ISSN:
Print - 2277 - 0593
Online - 2315 - 7461

Journal of Natural Science, Engineering and Technology

COMPARATIVE ANTIMICROBIAL ACTIVITY OF FIVE BRANDS OF CIPROFLOXACIN SOLD IN LAGOS STATE

[1, 2]B. A. ADENIYI, [1]*C. I. AYOLABI AND [1]E. J. UKONU

[1]Department of Microbiology, University of Lagos
[2]Pharmaceutical Microbiology, University of Ibadan, Ibadan
***Corresponding author:** cayolabi@unilag edu.ng; **Tel:** +2348037186163

ABSTRACT

The antimicrobial activity of five brands (A, B, C, D and E) of ciprofloxacin hydrochloride tablets commonly sold in Lagos Nigeria, were compared and assessed against susceptible clinical isolates (*Staphylococcus aureus, Escherichia coli* and *Salmonella enterica* serotypetyphi). Susceptibility test, minimum inhibitory concentration test and the bactericidal activity were determined. All sampled brands were within their shelf life. Most (60%) of the sampled brands were made in India while the remaining 40% were made in Nigeria. All the brands complied with the official specification in British Pharmacopeia (BP) for uniformity of weight as they show less than 5% deviation in weight. The mean antibacterial activities of the brands at 25µg/ml were found to be within the range of 38.0mm to 42.2mm zone of inhibition while the MICs range between 0.012µg/ml to 1.5µg/ml.

All the sampled brands were effective against all the test organisms to varying degree with brands A and E been more potent while brand D was the least effective. The order of MICs (decreasing order of potency) was D>B>C>A>E for *Staphylococcus aureus* and D>C>B>E>A for *Escherichia coli* while that of *Salmonella enterica* serotypeTyphiwas D>B>C>A>E. The bactericidal activity of each ciprofloxacin brand D and E are concentration-dependent; with brand E more active at all tested concentrations.

Keyword: antimicrobial activity,Ciprofloxacin, Minimum Inhibitory Concentration and sensitivity

INTRODUCTION

The introduction of generic drug product from multiple sources into the health care delivery system of many developing countries was aimed at improving the overall healthcare delivery systems in such countries. However, this has been accompanied by a variety of problems of which the most critical is the widespread distribution of fake and substandard drug products. There is growing trade in sub-standard and counterfeit drugs including antibiotics around the world (Masland and Marshall, 1990). The prevalence of such substandard drugs in the Nigerian markets has been worrisome to both regulatory agencies and all concerned. The availability of drugs, especially antibiotics in open markets have led to indiscriminate use and may contribute to high incidence of antibiotic resistance strains (Lester, 1990). Nigeria imports a large proportion of its pharmaceuticals from different countries.This has led to indiscriminate dumping of sub-standard products in the Nigerian market. Lagos drug market is one of the largest open drug markets in the country and served as the major source of drugs within the state and its neighboring environs including some West African countries.Ciprofloxacin is synthetic fluorinated

quinolone that is indicated in infections of the urinary, gastrointestinal and respiratory tracts, tissue infections, gonorrhea and septicemia caused by sensitive organisms (Solomkin, et al. 2010). It is effective in chronic Gram – negative infections such as osteomyelitis, recurrent cholangitis and acute exacerbations of pseudomonas infection as in cystic fibrosis (Bennett and Brown, 2003). It is suitable for the treatment of bacterial keratitis, lower respiratory tract infections especially of Gram – negative etiology (Lode *etal*., 1994). Ciprofloxacin is also widely used for several disease conditions and it is readily available in different brands even in the open markets. The insinuation by previous findings that some of the drugs circulating in Nigeria are of poor quality (Bernard *etal.,* 1999) prompted a call for urgent but independent laboratory investigation.

The need to select one or more products from among several generic drug products of the same active ingredients during the course of therapy is a cause of concern to healthcare practitioner.Therefore, the present study was undertaken to evaluate the drug sensitivity pattern, minimum inhibitory concentration (MIC) and bactericidal activity of ciprofloxacin brands against common clinical isolates encountered in Lagos.

MATERIALS AND METHODS

Three clinical isolates (*Staphylococcus aureus Escherichia coli* and *Salmonella enterica* serotypeTyphi)and five brands of ciprofloxacin hydrochloride tablets (A–Ciprogem; B - Cenox ;C- Prox ; D - Vitapro and E – Ciprotab) purchased from retail outlets in Akoka area of Lagos State were used. All media were autoclaved at 121^{o}C for 15 minutes after preparation according to manufacturer's specification and allowed to cool before pouring into Petri dishes. All inoculations, plate pouring, serial dilution and so on were done under the biosafety cabinet hood (BSL 2). Inoculating loop was flamed red hot over gas Bunsen burner before and after use.

Morphological and Biochemical Characteristics

Catalase test: - A loopful of the bacterial isolates from the stock culture was taken and emulsified with the aid of a wireloop on a sterile glass slide. 1 to 2 drops of 3% H_2O_2 was added. Effervescence indicated positive result (i.e. catalase +ve) and negative if otherwise (Fawole and Oso, 2004).

Coagulase test: A 0.5ml of citrated rabbit was added into a test tube, enough colony paste was inoculated to make a cloudy suspension and the tubes were incubated at 35^{o}C for 1-4hours in water bath. Positive result was indicated by the presence of clumping or agglutination (Fawole and Oso, 2004).

Starch hydrolysis: Starch agar plates were inoculated with different bacterial isolates and incubated at 35^{o}C for 2-3 days. At the end of the incubation period, each plate was flooded with Gram's iodine and a blue colouration indicates positive result.

Motility test: This test was carried out using Edwards and Wigs' motility medium. The semisolid medium was inoculated with the different bacteria isolates by stabbing with a sterile inoculating needle at the centre of the medium column to over half the depth. The motile organisms grew and spread out from the line of puncture while the non-motile organism grew only along the line of puncture (Cowan *etal*., 1990).

Nitrate reduction test: The test organisms

were inoculated separately into tubes containing nitrate peptone water and Durham tubes and incubated at 35°C for 2days. Test for nitrate reduction was determined by addition of 1ml each of reagents 1 and 2 of the modified Greiss-illosvay's reagent. The presence of nitrate was indicated by the development of a pink, purple or maroon colour within a few minutes. Presence of gas in the Durham tubes also suggested production of gaseous nitrogen and consequently a positive result (Cowan *etal.*, 1990).

Sugar fermentation test: Peptone water sugar used were prepared as follows; 1.5g of peptone water into 100ml of distilled water with 5mls of bromothymol sterilized at 121°C for 15minutes (separate broth for each sugar). Also 1g of sugar into 10mls of distilled water and sugar solution was sterilized by steaming for 30minutes. Aseptically, the sterile sugar solution and the sterile peptone water containing the indicator were added together and mixed well. The 5mls of the broths was aseptically dispensed into sterile test tubes containing an inverted Durham tube and corked tightly.

After cooling, each broth test tube containing Durham tube was inoculated with the test organism and a control experiment (an un-inoculated test tube) was made at this same time. After which all the test tubes were incubated aerobically at 37°C for 24hours. Acid production changed the colour of indicator from blue to yellow and gas production was shown by the space on top of the medium in the inverted Durham tube. The broth remains blue for no acid production and the Durham tube that was filled up indicated no gas production. Tubes with yellow colour were recorded as positive to sugar fermentation test while tubes with no colour change were recorded as negative to sugar fermentation test.

Uniformity of weight: Using an analytical weighing balance, five (5) tablets selected from each brand was weighed and the average weight calculated. In accordance with British Pharmacopeia (BP) and United State Pharmacopeia (USP), not more than two of the individual weights should deviate from the average weight by more than the percentage given in the pharmacopoeia. The BP and USP limits for tablet weight variation for tablets over 250 mg should not be more than 5%.

$$\text{Average weight} = \frac{\sum(x_1 + x_2 + \ldots x_n)}{N}$$

Where, x = weight of each tablet; N =total number of tablets.

$$\% \text{ deviation} = \frac{\text{Weight of tablet} - \text{Average weight of tablets}}{\text{Weight of tablet}} \times 100$$

Determination of Antimicrobial activity: The agar cup diffusion technique was used. A 0.2ml of logarithmic phase broth culture of each bacterium [optical density equivalent to 10^7-10^8 cfu/ml (i.e. 0.5 Mac Farland standard)] was used to seed sterile molten Mueller Hinton agar (MHA) medium maintained at 45°C. The seeded plates were allowed to dry in the incubator at 37°C for 20minutes. A standard cork borer (8mm in diameter) was used to cut uniform wells on the agar, into which was added 200µl of

25mg/ml, 12.5mg/ml and 6.25mg/ml solution of each brand. A pre-incubation diffusion of the drugs into the seeded medium was allowed for 1hour. Plates were incubated at 37^{o}C for 24-48 hours after which the diameters of the zones of inhibition were measured with meter rule (Adeniyi et al., 1996).

Determination of Minimum Inhibitory Concentration (MIC): One tablet equivalent to 500mg of each brand A, B, C, D, and E was dissolved in 10mls of sterile distilled water in a MacCartney bottle to obtain a stock concentration of 50mg/ml. Using a sterile micropipette, 5000µl (5ml) from each stock was aseptically transferred to 5ml of sterile distilled water in a sterile bijou bottle to half the drug concentration of each stock to 25mg/ml, which was the first working concentration. Subsequent double fold dilutions were made to give 12.5mg/ml, 6.25mg/ml, 3.125mg/ml, 1.563mg/ml, 0.7813mg/ml, 0.391mg/ml, 0.195mg/ml until 0.000012mg/ml (0.012µg/ml). The agar cup diffusion method (kavanaah, 1972 ; Adeniyi *etal.,* 1996) was used. A 1.5 x10^{8}cfu (colony forming unit) of overnight broth (nutrient, Lab M) culture of each bacterium was inoculated into a molten Mueller Hinton agar (Lab M) medium maintained at 45^{o}C. The plates were then allowed to set and wells were made on them using a sterile standard 8mm internal diameter cork borer. Each well was then filled with 200µl of every brand concentrations. The plates were allowed to stand on the bench for about 1hour for diffusion of the drug concentrations. The plates were all incubated at 37^{o}C for 24hours and read by taking note of zone of inhibition measured in millimeter (mm). Triplicate plates were made for each organism. Control experiment was made for sterility check of the agar medium.

Determination of Bactericidal activity

The bactericidal activity of two brands of ciprofloxacin (brand E and D) were determined in nutrient broth using the viable counting technique. (Howard *et al.*, 1993).The organisms, *Salmonella enterica* serotypeTyphi and *Staphylococcus aureus* were exposed to increasing concentrations of brand D and E, 200µl of logarithmic phase cells was inoculated into 5ml quantities of fresh warm (37^{o}C) nutrient broth containing the antibiotics (4.8ml). The initial viable counts were approximately 1.5 x 10^{8} cfu/ml for *S. aureus* and 1.8 x 10^{8} cfu/ml for *Salmonella enterica* serotypeTyphi. An appropriate quantity of suspension (0.2ml) was withdrawn and transferred aseptically into a McCartney bottle containing 5ml double strength Mueller Hinton broth and 4.8ml of the antibiotics and thereafter 0.1ml of the whole mixture was immediately (at time- 0 mins) transferred into a test tube (containing 9.9ml of distilled water) thereby obtaining 10^{-2} dilution. Further serial dilutions were made to obtain 10^{-4} and 10^{-6} after which 0.2ml of each dilution was plated out using pour plate method at different time intervals of 0, 30, 60, 120, 180 and 240 minutes respectively. Plates were then incubated at 37^{o}C for 24-48hours before counting. The results are expressed as percentage (%) viability against time.

RESULTS

The morphological and biochemical characteristic of a Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichiacoli* and *Salmonellaenterica* serotype Typhi) clinical isolates used are presented in Table 1. The result of weight variation determination of the five sampled brands is depicted in Table 2. Negligible variations were observed in the sampled brands with brand D giving the highest

variation of 3.0% which is higher than that of other brands but, it still fell within the required standard as stipulated in British pharmacopeia (2004). All the ciprofloxacin brands were very effective against all the testedorganisms to varying degree at 25µg/ml and the mean antibacterial activities of all the brands were found to be between 38.0mm and 44.0mm (Table 3).The Minimum Inhibitory Concentration (MIC) at which the five sampled brands of ciprofloxacin inhibited all the test organisms are presented in Table 4. Brand E was the most active having the least MICs against the test organisms except for *S. aureus* where brand A happened to have the least MIC. Based on the National Committee for Clinical Laboratory Standards (NCCLS, 1994) value for MIC of 1µg/ml or less against the three test organisms, brand A and E showed better result than the rest

Table 1: Morphological and Biochemical characteristics of the bacteria isolates used

Test	Staphylococcus aureus	Escherichia coli	Salmonella enterica serotype Typhi
Gram reaction	+	-	-
Cellular morphology	Cocci	Rods	Rods
Growth on MacConkey agar	N.D	Pink	Pale
Growth on mannitol	Yellow	N.D	N.D
Catalase test	+	+	+
Coagulase	+	N.D	N.D
Oxidase	-	-	-
Motility	-	-	+
Citrate	+	+	+
Indole	-	+	-
Urease	+	-	-
Methyl red	+	-	+
Voges proskaeur	-	-	0
Growth on Kligler Iron Agar (KIA) slant	Yellow	Yellow	Yellow
Butt	Yellow	Yellow	Yellow
Hydrogen sulphide production (H2S)	+	-	+
Gas production	+	+	+
Sugar fermentation glucose	A/G	A/G	A/G
Lactose	A	A	A/G
Sucrose	A	A	A/G
Mannitol	A	A	A
Maltose	A	A	A/G

Key: + = growth, - = no growth, A = acid no gas, N.D = Not done, A/G = acid and gas

Table 2: Weight and percentage deviation of tablet from each brand

Names	Weight (g)	% deviation
A	0.783 ±0.005	0.1%
B	0.766 ±0.063	1.3%
C	0.678 ±0.045	0.9%
D	0.937 ±0.075	3.0%
E	0.810 ±0.005	0.1%

Table 3: Mean Antibacterial activity of the sampled brands of Ciprofloxacin at 25µg/ml.

Bacterial strains	Sample No	Zone of inhibition (mm)				
		Brand A	Brand B	Brand C	Brand D	Brand E
Escherichia coli	1	44.0	42.0	42.0	40.0	44.0
	2	41.5	40.0	41.0	38.0	42.5
	3	40.0	39.0	38.0	36.0	40.0
	Average	41.8	40.3	40.3	38.0	42.2
Staphylococcus aureus	1	43.0	42.0	42.0	41.0	42.6
	2	41.0	40.0	40.0	39.0	40.2
	3	38.0	39.0	38.0	38.5	39.0
	Average	40.7	40.3	40.0	39.6	40.6
Salmonella enterica serotypeTyphi	1	42.0	42.0	42.0	40.0	43.0
	2	40.0	40.0	40.0	39.0	41.5
	3	39.2	38.0	38.0	38.0	40.0
	Average	40.4	40.0	40.0	39.0	41.5

Table 4:The Minimum Inhibitory Concentration (MIC) of the five brands of ciprofloxacin against the tested organisms (µg/ml)

Names	Staphylococcus aureus	Escherichia coli	Salmonella enterica serotypeTyphi
	µg/ml	µg/ml	µg/ml
A	0.095	0.048	0.19
B	0.19	0.19	0.38
C	0.76	0.19	0.38
D	1.5	0.38	0.38
E	0.19	0.012	0.024

The percentage survival of *S. enterica* serotype Typhi and *S. aureus* are shown in two brands of ciprofloxacin(Figures 1 and 2) (log 10 graphs). From the two graphs, the control confirms the effect of the two compared brands against the tested organisms at various concentrations (1xMIC, 2xMIC and 4xMIC) with the 2xMIC and 4xMIC having a sharp drop to the zero value as shown unlike at 1xMICs. The bactericidal activity of each ciprofloxacin against the tested organisms increased with increase in concentrations at 60minutes (Figure 3).

Figures3 and 4 depicted similar information but against *S. aureus*and*Salmonella enterica serotype Typhi.* Here, the bactericidal response of the two brands differs. The brand E at both 2MIC and 4MIC inhibited the proliferation of the test organism (*S. aureus*) to a zero values at 60 minutes. The reverse was the case for brand D as shown below; even at 4MIC at 60 minutes a significant percentage of the test organism still thrives under the influence of brand D. This shows that brand E is better and more active brand compared to brand D.

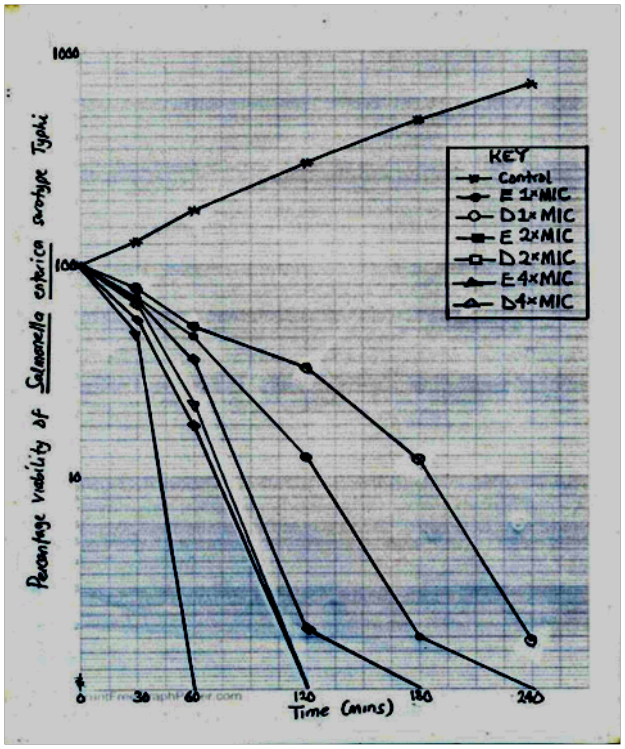

Figure 1: Kinetics of bactericidal studies of two brands of ciprofloxacin against *Salmonella enterica* serotypeTyphi

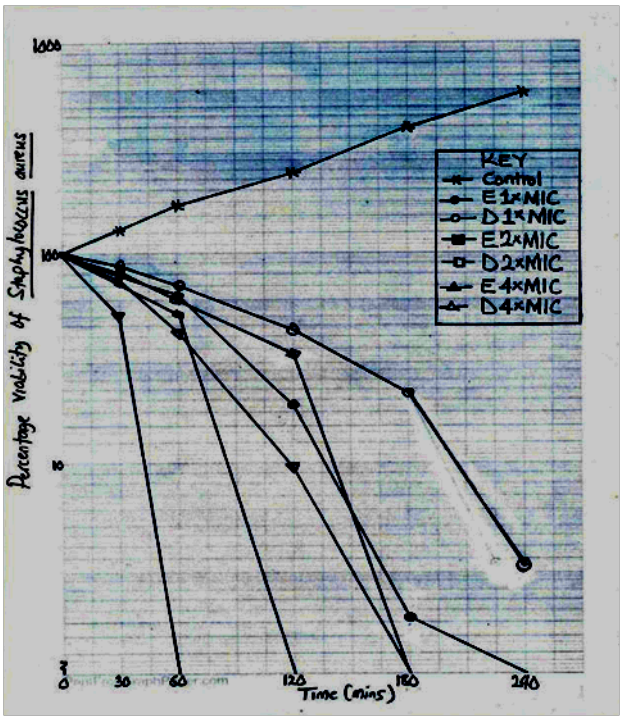

Figure 2: Kinetics of bactericidal studies of two brands of ciprofloxacin against *S. aureus*

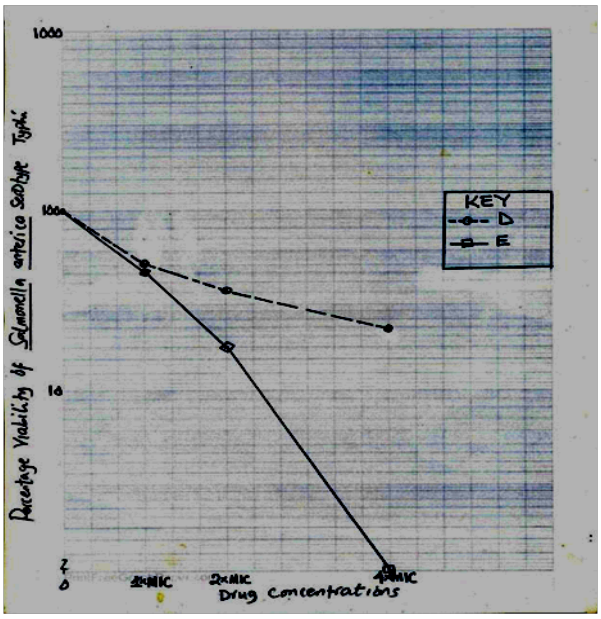

Figure 3: Bactericidal response of *Salmonella enterica* serotypeTyphi when exposed to increasing concentrations of ciprofloxacin (brand E and D) at 60mins

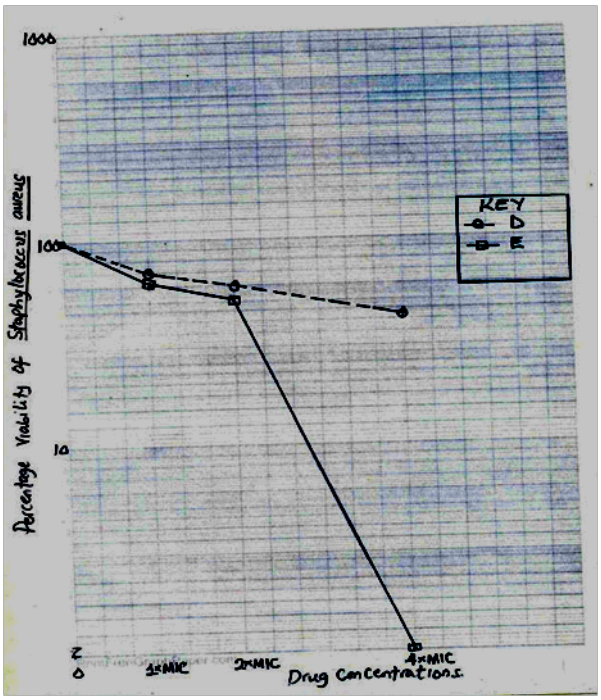

Figure 4: Bactericidal response of *S. aureus* when exposed to increasing concentrations of ciprofloxacin (brand E and D) at 60mins

DISCUSSION

Among the five different brands of oral formulation of ciprofloxacin sampled, brands E and A were the most commonly available during the period of the study. Owing to their higher demand rate in the market as this was confirmed by three out of the four visited registered pharmaceutical stores from whom those antibiotics were purchased within Akoka area in Lagos State. Most (60%) of the sampled brands were manufactured in India; these include E, C and B while 40% of the brands were manufactured in Nigeria namely A and D. Also, brand E and A were found to be more expensive out of all the sampled brands while each of the other three brands were halve the price of E and A. They were all available as 500mg per tablet caplet and were within their shelf live period at the time of the investigation. All the samples had batch number, NAFDAC registered number. The importance of NAFDAC certification of a particular antimicrobial agent cannot be over emphasized as researched work (Mukhtar *etal*., 2009) carried out on six brands of ciprofloxacin against four clinical isolates showed that five (certified) of the brands were of good and standard quality while one of them which was not certified by NAFDAC (without NAFDAC registration number) proved to be of a very low quality.

The uniformity of weight assay for drug products with the strength 500 mg (film coated tablets), a ±5% of the average pass the test for uniformity of weight. The results, thus, indicate that all the sampled products possess acceptable uniformity of weight within the pharmacopoeia limit. Though,

brand D has the highest detected variation in weight (3%) compared to its counterparts nevertheless, it still fell within the range limit.

In the mean antibacterial activity or susceptibility testing of the formulations against the test organisms,the drugs appeared with appreciable effects, but the drugs were of different manufacturers and so there are possibilities of difference in properties but may not be tangible enough to deviate from the required limit. Brand D shows the least diameter zone of inhibition of 38.0 mm on *Escherichia coli* at 25µg/ml indicating a good activity as the others. The brands displayed very similar potencies at higher concentrations but towards the least concentrations distinguished themselves. In terms of the minimum inhibitory concentration, brand A and E presented the least MIC against *Staphylococcus aureus* and *E. coli* with 0.095µg/ml, 0.048µg/ml and 0.19µg/ml, 0.012µg/ml for the respective brands against the test organisms and this fell within the required range specified in NCCLS; *in vitro* MIC value of ≤1µg/ml range, (NCCLS, 1994). The activity of all the sampled brands against *Salmonella enterica* serotypetyphi (etiological agent of typhoid fever) in terms of the MICs is somewhat appreciable.

Comparatively, brands, E and D showed the most and "least" effective bactericidal action respectively. As the concentration dependent antibiotics they are, both of them were very active against the tested organisms (*S. aureus* and *Salmonella enterica serotype typhi*) at higher MICs during the stipulated time interval of the experiment compared to lower MIC (1xMIC). The growth of the test organisms reduced gradually and drastically as the concentration of the sampled brands increases from 1xMIC, 2xMIC to 4xMIC. From the results obtained, brand E kills faster than its D counterpart. There was an observable difference in the viable organisms at 1xMIC, 2xMIC and 4xMIC of brand E and D. The kinetic responses of both *Salmonella enterica* serotypetyphi (G-ve) and *S. aureus* (G+ve)against brand D and E at 60 minutes at the various MICs shows that Brand E has a lesser number of test organisms thriving under its influence compared to brand D. This however makes brand E a better and more active compared to brand D. The results obtained showed that ciprofloxacins are more effective against *Salmonella enterica* serotypetyphithan *S. aureus* and therefore conforms to the findings by Bennet and Brown (2003) that ciprofloxacins are very effective but particularly against Gram-negative than Gram-positives. The reasons for the results above maybe due to the nature (structure) of Gram-negative organisms (e.g. *Salmonella enterica* serotypetyphi) cell wall which is very complex but made up of a thin peptidoglycan layer (2-7nm) compared to that of Gram-positive organisms which is very thick (20-80nm) (Willey *et al.,* 2008). Research has shown that ciprofloxacin is the 5th most prescribed antibiotics and is commonly used for the treatment of urinary and intestinal infections and was once considered a powerful antibiotic of last resort (Brunton *et al*., 2005).

In conclusion, the insinuation that some of the drugs circulating in Nigeria are of poor quality prompted a call for urgent but independent laboratory investigation of some of the widely consumed antibiotics in Lagos state.The *in vitro* analysis revealed that there is no significant difference in the results for different brands and from the result obtained, the antibacterial activities of all the sampled brands are within the acceptable limit making them drugs of choice in this

order (E>A>C>B>D). Therefore the sampled brands of ciprofloxacin oral formulations that were tested showed acceptable quality against the indicated susceptible organisms.

REFERENCES

Adeniyi, B. A., Odelola, H. A., Oso, B. A. 1996. .Antimicrobial potential of *Diospyros mespiliformis.African Journal of Medicine and Medical Sciences* **25**(2): 179- 184.

Adeniyi, B. A., Fong, H. H. S., Pezzuto, J. M., Luyengi, L., Odelola, H. A. 2000. Antibacterialactivity of diospyrin, isodiospyrin, and bisisodiospyrinfrom *Diospyros piscatoria* (Gurke) [Ebenaceae]. *Phytotherapy Research***14**:112-117.

Adeniyi, B. A., Odufowoke, R.O., Olaleye, S.B. 2006. Antibacterial and Gastro-protectiveproperties of *Eucalyptus torelliana* (Myrtaceae) crudeextracts. *Int. Journal of Pharmacol.* **2**(3):362-365

Bennett, P. N., Brown, M. J. 2003. Clinical Pharmacology 9th edition. Churchill Livingstone Edinburgh, London, Pp 232-233.

Bernard, P. P., Patrice, T., Jacques, P. 1999. Assess to Essential Drugs in Poor Countries. A Loss battle, *The Journal of the American Medical Association***281**: 361-67.

British Pharmacopoeia (B.P), 2004. Standard Method for analysis of antimicrobial. Her Majesty's Stationery Office, London, Vol. III.

Brunton, Laurence L.; Lazo, John S.; Parker, Keith, eds. 2005. Goodman & Gilman's The Pharmacological Basis of Therapeutics (11th ed.). New York: McGraw-Hill. ISBN 0-07-142280-3

De Sarro, A., De Sarro, G 2001. Adverse reactions to fluoroquinolones. An overview on mechanistic aspects. *Curr. Med. Chem.***8**(4): 371–**3**84.

Fawole, M. O., Osho, B. A. 2004. *Laboratory Mannual of Microbiology*. Spectrum books Limited, Ibadan. Pp. 22-28.

Guada, B. G., Seetharamppa, J. 2003. Extractive spectrophotometric determination of fluoroquinolones and antiallergic drugs in pure and pharmaceutical formulations. *Ind. Sci;***19**(**3**):161 –164

Hashimoto, H. 2000. Why Antimicrobial Agents Become Ineffective: Disease-causing Bacteria Are Evolving. Chuokoron- Shinsha, Inc. pp 61-65.

Howard, P. M., Pinney, R. J., Smith J. T 1993. Contributions of post antibiotic lag and repair-recovery to the post-antibiotic effects of ciprofloxacin on Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus and Streptococcus pyogenes. Chemotherapy 39 22-31.

Ivanov, D. V., Budanov, S. V 2006. Ciprofloxacin and antibacterial therapy of respiratory tract infections. *Antibiot. Khimioter.* **51** (5): 29–37.

Kavanagh, F., 1972. Analytical Microbiology. Vol. II, Academic Press, New York, USA., ISBN-13: 9780124035027, pp: 11.

Leibovitz, E., Dror, Y. 2006. The use of fluoroquinolones in children. *Curr Opin Pediatr* **18** (1): 64–70.

Lester, S. C., Del Pilar Pla, M., Wang, F., **Pe-**

rez Schael, I., Jiang, H., O'Brien, T. F. 1990. The carriage of Escherichia coli resistant to antimicrobial agents by healthy children in Boston, in Caracas, Venezuela, and in Qin Pu, China. *N Engl J Med* 323, 285–289.

Lode, H.S., Hokffkn, G., Borner, K. 1994. Use of Fluoroquinolones in Lower respiratory tract infections. *Int. Journal of Antimicrobial Agent**4**suppl.* 2: 547-552.

McConnell, J. M. 2008. Benzodiazepine tolerance, dependency, and withdrawal syndromes and interactions with fluoroquinolone antimicrobials. *Br J Gen Pract.* **58**(550): 365–366.

Mackie T. J., MacCartney J. E. 1989. Practical Medicinal Microbiology 13th Editorial Churchill Livingstone, New York Pp. 161-180.

Madigan, M. T., Martinko, J. M. 2006. Brock biology of microorganisms. (11th ed). Pearson.

Masland T., Marshall R. 1990. The pill pirates. Newsweek, 18–23pp.

Miles, A. A., Misra, S. S 1938. The estimation of the bactericidal power of the blood. *J. Hygiene* **38**: 732-49.

Mukhtar, M. D.,Chedi, B. Z. Aliyu, M. Umar, M. S., Abdullahi, A. A. 2009. In-Vitro Assessment of Some Oral Ciprofloxacin Brands Traded in Kano – Nigeria *International Journal of Pharmaceutical Sciences***2** (1): 13-17

National Committee for Clinical Laboratory Standards (NCCLS)(1994). Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Proposed Standard. NCCLS document M31-P (ISBN 1- 56238-258-6). NCCLS, 771 East Lancaster Avenue,Villanova, PA 19085, USA.

Owens, R. C., and Ambrose, P. G. (2005). Antimicrobial safety: focus on fluoroquinolones. *Clinical Infectious Diseases*. **41** Suppl 2: S144–57.

Singh, N. and Yu V. L. (2000). Emerging issue in Antibiotic Resistance in Blood borne infections. *American Journal Respir Crit Care Made*. **161**:1610-16.

Solomkin, J. S., Mazuski, J. E, Bradley, J. S., Rodvold, K. A., Goldstein E. J., Baron, E. J., O'Neil, P. J, Chow, A. W., Dellinger, E. P., Eachampati, S. R., Gorbach, S., Hilfilker M., May, A. K., Nathens, A. B., Sawyer, R. G., Bartlett, J. G. 2010. Diagnosis and management of complicated intra-abdominal infection in adults and children: guidelines by the Surgical Infection Society and the Infectious Diseases Society of America. *Clin. Infect. Dis.***50** (2): 133–164.

Willey, J. M., Sherwood, L. M. Woolverton, C. J. 2008. Prescott, Harvey and Klein's Microbiology (7th ed).Mc Graw-Hill companies New York. Pp 40-75.

WHO 1987. Standard Methods for testing drug quality efficacy and potency. Manual for Laboratory Investigations of Acute enteric infections, HO/CDS.

(Manuscript received: 23rd February, 2015; accepted: 18th February, 2016).