Evaluation Study of Antibacterial Efficacy of Ciprofloxacin Produced from Different Companies

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ﴾

{Allah will raise those who have believed among you and those who are given knowledge by degrees}

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Abstract

Ciprofloxacin is a broad-spectrum fluoroquinolone antibiotic widely prescribed in clinical and hospital settings. Due to its availability in various doses and dosage forms and its ability to achieve therapeutic concentrations in most body fluids and tissues, ciprofloxacin has become a valuable antibiotic with the most potent antibacterial activity against many Gram-negative and Gram-positive bacteria. In vitro antibacterial activity of three of the commonly sold brands of ciprofloxacin tablets was evaluated against *Staphylococcus* and *Pseudomonas* isolates by using Kirby-Bauer disk diffusion method. Interpretation of susceptibility and resistance was based on the presence and absence of a zone of inhibition surrounding the disk, and three different concentrations of the same antibiotic were evaluated against the pathogens. This method was also used to find the MIC for each one of the brands. The study revealed that ciprofloxacin tablets of brand A had relatively better efficacy than other brands as growth of microorganisms was inhibited with a low drug concentration of 25 µg/mL.

1. Introduction

Ciprofloxacin, a synthetic second-generation bactericidal antibiotic of fluoroquinolones (Thai et al., 2022) is one of the most important antibiotic medications needed in the basic healthcare system. It is available in the list of essential medicines of the World Health Organization (WHO) (World Health Organization, 2021). Ciprofloxacin was patented in 1983 by Bayer A.G. and approved in 1987 by the United States Food and Drug Administration (USFDA) (Sharma et al., 2010).

➢ Indication

Ciprofloxacin is a common antibiotic prescribed and usually well-tolerated. Ciprofloxacin has been shown to be active against isolates of various Gram-positive and Gram-negative bacteria, both in vitro and in vivo (Tamma et al., 2012). It is one of the antibiotics used for respiratory, urinary tract, intestinal and abdominal infections caused by various pathogens including *Escherichia coli, Haemophilus influenzae,* other *H. spp., Neisseria gonorrhoeae, N. meningitides, Moraxella catarrhalis, Klebsiella pneumoniae, Legionella pneumophila, Moraxella catarrhalis, Proteus mirabilis, and Pseudomonas aeruginosa,* methicillin-susceptible *Staphylococcus aureus, Streptococcus pneumoniae, Staphylococcus epidermidis, Enterococcus faecalis,* and *Streptococcus pyogenes* (Rodriguez et al., 2009, 2015; Vesga et al., 2010; Agudelo and Vesga, 2012; Tamma et al., 2012). Ciprofloxacin is one of the few oral antibiotics to treat *P. aeruginosa* infections (Rehman et al., 2019). Ciprofloxacin is FDA approved for the treatment of urinary tract infections, sexually transmitted infections (gonorrhea and chancroid, skin and soft tissue infection, bone, joint infections, prostatitis, pneumonia, typhoid fever, gastrointestinal infections, lower respiratory tract infections, inhalation anthrax (post-exposure prophylaxis), plague, and salmonellosis, acute bacterial exacerbation of chronic bronchitis (Meyerhoff et al., 2004; Apangu et al., 2017; Unemo et al., 2019). Ciprofloxacin and its derivatives are also being investigated for its action against malaria, cancers, and AIDS (Pietsch et al., 2017).

➢ Pharmacokinetics Characteristic of Ciprofloxacin

Quinolones have good oral bioavailability (Drlica, 1999). Unlike most classes of antibiotics, the bioavailability of oral quinolones (except norfloxacin) is comparable to quinolones administered intravenously (Just, 1993; von Rosenstiel and Adam, 1994). Therefore, often dose adjustment is not necessary while switching from intravenous to oral quinolones. The extent of absorption of orally administered quinolones is significantly decreased if co-administered with products containing positively charged cations such as zinc, aluminum, calcium, and magnesium. The positively charged cations form insoluble drug-cation complexes in the gastrointestinal tract (GIT). Usage

of antacids should be avoided, or at least administer ciprofloxacin either two hours before or six hours after antacids (Thai et al., 2023). Quinolones have a large volume of distribution and their distribution in urinary tract and respiratory tissues is of importance because they are effective against microorganisms commonly responsible for urinary tract and respiratory tract infections (Davis et al., 1996). Ciprofloxacin is structurally similar to nalidixic acid (Davis et al.,1996). Unlike nalidixic acid, ciprofloxacin contains the fluorine atom, due to this single change, ciprofloxacin is approximately 100 times more potent than nalidixic acid. Other changes in the structure resulted in ciprofloxacin's extended Gram-negative activity, higher potency, better tissue penetration, greater bioavailability, and longer plasma half-life (Sanchez et al., 1992; Tillotson, 1996).

➢ Administration and Dosage Forms

Ciprofloxacin is available orally at three different doses (250,500,750mg), intravenously, and in topical formulations (ophthalmic and otic). Ciprofloxacin is administered orally twice daily for 7 to 14 days or at least two days after signs and symptoms of the infection are over. The recommended oral dose regimen is 250 mg twice daily to treat mild to moderate and 500 mg twice daily for severe or complicated urinary tract infections. Therapy for mild to moderate respiratory tract or skin and soft-tissue infections require 500mg twice-daily dosing. Comparatively, a dosage of 750mg twice daily is recommended for severe or complicated infections (Davis et al., 1996).

Ciprofloxacin ophthalmic solution is FDA-approved for treating corneal ulcers and conjunctivitis caused by susceptible strains (McDonald et al., 2014; Mohamed et al., 2020). Ciprofloxacin otic solution is approved for treating acute otitis externa caused by susceptible strains of *Pseudomonas aeruginosa* or *Staphylococcus aureus* (Wiegand et al., 2019). Ciprofloxacin suspension gel is FDA approved for pediatric otitis media with effusion (Edmunds et al., 2017).

➢ Adverse effects

Adverse effects are mild at therapeutic doses and are mostly limited to gastrointestinal disruptions such as nausea, vomiting, diarrhea, and abdominal pain (Davis et al., 1996). Ciprofloxacin should be given with food to minimize gastrointestinal upset. The serious adverse effects of ciprofloxacin include prolonged QT interval, hyper or hypoglycemia, and photosensitivity (Blondeau, 1999).

➢ Mechanism of Action

Quinolones exert the potent antibacterial effect by binding to bacterial enzymes, DNA gyrase and topoisomerase IV. This binding results in the formation of quinolone–enzyme–DNA complex. Shortly after binding, the enzyme undergoes conformational changes. The enzyme breaks the DNA and the drug prevents re-ligation of the broken

DNA strands, thus preventing DNA replication. Ultimately, this results in the damage to bacterial DNA and thus cell death (Stefan et al.,2016). Reduced susceptibility to fluoroquinolones has become a major problem, mostly in Asia (Brown et al., 1996; Threlfall et al., 2001).

➢ Mechanism of Resistance

Mutation in DNA gyrase, plasmid-mediated and efflux pump-mediated resistance confers resistance to fluoroquinolones, including ciprofloxacin. For *E. Coli*, the primary resistance mechanism is generally the GyrA subunit of gyrase. (Güler and Eraç, 2016; Chang et al., 2021). There are several reports which alert on the customary dispensing of fluoroquinolones as over the counter drugs, which may lead to increased resistance of the pathogenic bacteria (Nagai et al., 2001). There is no cross resistance between fluoroquinolones and other classes of antibiotics, so it may be of clinical value when other antibiotics are no longer effective (Varshney et al., 2014).

Aim of the Study

All drug products must be evaluated for their quality, safety and efficacy. The clinical efficacy of drug products can be ensured when their quality is reliable and reproducible. The availability and use of substandard and spurious quality of oral ciprofloxacin formulations has been thought to have contributed to increased risk of treatment failure and bacterial resistance. With more appropriate use of ciprofloxacin there will be less development of resistance

The present study aimed to evaluate and compare the in vitro antibacterial activity of three of the mostly prescribed ciprofloxacin tablets to optimize the use of ciprofloxacin in community and hospital practice.

2. Materials and Methods

Table 1: Equipment and materials used in the lab work.

Equipment	Materials
Petri dish	Muelle-Hinton agar
Autoclave	Nutrient broth
Cotton swap	Antibiotic powder
L-shaped glass spreader	Distilled water
Bunsen burner	DMSO
Incubator	
Inoculation loop	
Test tubes	
Analytical balance	
Conical flask	
Forceps	

2.1 Test Organisms

Strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated and cultured by Clinical Laboratory Sciences Department, University of Basra.

2.2 Drugs, Media, And Reagents

Three commercially available brands of ciprofloxacin hydrochloride film-coated tablets, each with a label of 500mg, were purchased from a pharmacy local market. Detailed information about the brands is shown in table 2.

Table 2: Brands of Ciprofloxacin Tested for Efficacy.

Brand Name	Strength
Brand A	500mg
Brand B	500mg
Brand C	500mg

Notes: All brands contain 500 mg of ciprofloxacin in a tablet.

Both brand A and C have a shelf life of 3 years, while brand B have a shelf life of 5 years.

Media used in this study included Mueller– Hinton agar (Oxoid, UK) and Nutrient Agar (Kardan Azma, Iran). The media was prepared according to the manufacturer's specifications.

2.3 Preparation of Antimicrobial Agar Plates

The minimal inhibitory concentration (MIC) test was performed using Mueller- Hinton Agar (MHA), which is the best medium and gives satisfactory growth of most bacterial pathogens. The media was made by adding 9.5 g of Muller Hinton Agar in 250 mL of distilled water. The agar was mixed thoroughly in distilled water. If any residual amount of the agar powder remains undissolved, it can be heated on a flame with slight shaking until obtaining a clear solution. Next step is to sterilize the media in autoclave for 15 minutes at 121 °C. After 15 minutes the media was kept outside and allowed to cool to the extent that it should not solidify. The media was poured in sterile petri dishes. These plates were kept at room temperature and allowed to solidify. When the media was solidified then the plates were placed in incubator at 37 °C for 24 hrs.

2.4 Preparation of Antibiotic Stock Solution

A total of 10 film-coated tablets of ciprofloxacin from each brand were manually grinded to obtain a fine powder of the antibiotic. Afterwards, 5mg was weighed and dissolved in

a total volume of 5mL (2 ml of DMSO and 3mL of distilled water) to prepare a stock solution that contains (1mg/mL) or (1000µg/mL) concentrations.

2.5 Preparation of Different Concentrations of The Antibiotic

The stock solution was diluted to obtain a serial dilution of working solutions. The concentrations of the antibiotic solutions were expressed in µg/mL. The working solutions were prepared using the dilution formula:

$C_1 V_1 = C_2 V_2$

C_1 = the concentration of stock solution	V_1 = the volume of stock solution
C_2 = the concentration of diluted solution	V_2 = the volume of diluted solution

From the stock solutions, 500, 250,125, 50 and 25 µg/mL were obtained by using D.W as a diluent.

2.6 Preparation of Inoculum

Inoculum was prepared from a pure 24-hour bacterial culture on NA plates, isolated colonies were picked using a loop and sub-cultured to a tube having a volume of nutrient broth. The nutrient broth was made by adding a specific amount of NA in a known volume of distilled water. Then this mixture was autoclaved for 15 minutes at 121°C. Then the tube is incubated for the bacteria to grow. It is recommended that subcultures of the organisms to be tested be made the previous day in order for results to be valid.

2.7 Inoculation of the MH plates

A new sterile cotton swab was dipped into the inoculum tube, lifted out and the excess fluid was removed by pressing and rotating the swab against the wall of the tube. The swab should not be dripping wet. The swab was then used to inoculate the entire surface of MH agar plate by streaking the swab three times over the entire agar surface, rotating the plate approximately 60 degrees each time to ensure an even distribution of the inoculum. Discard the swab into an appropriate container. Leaving the lid slightly open, allow the plate to sit at room temperature for at least 3 to 5 minutes, but no more than 15 minutes, for the surface of the agar plate to dry before proceeding to the next step.

2.8 Procedure for MIC

MIC value is the least concentration of the antimicrobial agent that prevents visible growth of the microorganisms. It is a measure of the potency of the agent and the smaller the value the higher the antimicrobial efficacy.

The disk diffusion method is based on the determination of the zone of inhibition (ZI) proportional to the bacterial susceptibility to the antimicrobial present in the disk. The diameter of this ZI around the antimicrobial disk depends on the concentration of antibiotics in the disk and its diffusibility.

1. First, we must refresh the bacteria on the agar plates a day before the process of introducing the antibiotic solution into the agar plate.
2. The agar plate was inoculated with 200µL of the test bacteria and an L- shaped spreader was used to spread the microbial inoculum across the entire agar surface. The whole procedure was carried out under aseptic conditions near the flame.
3. MH agar plates were appropriately labelled for each organism to be tested with the names and concentrations of drug made. The labelling was made on the outer layer of the bottom of petri dish.
4. Placement of the antibiotic disks was carried out on the next day. Disks were added one at a time to the agar plate using forceps. Sterilize the forceps by cleaning them with a sterile alcohol pad and allowing them to dry or immersing the forceps in alcohol then igniting.
5. The lid of the petri dish was partially removed, and the disks were placed over the marked spots that were made earlier, and gently pressed to ensure complete adherence with the agar surface. The lid of the plate was replaced between disks to minimize exposure to airborne contaminants. Placing the disks close to the edge of the plate was avoided as the zones will not be fully round and can be difficult to measure.
6. The plates were then incubated at a temperature range of 35°C ± 2°C for 24 hrs.
7. After appropriate incubation, the plates were observed, and the inhibition zone diameter was measured and recorded for determination of MIC. This procedure was carried out for each of the 3 brands of antibiotic.

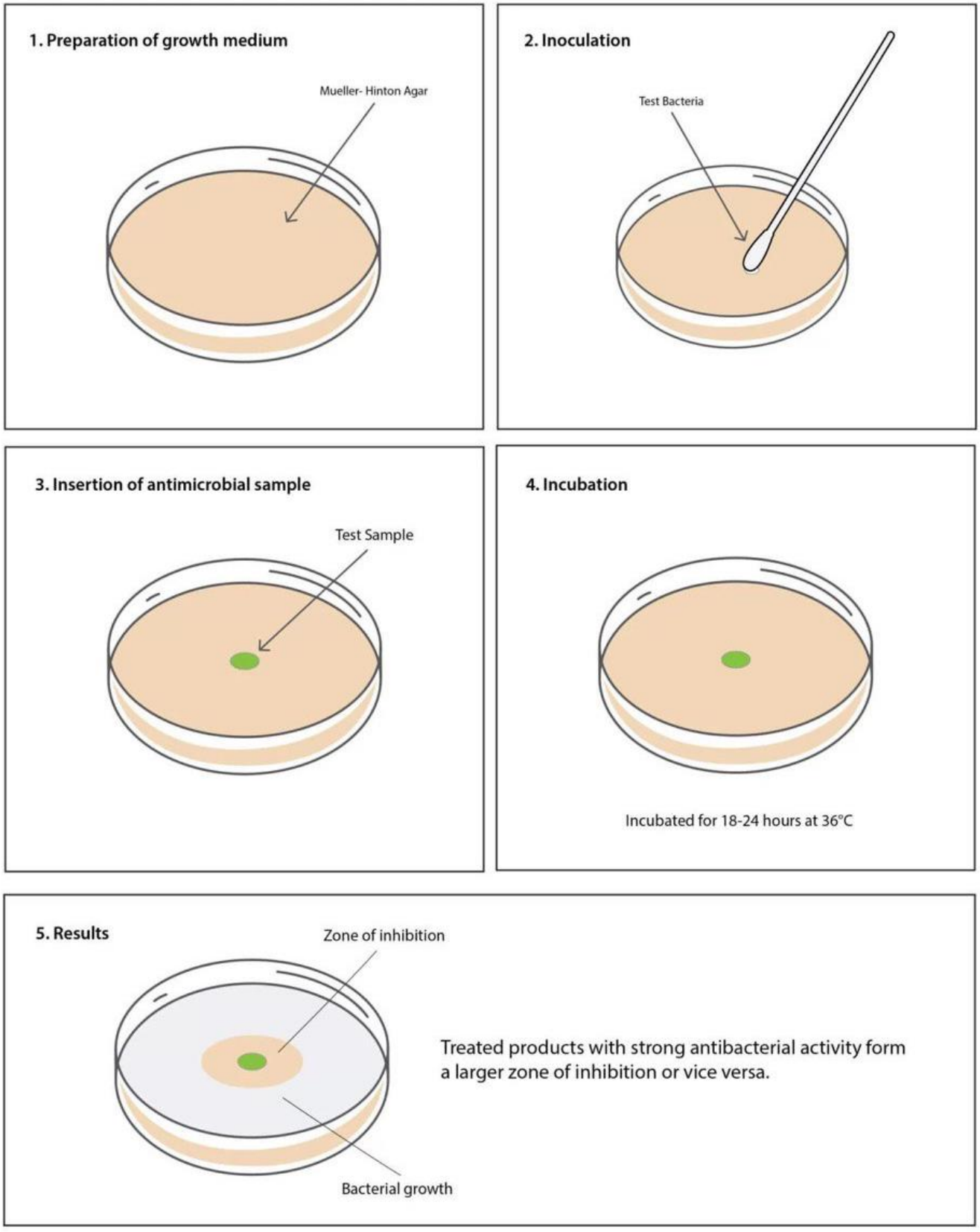

Fig. 1. Zone of Inhibition Test.

3. Results

Table 3: Results of the antibiotic under study of all 3 brands.

	Staphylococcus				***Pseudomonas***			
	Concentrations (µg/mL)							
Brand A	250	125	50	25	500	250	125	50
	Diameters (cm)							
	3.5	3.3	2.7	2.3	3.7	3.2	3	2.3

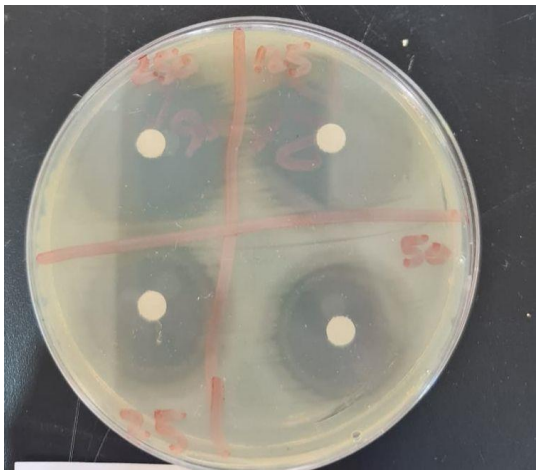

Fig. 2. Zones of inhibition with *staphylococcus*

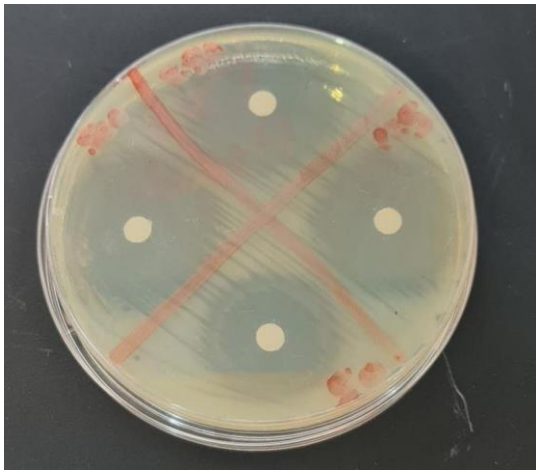

Fig. 3. Zones of inhibition with *Pseudomonas*

	Staphylococcus				***Pseudomonas***			
	Concentrations (µg/mL)							
Brand B	500	250	125	50	500	250	125	50
	Diameters (cm)							
	3.8	3.7	3.3	3.3	3.6	3.3	3.1	2.3

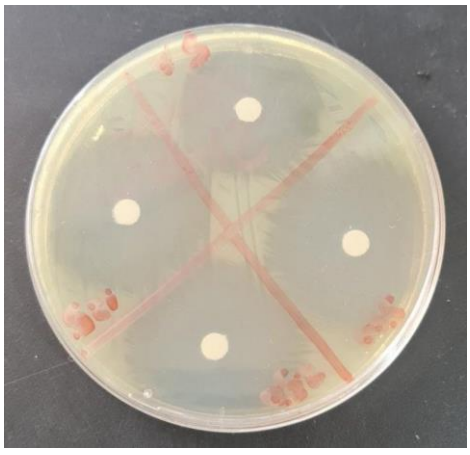

Fig. 4. Zones of inhibition with *Staphylococcus*

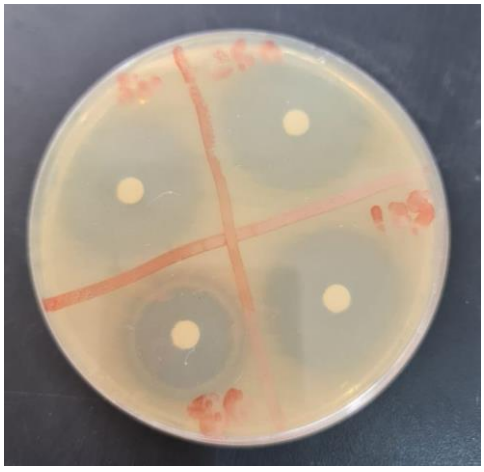

Fig. 5. Zones of inhibition with *Pseudomonas*

Brand C	***Staphylococcus***				***Pseudomonas***			
	Concentrations (µg/mL)							
	500	250	125	50	500	250	125	50
	Diameters (cm)							
	4	3.6	3.5	3	3.2	3.2	3	2.2

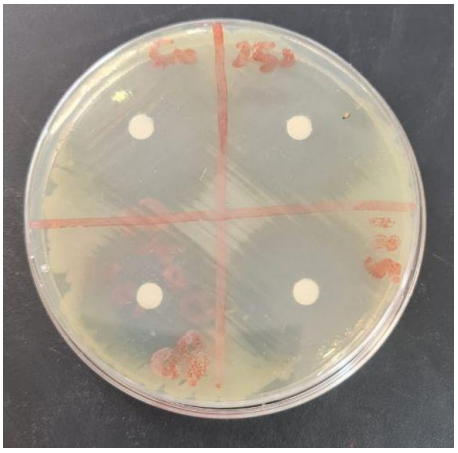

Fig. 6. Zones of inhibition with *staphylococcus*

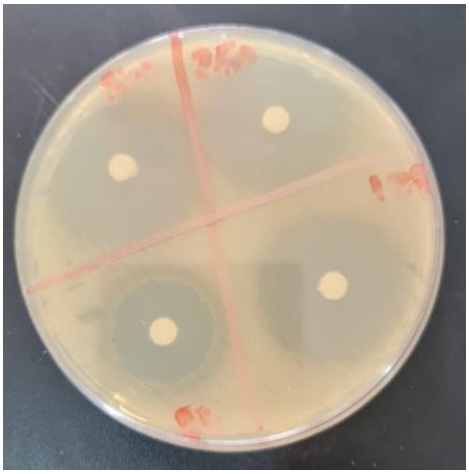

Fig. 7. Zones of inhibition with *Pseudomonas*

4. Discussion

Antibiotics must be safe and effective and of acceptable quality to use in both human and veterinary medicines (FDA, 2017; Bihari, 2022; Eshetie et al., 2020; FDA, 2022). Sub-standard antimicrobial agents are a serious public health problem with disappointing consequences on patients and advancement of antimicrobial resistance.

Ciprofloxacin resistance is becoming one of the serious health concerns. Many studies worldwide reported a clear upsurge in ciprofloxacin resistance. This could be partly attributed to high prevalence of substandard drugs and/or irrational use. Administering fake and/or low-quality drugs could result in therapeutic failure, toxicity or allergic reactions due to their content, drug resistance, prolonged illness, high cost of treatment and even mortality which all can directly or indirectly influence public health (Gelata and Tufa, 2023).

Biological essay of antibiotics is especially important in the evaluation of antibacterial products. The disk diffusion test is quite common for antimicrobial susceptibility testing based on size of inhibition zone. In our study, a total of 3 different brands of ciprofloxacin tablets were assayed against 2 strains of bacteria which are *S. aureus* and *P. aeruginosa*. The efficacy of different brands of ciprofloxacin tested in this study was directly related to the percent of inhibition to the test organism i.e., the more the drug inhibits the test bacteria, the higher the efficacy of the drug (Paul, 2009). All brands were relatively effective against the test organisms producing clear ZI with all

concentrations used which indicates quite good susceptibility of these organisms and no resistant microorganisms were found. The mean of ZI diameters of all brands was 4-2.2 cm which means there is barely slight variation in efficacy of ciprofloxacin. As it is evident from the results in table 3, the use of increasingly higher concentrations of antibiotic used resulted in larger zones of growth inhibition. Different factors might be responsible for the variation in efficacy. Basically, it could be due to the difference in the quality of active ingredients found in these different brands of ciprofloxacin. Also, it could be due to the difference in their manufacturing process although the active ingredient of the drugs is believed to be the major factor. Furthermore, the diffusability of the active ingredients of brands of ciprofloxacin impregnated on disks may vary as the dispersion of the particles of each brand are affected by the diluents in the tablet. This may also have effects on efficacy evaluation findings. As a general belief, higher antibacterial activity is linked to higher prices of drug. However, in our study, no correlation between price and antibacterial effects of ciprofloxacin brands was found. The most expensive brand, brand B, had nearly equal activity against both test bacteria compared to that of brand C, the brand with lowest price among the 3 brands. On the contrary, brand C at a concentration of 500 µg/mL produced a larger ZI of 4 cm compared to a ZI of 3.8 cm by brand B. Moreover, brand A, the brand with intermediate price exhibited good efficacy and inhibited the growth of *S. aureus* at a concentration as low as 25 µg/mL. This is very fortunate for low-income earners who may not afford expensive drugs since these alternative brands with lower prices and equal efficacy are commercially available. The susceptibility of tested bacteria toward ciprofloxacin is a good predictor that ciprofloxacin is still the drug of choice for treatment of *S. aureus* and *P. aeruginosa* associated infections.

5. Conclusion

- All three brands of ciprofloxacin tablet exhibited potent antibacterial effect as indicated by production of zones of growth inhibition against both test bacteria. The proximity in diameters of ZI produced is a good marker that the three brands are efficacious.
- No resistant bacteria were encountered during our tests.
- No correlation between price of drugs and antibacterial effect was found which could serve as an indicator that no difference in quality of antibiotics is created by the great difference in the price.
- Cheaper, yet efficacious brands of ciprofloxacin are commercially available in the pharmaceutical market.
- As a recommendation, brand A use is considered rational as it combines good intermediate price with potent activity.

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