for the sensitivity test.

2.7 Preparation of the test plates

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial suspension was immediately transferred to the sterilized Petri dishes. The Petri dishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media.

2.8 Preparation of dried filter paper discs

Three types of discs were used for antimicrobial screening.

- Standard discs

  These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test sample. In this procedure, Ciprofloxacin Crude Drug (supplied from Delta Pharmaceuticals) was used.

- Blank discs

  These were used as negative controls which ensure that the residual solvent (left over the discs even after air-drying) and the filter paper were not active themselves.

- Preparation of sample discs with test samples

Calculated concentration of tablet solution was taken and it was poured on the disk to prepare the sample disks with varying concentrations under laminar airflow and the disks were dried.

2.9 Placement of the antibiotic and blank disc

Before the placement the antibiotic disc (Ciprofloxacin) a parameter marker was used to marks the bottoms of the test plates with sections according to the name and concentration of the sample.

The sections were labeled accordingly

Antibiotic Ciprofloxacin of 500µg, 750µg and 1000µg discs were manually placed on the agar plate .

(a) The Muller-Hinton agar plate pre-inoculated with the test bacteria, was placed over the disc template.
(b) One disc was removed from the cartridge using forceps that had been sterilized.
(c) The plate was lifted and the disc was placed over one of the positioning marks.

The disc was pressed with the forceps to ensure complete contact with agar surface. The lid of the plate was replaced between discs to minimize exposure to air borne contaminants. All of these steps were done under laminar airflow.

2.10 Incubation of the plates

1. A temperature range of 35°c ±2°c was maintained.
2. Temperature above 35°c might not allow the detection of growth and zone of inhibition.
3. Results were read after 24 hours of incubation.

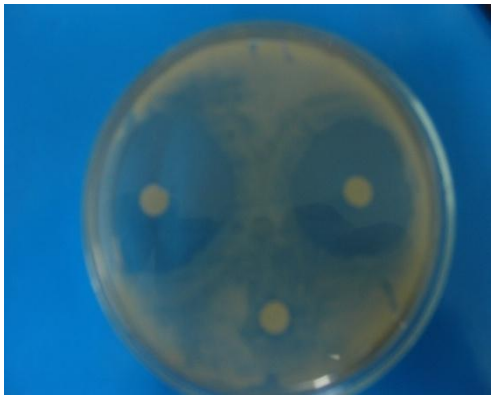

Fig - 2.11 Sample Zone of Inhibition

2.11 Mesuring zone sizes

1. Following incubation, the zone sizes were measured to the nearest millimeter using a ruler. The diameter of the disc was included in the measurement.
2. When measuring zone diameter, it was round up to the next millimeter.
3. The plate was held a few inches above a black, nonreflecting surface illuminated with reflected light.
4. The plate was viewed using a direct, vertical line of sight to avoid any parallax that might result in misreading.
5. The zone size was recorded on the recording sheet.
6. If the placement of the disc or the size of the zone did not allow to read the diameter of the zone, then it was measured from the centre of the disc to a point on the circumference of the zone where a distinct edge was present(the radius) and the measurement was multiplied by 2 to determine the diameter.

2.12 Procedures

1. 800ml media(Agar) was prepared with Distilled Water. Where Nutrient Agar needed 22.4gm.
2. Media and other equipments were sterilized in the Autoclave for 1 hour. Where temperature range is 60°C To 121°C And Pressure is 1 15-lbs./sq. inch.
3. Petridish cleaned and kept in the Hot Air Woven to dry and sterilize .
4. Bacterial subculture was made before experiment at least 1 day.
5. There were six types of Bacterial subcultures prepared.

6. The bacterial subcultures were used to prepare 6 different test plates using the pre-sterilized agar media, with different organism in each and were labelled accordingly.
7. Seven sets of test plates were prepared for each type of bacteria, 5 for different samples, 1 for positive control and one for negative control

8. The sample discs were prepared accordingly, with three concentrations 500, 750 and 1000 µg disc and for 6 different bacteria 18 sets of discs for each different brand were prepared with varying concentrations.
9. Only one brand sample was used each time.
10. The discs were placed carefully on the plates under aseptic conditions and with minimal air contact.
11. The plates were incubated soon after placing the disc.
12. The temperature range of 35°c ±2°c is normally required for incubation and the incubation time was 24 hours which were considered as standard for this test.
13. Lastly the zone of inhibitions were measured for each concentrations and the results were noted very carefully.

3. Antimicrobial Sensitivity test Result

Antimicrobial sensitivity test was performed for 5 different brands of Ciprofloxacin antibiotics -

- **DFX (Delta)**
- **Neofloxin (Beximco)**
- **Flontin (Renata)**
- **Beuflox (Incepta)**
- **Ciprocin (Square)**

The tests were performed against Standard Ciprofloxacin Solution supplied from Delta Pharmaceuticals Ltd., which was used as the Reference Standard and also the positive control.

For every test a Blank Disc was used as the negative control.

The tests were performed using 6 different types of microorganisms -

- ***Salmonella typhi***
- ***Staphylococcus aureus***
- ***E. coli***
- ***Bacillus subtilis***
- ***Vibrio mimicus***
- ***Shigella dysentery***

The test result was determined by comparing the sensitivity between the Reference Standard and the Samples Ciprofloxacin solutions against the microorganisms.

The test results are tabulated in the following with their corresponding graphical representations.

Table 3.1 Test Results against *Salmonella typhi*

SI NO	Brands of Ciprofloxacin	Zone of Inhibition (mm)			
		Blank	500 µg/disc	750 µg/disc	1000 µg/disc
01	DFX	No Growth	40	48	49
02	Neofloxin	No Growth	41	43	48
03	Flontin	No Growth	38	43	47
04	Beuflox	No Growth	36	42	48
05	Ciprocin	No Growth	38	43	50
06	Standard	No Growth	40	45	51

From the table 3.1, it is observed that, all the six samples showed similar sensitivity like the standard against *Salmonella typhi*

Figure 3.1 Comparison between different brands of Ciprofloxacin against *Salmonella typhi*

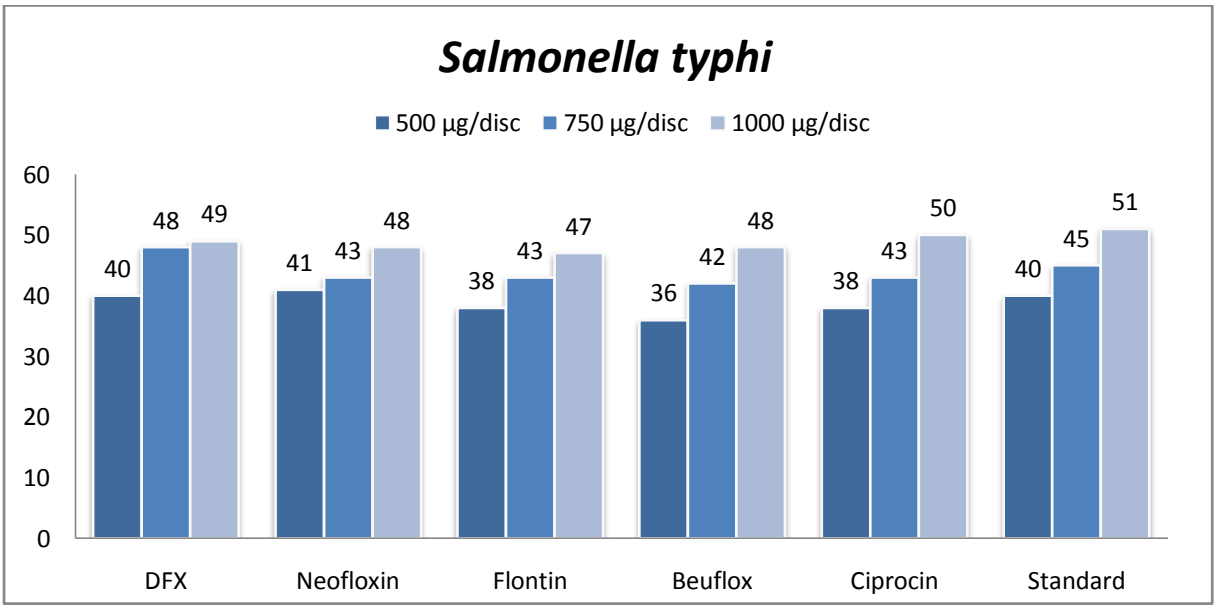

Figure 3.1 - it shows that the samples showed similar activity like the standard

Table 3.2 Test Results against *Staphylococcus aureus*

SI NO	Brands of Ciprofloxacin	Zone of Inhibition (mm)			
		Blank	500 µg/disc	750 µg/disc	1000 µg/disc
01	DFX	No Growth	42	46	52
02	Neofloxin	No Growth	37	40	50
03	Flontin	No Growth	36	45	51
04	Beuflox	No Growth	37	45	52
05	Ciprocin	No Growth	40	42	40
06	Standard	No Growth	40	45	54

From the table 3.2, it is observed that, all the six samples showed similar sensitivity like the standard against *Staphylococcus aureus*

Figure 3.2 Comparison between different brands of Ciprofloxacin against *Staphylococcus aureus*

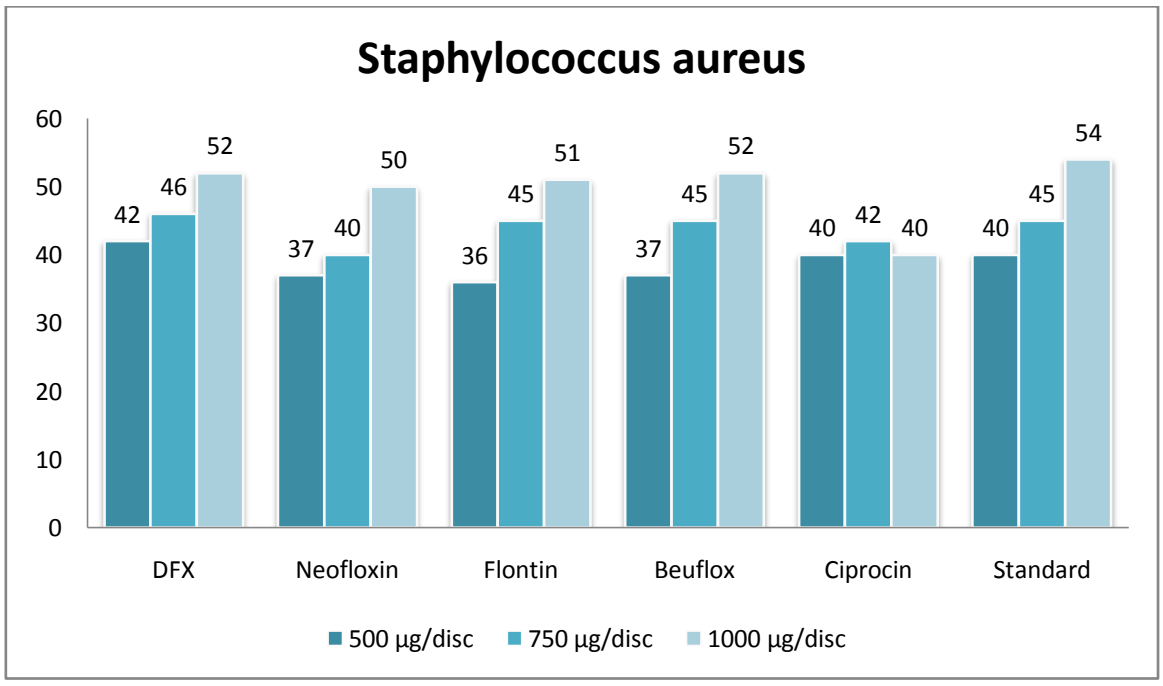

Figure 3.2 - it shows that the samples showed similar activity like the standard

Table 3.3 Test Results against *E. coli*

SI NO	Brands of Ciprofloxacin	Zone of Inhibition (mm)			
		Blank	500 µg/disc	750 µg/disc	1000 µg/disc
01	DFX	No Growth	38	51	51
02	Neofloxin	No Growth	42	47	47
03	Flontin	No Growth	39	42	49
04	Beuflox	No Growth	40	44	49
05	Ciprocin	No Growth	36	45	56
06	Standard	No Growth	40	45	55

From the table 3.3, it is observed that, all the six samples showed similar sensitivity like the standard against *E. coli*

Figure 3.3 Comparison between different brands of Ciprofloxacin against *E. coli*

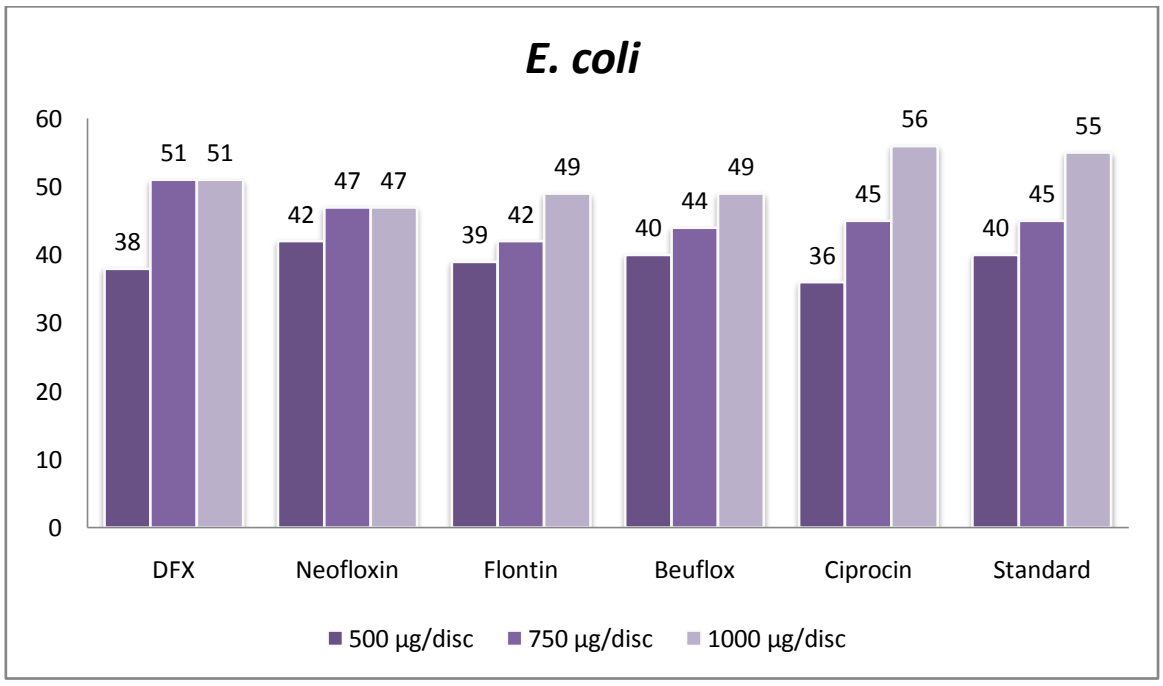

Figure 3.3 - it shows that the samples showed similar activity like the standard

Table 3.4 Test Results against *Bacillus subtilis*

SI NO	Brands of Ciprofloxacin	Zone of Inhibition (mm)			
		Blank	500 µg/disc	750 µg/disc	1000 µg/disc
01	DFX	No Growth	44	48	52
02	Neofloxin	No Growth	38	41	49
03	Flontin	No Growth	42	46	55
04	Beuflox	No Growth	39	40	52
05	Ciprocin	No Growth	42	44	49
06	Standard	No Growth	40	45	55

From the table 3.4, it is observed that, all the six samples showed similar sensitivity like the standard against *Bacillus subtilis*

Figure 3.4 Comparison between different brands of Ciprofloxacin against *Bacillus subtilis*

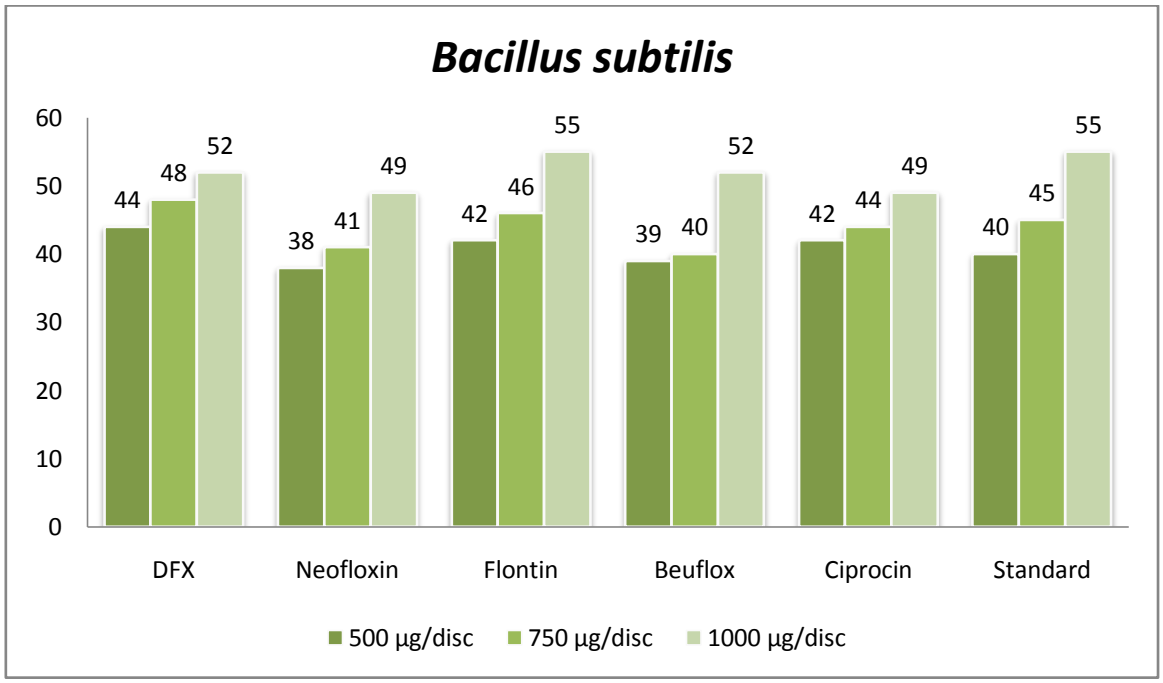

Figure 3.4 - it shows that the samples showed similar activity like the standard

Table 3.5 Test Results against *Vibrio mimicus*

SI NO	Brands of Ciprofloxacin	Zone of Inhibition (mm)			
		Blank	500 µg/disc	750 µg/disc	1000 µg/disc
01	DFX	No Growth	43	47	55
02	Neofloxin	No Growth	42	44	53
03	Flontin	No Growth	37	41	50
04	Beuflox	No Growth	40	46	51
05	Ciprocin	No Growth	40	42	52
06	Standard	No Growth	40	45	55

From the table 3.5, it is observed that, all the six samples showed similar sensitivity like the standard against *Vibrio mimicus*

Figure 3.5 Comparison between different brands of Ciprofloxacin against *Vibrio mimicus*

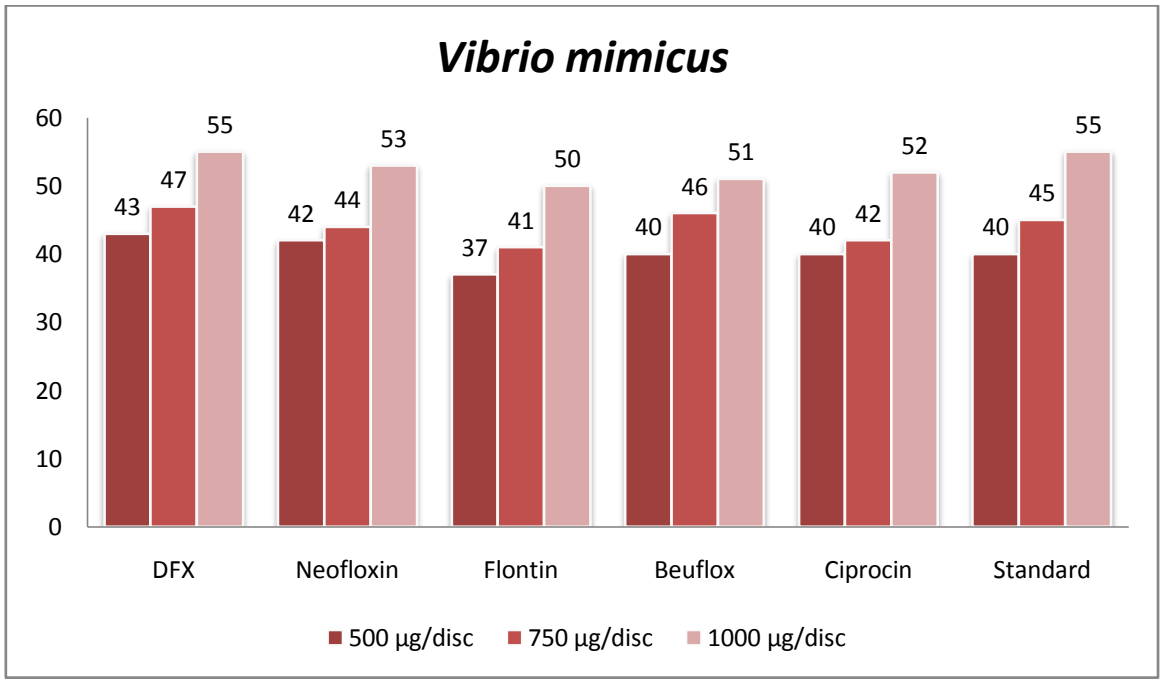

Figure 3.5 - it shows that the samples showed similar activity like the standard

Table 3.6 Test Results against *Shigella dysentery*

SI NO	Brands of Ciprofloxacin	Zone of Inhibition (mm)			
		Blank	500 µg/disc	750 µg/disc	1000 µg/disc
01	DFX	No Growth	39	54	53
02	Neofloxin	No Growth	40	45	55
03	Flontin	No Growth	41	44	52
04	Beuflox	No Growth	35	41	54
05	Ciprocin	No Growth	35	41	56
06	Standard	No Growth	40	45	55

From the table 3.6, it is observed that, all the six samples showed similar sensitivity like the standard against *Shigella dysentery*

Figure 3.6 Comparison between different brands of Ciprofloxacin against *Shigella dysentery*

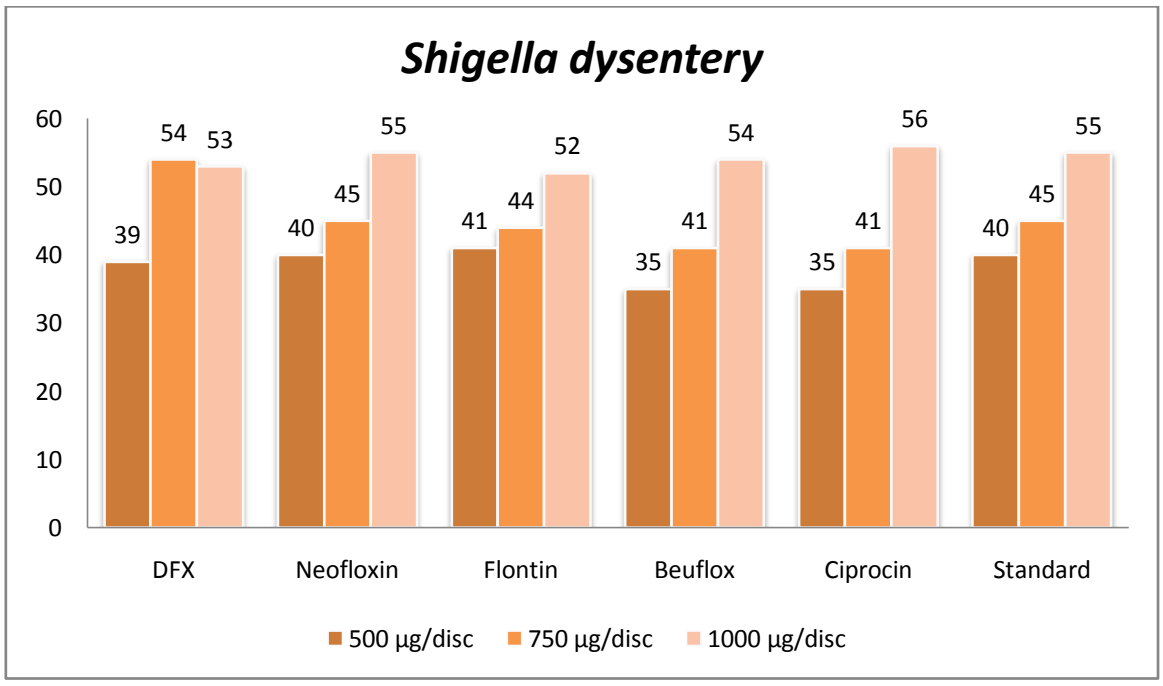

Figure 3.6 - it shows that the samples showed similar activity like the standard

DISCUSSION

Ciprofloxacin is one of the most popular antibiotic available in the market today; its unusual pharmacokinetic characteristics and potent antimicrobial activities have been reported in results in different antibiotic sensitivity test. It shows the effectiveness for a broad range of infections.

In the tests above, it was observed that all the five brands were active against all the six strains of microorganisms.

In this study, the test parameters were stated by the Reference Standard which were 40 mm, 45mm and 51 mm; for 500 µg/disc, 750 µg/disc and 1000 µg/disc respectively. From the tables it has been observed that all the five brands DFX, Neofloxin, Flontin, Beuflox and Ciprocin showed similar activity as the standard against all the six microorganisms, *Salmonella typhi, Staphylococcus aureus, E. coli, Bacillus subtilis, Vibrio mimicus and Shigella dysentery,* respectively. This shows that the samples met the test parameters.

The Figures show comparative graphs between all the 5 brands and the standard against all the microorganisms. From the figures it is clearly visible that the samples had the similar sensitivity pattern as the reference standard.

The blanks for each test showed no growth, which proved that the reagents or materials did not contain any antimicrobial property.

From all the tables above, it can be observed that all the five brands showed similar activity as the Reference Standard. Their pattern of activity for all three concentrations were almost similar as the Standard.

These properties of ciprofloxacin promised that it would have a high degree of efficacy in the treatment of infections, especially those caused by intracellular pathogens. The results of many preclinical and clinical trials have suggested the usefulness of a short - term course of administration of this drug to patients with bacterial and nonbacterial infections. Therefore in this study it was predetermined that all the tablets would show desired activity. All the 5 different sample tablets of ciprofloxacin showed good sensitivity against all 6 microorganisms when compared to the standard. The blanks were always found to be resistant to inhibition and the standard discs stated the standard parameters for the tests.

Conclusion

After performing all the quality control parameter tests for five different brands of Ciprofloxacin - DFX 500mg, Neofloxin 500mg, Flontin 500mg, Beuflox 500mg and Ciprocin 500mg tablets; manufactured by Delta Pharmaceuticals Ltd, Beximco Pharmaceutical Ltd, Renata Pharmaceuticals Ltd, Incepta Pharmaceuticals Ltd and Square Pharmaceuticals Ltd. Here in this study three concentrations of 500µg/disc, 750µg/disc and 1000µg/disc were used to test their antimicrobial sensitivity. The results showed that all the three concentrations of each of the five brands showed satisfactory sensitivity towards all of the six microorganisms.

So we can conclude that all of our sample brands met the quality control parameter which was set by the standard Ciprofloxacin powder provided by Delta Pharmaceuticals Ltd.

The results did not show any significant difference with the reference standard for the antimicrobial sensitivity for the microorganisms.

Therefore it can be concluded that, all of the six brands of Ciprofloxacin tablet, were manufactured according to the specification given by the USP/BP and their quality was adequately maintained.

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