MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

SCHOOL OF PHARMACY

TITLE: ASSESMENT OF ANTIBACTERIAL ACTIVITIES OF DIFFERENT BRANDS OF CIPROFLOXACIN TABLETS.

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1. AKNOWLEDGEMENTS

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2. ABSTRACT

The present study was carried out to evaluate and compare the antibacterial susceptibility of bacteria to different brands of Ciprofloxacin tablets.

The following three bacterial strains were used: *Staphyloccocus aureus* [ATCC 25923], *Escherichia coli* [ATCC 25922] and *Pseudomonas aeruginosa* [ATCC 27853]. Standard commercial discs of definite potency are used as reference standard (Ciprofloxacin 1µg). The test products 500 mg tablets of the following brands: Ciprobay, Bactiflox, Ciprodenk, Ladinin, Cipron, Zindolin, Bactiflox, ladinin and Ciproflox.

There was a slight increase in the ZI of different brand at different time interval. This means that the solubility of the drugs invitro is increasing with time which in turns increases the zone of inhibition.

Ciprobay produced more consistent ZI as compared to other brands, while no significant ($p<0.05$) differences were observed among other brands in respect of ZI.

All tested brands of Ciprofloxacin yielded ZI within the acceptable ranges (as per USP). However, there were significant differences among them.

3. INTRODUCTION.

Antimicrobial susceptibility tests measure the ability of an antibiotic or other antimicrobial agents under suitable conditions to inhibit bacterial growth *in vitro* (Inhibitory effect on micro-organism) (Bauer *et al.* 1966).

For evaluating the safety and effectiveness of antibiotic products, several types of antimicrobial susceptibility (sensitivity) tests are recommended. The choice of the method depends on local needs and resources, However, the disk diffusion test has a long and successful track record; it is still the most common test used for antimicrobial susceptibility testing. In this method, the paper discs impregnated with a defined quantity of antimicrobial agent are placed on agar medium uniformly seeded with test organism. A concentration gradient of the antibiotic forms by diffusion from the disc and growth of test organism is inhibited at a distance from the disc that is related among other factors to the susceptibility of the organism.

The modified "Kirby Bauer Method" is the recommended method by National Committee on Clinical Laboratory Services (NCCLS-USA) subcommittee on Antimicrobial Susceptibility testing (Bauer *et al.* 1966). The Bauer Kirby procedure has been standardized to correlate the zone diameter produced by the fixed amount of antimicrobial agent in the disc with an MIC for the drug–organism combination. The results may be interpreted as resistant, intermediate, moderately susceptible or susceptible. The term intermediate is important. It generally means that the result is inconclusive for that drug-organism combination. The term moderately susceptible is applied to those situations where a drug may be used for infections in a particular body site, e.g. cystitis, because it is highly concentrated in the urine.

Ciprofloxacin (Cipro®) was discovered in 1960s by Bayer. Its discovery stemmed from researchers in the 1960s looking for an alternative treatment to malaria. Cipro® was approved in 1987 by the U.S. Food and Drug Administration as a

broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. Since then it has been prescribed to over 500 million patients worldwide. Cipro® has been approved for the treatment of 14 types of infection including respiratory and urinary tract infections, skin, and other gastro-intestinal infections (SIS, 1987). Cipro® is the most widely used fluoroquinolone antibiotic in the world, which testifies to its wide range of uses. It is also the first antibiotic to be approved specifically for an indication associated with the intentional use of a lethal biological weapon (Hilliard *et al.* 1995). Cipro is available in three different forms: Tablets, Oral Suspension (strawberry-flavored liquid to be taken by mouth), and I.V. (which a doctor or nurse injects directly into the bloodstream) (Drusano *et al.* 1986).

Because of its general safety, potency and broad spectrum activity, Ciprofloxacin was initially reserved as a "last-resort" drug for use on difficult and drug-resistant infections. As with any antibiotic, however, increasing time and usage has led to an increase in Ciprofloxacin-resistant infections, mainly in the hospital setting. Also, implicated in the rise of resistant bacteria is the use of lower-cost, less potent fluoroquinolones, and the widespread addition of Ciprofloxacin and other antibiotics to the feed of farm animals, which leads to greater and more rapid weight gain, for reasons which are not clear (Brouwers, 1992).

Resistance to ciprofloxacin develops slowly by multiple step mutations. Because of using sub-standard (under dose) or inappropriately manufactured drugs spelling to poor release of active ingredients.
Moreover, some intrinsic microbial factors may also attribute to emergence of drug resistance:

1. Conversion of active drug to inert product by enzyme found in resistant MO.
2. Change in antibiotic target site leading to resistance. Acquisition via gene transfer, of a resistant form of target enzyme.
3. Reduction in cellular permeability to the antibiotic leading to exclusion

The toxicity of drugs that are metabolised by the cytochrome P450 system is enhanced by concomitant use of some quinolones (Janknegt, 1990). They may also

interact with the GABA A receptor and cause neurological symptoms; this is further augmented by certain non-steroidal anti-inflammatory drugs (Krishek and Smart, 2001).

The present study was carried out to evaluate and compare the antibacterial susceptibility of Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia-coli* and *Pseudomonas aeruginosae*) bacterial strains to different brands of ciprofloxacin 500 mg tablets available in the Dar-es-salaam market.

4. PROBLEM STATEMENT AND RATIONALE

It has been noted that there is bacterial resistance to ciprofloxacin. One of the reason for the development of resistance could be, due to the drug not reaching the therapeutic concentration in plasma.

Currently, our country faces prevalence of counterfeit products, medicines inclusive. This has led to people developing habit of preferring medicines from specific countries/sources and rejecting from others. Antibiotic (Ciprofloxacin) is just one of the products.

Even price differences seem not to bother them. In this study it is intended to determine the safety and efficacy of various brands of ciprofloxacin against standard isolates of bacteria.

This needs to be addressed to ''reality'' on this perception.

5. BROAD OBJECTIVES

To assess the *in vitro* antibacterial effect of different brands of ciprofloxacin tablets that are available in Dar es Salaam market.

5.1. Specific objectives

i. To determine the antibacterial activity of different brands of ciprofloxacin against reference strains of bacteria.

ii. To compare antibacterial activity of brands of ciprofloxacin against standard strains of microorganism with time.

iii. To carry out a comparative assessment of antibacterial activity of brands of ciprofloxacin to that of pharmacopeia specifications.

iv. To compare antibacterial activity of each brand of ciprofloxacin against price.

6)METHODOLOGY

6.1 Study area

This study was conducted at School of Pharmacy -Microbiology Laboratory (MUHAS).

6.2 Sampling procedure

- Probability (cluster sampling) technique was employed.
- Being the first study of its kind , a sample size of 15 different brands of Ciprofloxacin tablet 500mg was used based on the following assumption: Each of the brand was purchased more than twice, but assayed separately for comparisons purposes based on the manufacturer or selling premises.

6.3 Acquisition of chemical and biological materials.

- Reference strains of bacteria namely *Staphylococcus aureus* (ATCC25923) , *Pseudomonas aureginosa* (ATCC27853) and *Escherichia coli* (ATCC25922) was obtained from Microbiology/Immunology Lab-School of medicine-MUHAS.
- All samples and the test microbes were inoculated onto Mueller-Hinton agar and then the agar plates was incubated at 37°C for 24hr.
- Various brands of ciprofloxacin bought were tested for antimicrobial activity. The test ciprofloxacin tablets were randomly bought from pharmacies and medical store located within Dar es salaam city.

6.4 Preparation of antibiotics discs

Each of the purchased antibiotics was dissolved in 0.1M HCl (to mimic the stomach conditions) and embedded in Whatman no. 1 filter papers from which disks of about 5mm was cut out and employed for antimicrobial activity testing.

The sensitivity discs was prepared as per the Clinical Laboratories Standards Institute (CLSI, 2002) guidelines to contain the concentrations1 µg equivalent to the standards (commercial disc of 1µg).

In order to get 1 µg from 500mg of the antibiotic, each tablet was weighed and then crushed in a mortar.A given amount of the resultant powder was dissolved in 0.1M HCl of a given volume making a dilution of 1:20.
Then the solution was transferred into a test tube and placed in a shaker at a speed of 2hrs.

At different time intervals (0 min, 15 min, 30 min, 45 min and 24hrs) the disc was inoculated with the solution using a calibrated pipette (20 µl).

6.5 Equipment and Material used

Electric shaker
Calibrated pipette
Sterile cottonwool
Filter paper
Autoclave
Mcfarland 0.5M

6.6 Antibacterial activity assaying on the test organisms.

The antibiotic susceptibility patterns of the isolates to ciprofloxacin tablets that are available in the market was evaluated using the Kirby Bauer disc diffusion technique.

Discrete colonies of the different identified isolates was inoculated into 5ml of the broths and incubated at 35°C for 24hr.The resultant microbial suspension was adjusted to match standard turbidity (McFarland 0.5M) prior subjecting them to susceptibility tests as per CLSI (2006) recommendations.

Each of the isolates was uniformly and aseptically inoculated into a different agar plates by spread plate method using sterile cotton wool.The appropriate antibiotic discs (1µg) of the test antibiotics in duplicates was aseptically placed on the agar using sterile foreceps.
The discs was evenly distributed at 24mm distance ensuring that are at least 15mm from the edge of the Petri dish. The plate was then incubated at 37 C for 24 hr.

Interpretation of results was based on comparison of the inhibition zones (ZI) yielded by reference strains of bacteria against the control antibiotic discs and test antibiotics.

7. RESULTS AND DISCUSSION

The study was conducted to compare the antibacterial susceptibility of different brands of Ciprofloxacin namely Ciprobay, Bactiflox, Ecoflox) 500 mg tablets with the standard Ciprofloxacin disc of 1µg at different time interval (0 min, 15 min, 45 min, 90 min and 24hrs).

The results are expressed as mean of inhibition zone diameters(mm) produced by the 1 µg potency discs at different time interval is given in the **Tables 1-3** for *P. auriginosa*, *Escherichia coli* and *Staphylococcus aureus* respectively.

Mean of zone of inhibition produced by the standard disc (1µg of commercial disc of ciprofloxacin) for *P. aureginosa* was 32mm,for E. coli (37mm) and for *S. aureus* was 23mm.

Zone of inhibition (ZI) produced 0.1M HCL were 7mm for *P. aureginosa ; 8*mm for *E. coli* and 7mm for *S. aureus*.
This indicates the insignificant antibacterial effect exerted by HCl on the bacteria as compared to that of standard disc and tested brands.

The control limits for monitoring ZI with 1 µg disc content of Ciprofloxacin for the bacterial the tested bacterial strains is given below:

Organism	**Strain Code**	**Range of Inhibition Zone Diameter (mm)**
E. coli	ATCC 25922	30 to 40
P. aeruginosa	ATCC 27853	25 to 33
S. aureus	ATCC 25923	22 to 30

Antimicrobial susceptibility testing of different brands of Ciprofloxacin 500 mg tablets for Pseudomonas aureginosa(Table 1)

TIME	CIPROBAY	BACTIFLOX	LADININ	ECOFLOX	ZINDOLIN	CIPRON	CIPRO-DENK	C-FLOX	CIPRO-FLOX
0min	27.7	26.0	24.3	24.3	27.0	26.0	27.0	24.3	25.7
15min	28.7	27.3	25.3	25.0	27.3	27.7	27.7	25.7	25.7
45min	29.0	27.7	26.3	25.7	28.0	27.7	28.3	25.0	25.7
90min	29.0	28.7	27.3	27.7	28.0	28.7	28.3	26.3	26.7
24hrs	30.3	30.0	29.0	29.0	29.3	30.0	29.7	27.3	28.7
Avg	28.9	27.9	26.9	26.3	27.9	28.0	28.2	25.7	26.5
STDEV	0.8091	0.4461	0.446	0.659	0.5151	0.581	0.47	0.7	1.25

As seen from the table above that there is an increase in the zone of inhibitions(ZI) of different brands of ciprofloxacin at different time interval (0min, 15min, 45min, 90min and 24hrs).Ciprobay shows large ZI followed by bactiflox,ladinin,ciprodenk etc

Antimicrobial susceptibility testing of different brands of Ciprofloxacin 500 mg tablets for E.coli(Table 2)

TIME	CIPRO BAY	BACTIFL OX	LADINI N	ECOFL OX	ZINDOL IN	CIPRO N	CIPRO-DENK	C-FLOX	CIPRO-FLOX
0min	29.0	27.3	26.3	26.7	27.7	26.0	27.3	26.3	27.7
15min	29.3	28.0	28.0	26.0	27.0	27.7	29.0	27.0	28.7
45min	30.0	29.7	28.7	28.3	29.0	27.7	30.7	27.7	29.7
90min	32.0	30.3	29.7	30.3	29.7	29.3	32.0	28.7	29.7
24hrs	33.7	31.7	31.3	30.3	31.0	31.7	32.7	30.3	31.0
Avg	30.8	29.4	28.8	28.7	28.9	28.5	30.3	28.0	29.3
STDEV	0.6094	0.5404	1.15	1.343	0.936	0.85	0.79	1.41	0.69

Antimicrobial susceptibility testing of different brands of Ciprofloxacin 500 mg tablets for Staphylococcus aureus(Table3)

TIME	IPROBAY	ACTIFLOX	ADININ	COFLOX	INDOLIN	IPRON	IPRO-ENK	-FLOX	IPRO-LOX
0min	18.3	18.0	17.3	16.7	15.3	15.3	16.7	16.3	16.0
15min	19.0	18.3	18.0	15.3	16.3	16.0	16.7	15.7	16.0
45min	19.3	18.7	18.7	17.3	17.7	16.7	17.3	17.7	16.7
90min	20.0	20.0	18.7	18.3	19.0	18.3	18.0	18.3	19.0
24hrs	20.7	20.3	20.0	19.0	19.7	19.4	19.7	19.0	19.7
Avg	19.5	19.1	18.5	17.3	17.6	17.2	17.7	17.4	17.7
STDEV	0.4461	0.3772	0.7037	0.5404	0.6347	0.5402	0.728	0.47	0.8485

As expected, there was a slight increase in the ZI of different brand at different time interval. This means that the solubility of the drugs invitro is increasing with time which in terms increases the zone of inhibition.

Difference of the solubility between different brands with time can be due to various factors like type of binders, diluent,and lubricant used.

Apparantly, Ciprobay produced more consistent ZI as compared to other brands (Tabels 1-3) and Figures 1-3.While no significant ($p<0.05$) differences were observed among these brands in respect of ZI: Bactiflox, Ciprodenk, Ladinin,Cipron, Zindolin and Ciproflox.

Comparative analysis of the tested brands to the specified limit using the control disc (1ug) (CLS); all brands qualify since the produced ZI are within the acceptance ranges.

With respect to price ,there is a positive correlation with different brands of ciprofloxacin tablets, however, the lingering question of whether or not price dictates quality. As with most other things, there are both positive and negative points to both sides, with the decision usually falling squarely on the shoulders of the person deciding.

Cheap generic drugs, provided they're made by reputable pharmaceutical companies, have the same exact ingredients as their branded counterparts. The core ingredients, the compounds in the medication that make it work and fix problems in the body, are the same both both cheap generic drugs and the more expensive, brand-named ones. There are typically very few differences between the two. For example, the active ingredients are of a lower concentration in the generic ones than their prescription or branded counterparts. In most cases, however, the only real difference between the two would be the packaging. A number of cheap generic drugs are made by the same pharmaceutical companies that sell the trademarked medications.

Cheap generic drugs are not designed to be any less effective than their branded counterparts. There are some cases where the active ingredient is in a lower concentration, but this usually occurs if the generic version is over-the-counter, while the branded one is available only by prescription. They aren't less effective and they don't take longer to work than the branded ones.

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 widespread 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 those 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 were commonly prescribed for medical conditions that were not even bacterial to begin with, such as viral infections, or those to which no proven benefit existed.

8.CONCLUSION

All tested brands of Ciprofloxacin yielded ZI within the acceptable ranges (as per USP). However, there were significant differences among them.

The strain of *E. coli* was the most susceptible followed by *P. aeruginosa* to the tested brands of ciprofloxacin.

Generally, Ciprobay exerted the highest (largest ZI) antibacterial effect on all reference strains; this was evidenced from time 0min-24hours assays.

A positive correlation was observed between price of brand and antibacterial effect ($r= 0.564$; $p<0.05$)

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