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Comparative Antibacterial Activity of Five Brands of Ciprofloxacin Injectables in Nigeria

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Ciprofloxacin, a widely used antimicrobial agent, is available in Nigeria in various tablet and injectable dosage forms. In this era of fake and substandard drugs, it is important to be sure of the quality and antimicrobial potency of ciprofloxacin injectables imported and sold in Nigeria.

Objectives: This study is aimed at evaluating and comparing the antibacterial efficacy of five different brands of ciprofloxacin infusion available in Nigeria against clinical bacterial isolates.

Method: Ultraviolet-visible (UV-VIS) spectroscopy was used to determine the percentage content of active pharmaceutical ingredient in each brand, while antibacterial activities were compared against five bacterial strains including: *Escherichia coli* (E), *Klebsiella pneumoniae* (K), *Pseudomonas aeruginosa* (Ps), *Proteus mirabilis* (Pr), *Staphylococcus aureus* (St) and *Salmonella typhi* (Sa). Minimum inhibitory concentrations (MICs) and the minimum bactericidal concentration (MBCs) were determined by broth dilution method. Statistical analysis of the results obtained was done by ANOVA.

Result: The percentage content of all the five brands of ciprofloxacin injection was not less than the specification (95-105%) of the British Pharmacopoeia, BP (2009). The susceptibility test showed that 16 out of 30 (53.3%) isolates were multidrug resistant. Ciprofloxacin was active on most of the clinical isolates which justify its wide use in treating infections in Nigeria; however, resistance to ciprofloxacin is increasing.

Conclusion: Comparing the different brands, there was slight variation but no significant difference ($p > 0.05$) in their antibacterial activity ($p = 0.96$, 0.999 for sensitivity at 10µg/ml and MIC respectively).

Keywords: Ciprofloxacin brands, Fake drugs, Physicochemical analysis, Antibacterial, Antimicrobial resistance

INTRODUCTION

Ciprofloxacin hydrochloride is a second-generation fluoroquinolone antimicrobial agent, which chemically is 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid, chemical formula $C_{17}H_{18}FN_3O_3$ and a molecular weight of 331.346g/mol (Fig. 1) (Prabodh *et al.,* 2010). Like other fluoroquinolones, ciprofloxacin is active against a wide range of Gram-positive and Gram-negative organisms and it is used to treat various infections (Dana *et al.,* 2000). Bayer A. G., given patent, was the first manufacturer of ciprofloxacin branded Cipro® in 1983; but since the expiration of the patent in 2003, over 300 brands of ciprofloxacin, that are cheaper have been marketed worldwide.

Ciprofloxacin antibacterial activity is brought about by it targeting the DNA gyrase and topoisomerase IV for mainly Gram-negative and Gram-positive bacteria respectively. The inhibition of gyrase-mediated DNA super coiling results in DNA destruction (Zhao *et al.*,1997; Boothe, 2001). The drug is distributed widely throughout the body; tissue concentrations often exceed serum concentrations, especially in kidneys, gallbladder, liver, lungs and prostatic tissues. It is metabolized in the liver and excreted via the urine predominantly and through the faeces (Goodman and Gilman, 2008). All these therapeutic properties of efficacy, broad spectrum activity, tolerability, dosage forms, availability and affordability make ciprofloxacin one of the most widely used drug worldwide (Dana *et al*., 2000; Prabodh *et al*., 2010).

There is a large market for drugs in Nigeria with a population of about 200 million people. However, due to low capacity utilization of pharmaceutical industries in Nigeria and inability to produce enough medicine to meet the health needs of the populace, about 70% of the drugs are imported (Okoli, 2000). This has made the country to become more or less a dumping ground for various drugs, most of which invariably are substandard and less effective, no thanks to the prevailing poor economic situations of majority of the populace (Chika *et al.,* 2016). The availability and circulation of substandard medicines in the developing world is a serious clinical and community health concern (Buowari, 2013) and according to an estimate by WHO, about 10% of drugs circulating worldwide and 25% or more in less developed countries are fake (Amadi *et al.,* 2014). In the developing countries, an estimated $30.5 billion is spent on substandard and falsified drugs, accounting for 10.5% of medicines samples in the supply chain in these countries (WHO, 2020). Nigerian health officials estimated that up to 70% of drugs in circulation in the country till 1990s were either fake or adulterated (Wertheimer and Wang, 2012). However, the menace of fake drugs has significantly diminished in Nigeria; from 41% in 2002 through 16.7% in 2005 to 6.4% in 2012, and 4.3% in 2017 (Amadi *et al.,* 2014, NAFDAC, 2019); thanks to the concerted efforts of NAFDAC. However, the war against fake drugs in Nigeria must be sustained to forestall any resurgence, and achieve 99% eradication.

Substandard drugs contribute a lot to the development of antimicrobial resistance, and in developing countries, antimalarial (19.6%) and antimicrobial (16.9%) drugs were found to be the mostly counterfeited medicines between 2013-2017 (WHO, 2017). The problems of antimicrobial resistance are global and affect almost all agents including ciprofloxacin and other fluoroquinolones. Gram-positive and Gram-negative bacteria have been reported to be resistant to quinolones (Hooper and Jacoby, 2015; Correia *et al.*, 2017Asna *et al.,* 2003; Cooke *et al.*, 2006; Ochiai, 2008). Although newer generation fluoroquinolones such as gatifloxacin are currently active against nalidixic acid resistant strains (Pandit *et al.,* 2007; Dolecek, 2008), but with time. resistance to newer agents may soon appear and become widespread if indiscriminate and inappropriate use of fluoroquinolones (and other antimicrobials) continue (Turner *et al.,* 2006). The accumulation of different bacterial mutations has been associated with the development of very high minimum inhibitory concentrations to ciprofloxacin in isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterobacteriaceae* species (Acar and Goldstein, 1997). Resistance to ciprofloxacin was reported to have increased slowly from 1.2% in 1998 to 2.5% in 2001 (Karlowsky, 2002), and from 1.8% in 1997 to 15.9% in 2007 (Blaettler, 2009) and as reviewed by Fasugba *et al.*, (2015). The menace of antimicrobial resistance associated with indiscriminate use of antimicrobials and the increase in the circulation of substandard drugs thus call for concerted, proactive and global pharmacovigilance. There is a need for constant and regular evaluation of drugs especially antimicrobial agents.

Some research works on the quality of ciprofloxacin tablet brands have been reported; Adegbolagun *et al.*, (2007) on the physico-chemical quality and Igboasoiyi *et al*., (2018) on the physical and pharmaceutical properties.

This study was aimed at performing comparative physico-chemical quality and antibacterial efficacy of five brands of ciprofloxacin infusion against clinical isolates of bacteria that cause infections for which ciprofloxacin is commonly indicated.

METHODOLOGY

Materials

Drug samples

Five brands of readily accessible generic brands of ciprofloxacin injections manufactured in India and China were purchased in May 2016 from various registered pharmacies in Ibadan, Oyo state, Nigeria. All the drug samples were within their expiration dates, stored at 4ºC and analyzed within their expiration dates. Table 1 provides the description of the ciprofloxacin brands sampled.

Microorganisms Used

Thirty bacterial isolates comprising five strains each of *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi* and *Staphylococcus aureus* were collected from Microbiology unit of University College Hospital (UCH), Ibadan. The organisms were from different sources such as urine, wound and stool, were collected on sterile nutrient agar slants and confirmed using standard biochemical tests including Gram staining, catalase, indole, oxidase and citrate tests (Prescott, 2013). Fresh cultures of the confirmed isolates were then sub-cultured on fresh nutrient agar slants and stored in the refrigerator at 4°C. *Escherichia coli* ATCC 25922 was used as a reference strain.

Method

Analyses carried out on the generic brands were physicochemical and microbiological as described below.

Physicochemical analysis: Determination of percentage content of active ingredient

The primary and secondary packages of the various brand samples were visually examined carefully to check for compliance with requisite labeling and packaging information.

Standard ciprofloxacin stock solution of 0.05mg/ml was prepared by dissolving 0.02g of the powder in 10ml distilled water and was made up to a 100ml in volumetric flask with distilled water. From this stock solution, standard solutions of concentrations (0.002, 0.004, 0.006, 0.008, 0.010, and 0.012 mg/ml) were prepared. Distilled water was used as the blank for the standard solutions. Also, test solution of each drug sample at 0.004mg/ml was prepared by making up a 10ml aliquot ciprofloxacin infusion to 50ml in a volumetric flask with distilled water. A 0.5ml of the resulting solution was then transferred into another 50ml volumetric flask and made up to the mark with distilled water. Sodium chloride at 0.018mg/ml was used as blank control. The absorbance of these solutions was then measured at a wavelength of 326 nm using an ultraviolet spectrophotometer.

Antibiotics susceptibility of test organisms

Antibiogram of the organisms was determined by Kirby-Bauer disc diffusion method on Mueller Hinton agar. A 0.1ml of a 10^{-2} dilution of an overnight broth culture of each organism (containing an inoculum size 1.0 x 10^8 cells/ml based on compared turbidity with 0.5 McFarland standard) was seeded into molten but cooled 20 ml Mueller Hinton agar. The following antibiotic multi-discs (Abtek Biologicals) were used: **Gram-negative**, Augmentin (AUG) 30µg, Ofloxacin (OFL) 5 µg, Gentamicin (GEN) 10 µg, Nalidixic acid (NAL) 30 µg, Nitrofurantoin (NIT) 200 µg, Cotrimoxazole (COT) 25 µg, Amoxicillin (AMX) 25 µg, Tetracycline (TET) 25 µg (Table 3); **Gram positive**, Augmentin (AUG) 30mg, Amoxicillin (AMX) 25mg, Erythromycin (ERY) 5mg, Tetracycline (TET) 10mg, Cloxacillin (CXC) 5mg, Cotrimoxazole (COT) 25 mg, Gentamicin (GEN) 10 mg and Chloramphenicol (CHL) 30mg (Table 4).

The antibiotic discs were placed on the set agar and allowed to diffuse for 1 hour before incubation at 37ºC for 24hr. The diameter of the zones of inhibition were measured in millimetres and values were interpreted according to standards (CLSI, 2016).

Susceptibility testing of the isolates to ciprofloxacin brands

Two concentrations (10 and 5µg/ml) of the different brands of ciprofloxacin infusion and ciprofloxacin powder (reference standard) were prepared using sterile distilled water. An overnight culture of bacterial suspension adjusted to 0.5 McFarland standard (1.0 x 10^8 cells/ml) was prepared and a 10^{-2} dilution of the stock bacterial suspension was made using sterile distilled water. Using a sterile cotton swab, the entire surface of freshly prepared Nutrient agar was inoculated. The different concentrations of the different brands of ciprofloxacin infusion were then introduced into holes bored into the set agar using an 8mm cork-borer. The antibiotics were left to diffuse for about 30 minutes and the plates were then incubated at 37°C for 24 hours. The diameters of the zone of inhibition were then measured to the nearest millimeter (mm).

Determination of minimum inhibitory concentration (MIC)

The MIC was determined using broth dilution method (Andrews, 2001). Using serial dilution, concentrations (10, 5, 2.5, 1.25, 0.625 and 0.3125µg/ml) of the ciprofloxacin reference and generic samples were

prepared in test tubes. Four additional concentrations of 100, 50, 25, and 12.5µg/ml were prepared for *Proteus mirabilis* to make allowance for higher resistance. Thereafter, 0.1ml of a 10^{-2} dilution of an overnight broth culture of each bacterial strain was added to the tubes. The tubes were then incubated at 37°C for 24 hours and the minimum concentration that inhibited the growth of each organisms was taken as the MIC.

Determination of minimum bactericidal concentration (MBC)

The tubes that did not show any visible growth in the MIC determination were streaked on a set Nutrient agar plate and incubated at 37°C for 24 hours. The minimum concentration where no visible growth of the organism occurred was taken as the MBC.

RESULTS AND DISCUSSION

The comparative analysis carried out among the five brands encompassed both physico-chemical and microbiological evaluations as follows.

Ciprofloxacin hydrochloride content

A calibration plot of pure ciprofloxacin powder which was linear over the concentration range of 0.002 to 0.012mg/ml, with a slope of 64.34, intercept of 0.082 and coefficient of determination of 0.992 obtained is shown in Figure 1. The concentration of ciprofloxacin in each of the samples obtained by extrapolation from the Beer-Lambert's plot is presented as percentage content of active ingredient in the five samples (Table 2). From the assay, it was observed that the percentage content of each sample was not less than the BP specifications of 95.0 – 105.0%, but were actually higher ranging from 108.4 – 179.5%.

Antibiotics susceptibility of the isolates

The cultural and biochemical tests confirmed the identity of the collected bacterial isolates that they were as labelled.

All the Gram-negative organisms showed outright resistance to tetracycline, amoxicillin, augmentin and cotrimoxazole, showing that they are multidrug resistant (MDR), having shown resistance to 3 or more antibiotics belonging to different classes (Tables 3 and 5). However, a strain of *Escherichia coli*, *Salmonella typhi*, and two strains of *Klebsiella pneumoniae* were sensitive to nitrofurantoin. Only a strain of *Salmonella typhi* was sensitive to nalidixic acid, two strains of *Escherichia coli* and four strains of *Salmonella typhi* were sensitive to gentamicin and a strain of *Escherichia coli*, three strains of *Salmonella typhi* and a strain of *Pseudomonas aeruginosa* were sensitive to ofloxacin. *Staphylococcus aureus* showed no zone of inhibition for the eight antibiotic discs used, indicating that they are MDR strains (Table 4 and 5).

Ciprofloxacin Brands Tested

All the tested brands of ciprofloxacin were imported; three from China and two from India (Table 1). It has been reported that about 70% of drugs in Nigeria are imported (Okoli, 2000), over 90% of which are from Asia (Chika *et al.*, 2016; NAFDAC, 2019). It was also emphasized that majority of fake or substandard drugs in Nigeria were imported from India, China or Pakistan (Wertheimer and Wang, 2012; Bate, 2012). This also informed the choice of brands of ciprofloxacin tested in this study; all were imported from Asia. Further, infusions rather than tablets were considered for this study owing to the onset of action of parenteral drugs and the drastic implications of injecting substandard or fake drugs; hence must be of required quality.

Table 1: Details of the ciprofloxacin brands sampled

Sample	Country of origin	NAFDAC number	Manufacture date	Expiry date
A	India	Yes	February 2015	January 2018
B	India	Yes	September 2015	August 2018
C	India	Yes	August 2015	July 2018
D	China	Yes	October 2015	October 2018
E	China	Yes	October 2015	September 2018

Table 2: Absorbance of 0.004mg/ml ciprofloxacin NaCl infusion samples tested

Concentration (mg/ml)	Absorbance 1	2	3	Average absorbance	Corrected absorbance	% content of drug
A	0.494	0.495	0.495	0.495	0.432	136.0
B	0.590	0.590	0.591	0.590	0.527	172.9
C	0.424	0.424	0.424	0.424	0.361	108.4
D	0.608	0.607	0.607	0.607	0.544	179.5
E	0.434	0.435	0.435	0.435	0.372	112.7
Blank (NaCl)	0.063	0.063	0.063	0.063	0.063	

Table 3: Sensitivity of test organisms (Gram-negative) to standard antibiotic discs

Isolates	AUG 30µg	OFL 5µg	GEN 10µg	NAL 30µg	NIT 200µg	COT 25µg	AMX 25µg	TET 25µg
E1	R	S	S	I	S	S	R	S
E2	R	R	R	R	R	R	R	R
E3	R	S	S	R	I	R	R	R
E4	R	R	R	R	R	R	R	R
E5	R	R	R	R	R	R	R	R
E*	R	S	R	S	I	R	R	R
Sa1	R	S	S	R	I	R	R	R
Sa2	R	R	R	R	R	R	R	R
Sa3	R	S	S	I	S	R	R	R
Sa4	R	S	S	S	R	R	R	R
Sa5	R	R	S	R	R	R	R	R
Ps1	R	R	R	R	R	R	R	R
Ps2	R	R	R	R	R	R	R	R
Ps3	R	R	R	R	R	R	R	R
Ps4	R	R	R	R	R	R	R	R
Ps5	R	S	R	R	R	R	R	R
Pr1	R	R	R	R	R	R	R	R
Pr2	R	R	R	R	R	R	R	R
Pr3	R	R	R	R	R	R	R	R
Pr4	R	R	R	R	R	R	R	R
Pr5	R	R	R	R	R	R	R	R
K1	R	R	R	R	R	R	R	R
K2	R	R	R	R	S	R	R	R
K3	R	R	R	R	S	R	R	R
K4	R	R	R	R	R	R	R	R
K5	R	R	R	R	R	R	R	R

KEY: E= *Escherichia coli*; E*= ATCC 25922; Sa= *Salmonella typhi*; Ps= *Pseudomonas aeruginosa*; Pr= *Proteus mirabilis*; K= *Klebsiella pneumoniae;* St= *Staphylococcus aureus*; AUG= Augmentin; OFL= Ofloxacin; GEN= Gentamicin; NAL= Nalidixic acid; NIT= Nitrofurantoin; COT= Cotrimo+xazole; AMX= Amoxycillin; TET= Tetracycline; S= Susceptible; I= Intermediate; R= Resistant

Table 4: Sensitivity of test organisms (Gram-positive) to standard antibiotic discs

Isolates	AUG 30mg	AMX 25mg	ERY 5mg	TET 10mg	CXC 5mg	GEN 10mg	COT 25mg	CHL 30mg
St1	R	R	R	R	R	R	R	R
St2	R	R	R	R	R	R	R	R
St3	R	R	R	R	R	R	R	R
St4	R	R	R	R	R	R	R	R
St5	R	R	R	R	R	R	R	R

KEY: E= *Escherichia coli*; Sa= *Salmonella typhi*; Ps= *Pseudomonas aeruginosa*; Pr= *Proteus mirabilis*; K= *Klebsiella pneumonia;* St= *Staphylococcus aureus*; AUG= Augmentin; GEN= Gentamicin; COT= Cotrimoxazole; AMX= Amoxycillin; TET= Tetracycline; ERY= Erythromycin; CXC= Cloxacillin; CHL= Chloramphenicol; S= Susceptible; I= Intermediate; R= Resistant

Table 5: Susceptibility of test organisms to brands of ciprofloxacin infusion at 10µg/ml and 5µg/ml

Sample	A		B		C		D		E		Cip. HCl	
Conc µg/ml	10	5	10	5	10	5	10	5	10	5	10	5
E1	R	I	R	S	I	R	R	R	R	R	I	I
E2	R	R	R	R	R	R	R	R	R	R	R	R
E3	S	R	S	R	S	S	S	S	S	I	S	S
E4	R	R	R	R	R	R	R	R	R	R	R	R
E5	S	R	R	S	R	S	R	R	R	R	I	S
E*	S	R	S	R	S	I	S	S	S	I	S	R
Sa1	I	R	I	R	R	R	R	R	R	R	S	R
Sa2	R	R	S	R	I	R	S	R	I	R	I	R
Sa3	R	R	R	R	R	R	R	R	R	R	I	R
Sa4	R	R	R	R	R	I	R	I	R	R	I	I
Sa5	I	R	R	I	I	R	I	R	I	R	S	I
Ps1	R	R	S	R	R	R	R	R	I	R	S	R
Ps2	R	R	S	R	R	R	R	R	R	R	S	I
Ps3	S	R	R	R	S	R	S	R	S	R	S	R
Ps4	I	R	I	R	R	R	R	R	I	R	S	I
Ps5	R	R	R	R	S	R	S	R	S	R	S	R
Pr1	S	R	S	I	S	I	S	R	I	R	I	S
Pr2	I	R	I	R	I	R	I	R	R	R	S	I
Pr3	R	S	R	S	R	S	R	R	R	R	R	R
Pr4	I	I	I	I	R	S	R	S	S	R	R	S
Pr5	R	R	R	R	R	R	R	R	R	R	R	R
K1	I	R	I	R	I	R	R	R	R	R	S	S
K2	R	R	R	R	R	R	R	R	R	R	R	R
K3	R	R	R	R	R	R	R	R	R	R	R	R
K4	R	R	R	R	R	R	S	R	S	R	R	R
K5	R	R	R	R	S	R	I	R	R	R	R	R
St1	S	R	S	R	S	R	I	R	S	R	S	R
St2	R	R	I	R	S	R	R	R	I	R	S	R
St3	R	R	S	R	I	R	I	R	I	R	S	S
St4	I	R	I	R	R	R	R	R	S	R	S	R
St5	R	R	R	R	R	R	R	R	R	R	R	R

KEY: E= *Escherichia coli*; E*= ATCC 25922; Sa= *Salmonella typhi*; Ps= *Pseudomonas aeruginosa*; Pr= *Proteus mirabilis*; K= *Klebsiella pneumonia;* St= *Staphylococcus aureus*; S= Susceptible; I= Intermediate; R= Resistant

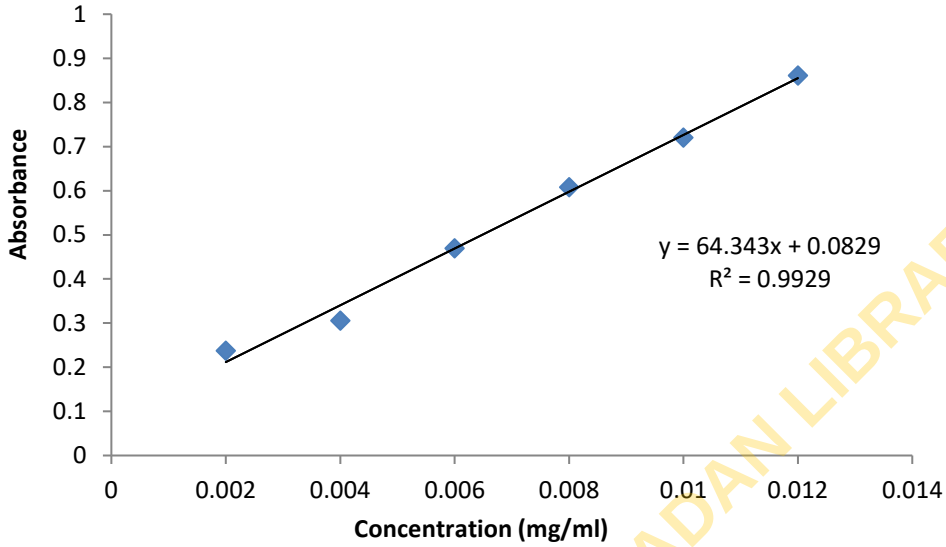

Figure 1: Calibration curve for determination of the percentage ciprofloxacin content in the infusions

Susceptibility testing of bacterial isolates to ciprofloxacin samples

There were variations in antimicrobial potency of the samples tested as shown by the difference in the percentage of micro-organisms that were resistant to each brand of the ciprofloxacin infusions used (Table 6). The higher the percentage of resistance, the lower the antimicrobial activity of the ciprofloxacin brands. When compared with the standard ciprofloxacin, the brands were found to be less active. The drug samples showed varying antibacterial activities; the variation at 5µg/ml was more than at 10µg/ml, but the difference was statistically not significant (p> 0.05). Antibacterial activity of ciprofloxacin is concentration dependent (Dana *et al*., 2000; Prabodh *et al*., 2010), hence the tests were done at two different concentrations, 5 and 10µg/ml. As expected, the activity of the tested drugs was much higher at 10 than 5µg/ml. The implication is that a little variation in the concentration of ciprofloxacin in the samples may have a profound effect on the potency, therefore strict adherence to the BP specification of 95-105% is important. However, all the drug samples had contents in excess of 105%, for example samples B and D had 172.9% and 179.5% respectively (Table 2). These percent contents are too high and may be of concern in term of toxicity.

Among the bacterial isolates tested, *E. coli* and *Salmonella* were the most susceptible at about 20% sensitivity; while *Staphylococcus aureus* and *Proteus mirabilis* were 100% resistant. The bacteria isolates showed the highest percentage resistance to samples E (96.7%) at 5µg/ml and to D (63.3%) at 10µg/ml, indicating least activity. The least activity shown by sample D may not be surprising since, even at physical examination stage, the labeling did not disclose the entirety of its contents, which is unacceptable in pharmaceutical specifications on drug labelling (BP, 2009).

The isolates showed the least percentage resistance to ciprofloxacn standard which is the pure active pharmaceutical ingredient. Resistance to quinolones can develop rapidly, even during a course of treatment by pathogens especially gram-positive organisms including *Staphylococcus aureus* (Singh and Yu, 2000). This could account for the high level of resistance observed with *Staphylococcus aureus* and all the other isolates used generally.

This study also gives the susceptibility patterns of various clinical isolates of *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis*, *Pseudomonas*

aeruginosa and *Staphylococcus aureus* to ciprofloxacin. On a general note, the Gram-negative organisms: *Escherichia coli*, *Salmonella typhi* and *Proteus mirabilis*, showed higher percentage of susceptibility to the ciprofloxacin brands. This conforms to the report of Bennet and Brown, (2003) that ciprofloxacin is effective against a range of bacteria but particularly the gram-negative organisms.

Table 6: Number (Percentage) of isolates resistant to each brand of Ciprofloxacin infusion at 5 and 10 µg/ml

Sample	***E*** **(n=5)**		***K (n=5)***		***Ps*** **(n=5)**		***Pr*** **(n=5)**		***St*** **(n=5)**		***Sa*** **(n=5)**		**Total / (Average)**	
	5 µg/ml	**10 µg/ml**	**5 µg/ml**	**10 µg/ml**	**5 µg/ml**	**10 µg/ml**	**5 µg/ml**	**10 µg/ml**	**5 µg/ml**	**10 µg/ml**	**5 µg/ml**	**10 µg/ml**	**5 µg/ml**	**10 µg/ml**
A	4 (80)	3 (60)	5 (100)	4 (80)	5 (100)	3 (60)	3 (60)	2 (40)	5 (100)	3 (60)	5 (100)	3 (60)	27 (90)	18 (60)
B	3 (60)	4 (80)	5 (100)	4 (80)	5 (100)	2 (40)	2 (40)	2 (40)	5 (100)	1 (20)	4 (80)	3 (60)	24 (80)	16 (53.3)
C	3 (60)	3 (60)	5 (100)	3 (60)	5 (100)	3 (60)	2 (40)	3 (60)	5 (100)	2 (40)	4 (80)	3 (60)	24 (80)	17 (56.7)
D	4 (80)	4 (80)	5 (100)	3 (60)	5 (100)	3 (60)	4 (80)	3 (60)	5 (100)	3 (60)	4 (80)	3 (60)	27 (90)	19 (63.3)
E	4 (80)	4 (80)	5 (100)	4 (80)	5 (100)	1 (20)	5 (100)	2 (40)	5 (100)	1 (20)	5 (100)	3 (60)	29 (96.7)	15 (50)
Cipr. HCl	2 (40)	2 (40)	4 (80)	4 (80)	3 (60)	0 (0)	2 (40)	3 (60)	4 (80)	1 (20)	3 (60)	0 (0)	18 (60)	10 (33.3)

KEY: E= *Escherichia coli*; Sa= *Salmonella typhi*; Ps= *Pseudomonas aeruginosa*; Pr= *Proteus mirabilis*; K= *Klebsiella pneumonia;* St= *Staphylococcus aureus*

Table 7: MIC and MBC of Ciprofloxacin brands and standard on selected test organisms

Sample	**A (µg/ml)**		**B (µg/ml)**		**C (µg/ml)**		**D (µg/ml)**		**E (µg/ml)**		**Cipr. (µg/ml)**	
Isolates	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E1	0.31	1.25	0.63	ND	1.25	ND	0.31	ND	1.25	5.00	0.31	5.00
E3	1.25	2.5	0.63	ND	0.31	ND	0.31	ND	0.31	0.31	0.31	0.31
K1	2.50	10.0	0.63	ND	1.25	10.0	0.63	ND	0.63	1.25	0.31	0.31
K5	0.63	ND	0.63	ND	0.31	ND	0.31	ND	0.31	0.63	0.63	10.0
Pr1	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Pr2	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Ps3	0.63	ND	0.63	2.50	0.63	ND	0.31	ND	0.31	0.31	0.31	0.31
Ps5	0.31	ND	0.63	10.0	0.31	ND	0.31	ND	0.31	1.25	0.31	5.00
St1	0.63	ND	0.63	ND	1.25	5.00	0.63	ND	0.63	1.25	0.31	0.313
St3	1.25	5.00	1.25	1.25	1.25	5.00	1.25	10.0	2.50	2.50	0.31	5.00

Determination of MIC and MBC

In terms of the minimum inhibitory concentration, all the brands, including the active pharmaceutical powder, showed activity against all the organisms at MIC range of 0.31-2.50 µg/ml, except for *Proteus mirabilis*, which was resistant even at higher concentrations of 100µg/ml. This shows that ciprofloxacin has good broad spectrum of activity and potency at low concentrations. The MBC/MIC ratio called MIC Index calculated from Table 7 is generally less than 4 which confirmed the bactericidal nature of ciprofloxacin at low concentrations (Prabodh, 2010). This is of great therapeutic advantage as a patient would not need a high level of drug exposure to ensure achievement of a predefined pharmacokinetic or pharmacodynamics level associated with maximal efficacy. For *Proteus mirabilis*, Wang *et al*. (2014) in their study found that there was a significant decrease in susceptibility of *Proteus mirabilis* to 3rd-generation cephalosporins and ciprofloxacin. This could explain its growth even at high concentrations of the drug.

CONCLUSION

The physicochemical tests carried out on the drug samples revealed variations in ciprofloxacin content, which were higher than the BP standard. This may be a cause for concern in terms of toxicity for a drug of narrow therapeutic index like ciprofloxacin.

Considering the antibacterial potency, all the drug samples showed good activity comparable with the standard. Also, the study showed that ciprofloxacin is still found to be active on susceptible and some resistant clinical isolates which justify its wide use in treating infections in Nigeria. However, since resistance to ciprofloxacin is increasing, there is a great need to discourage the improper use of ciprofloxacin to combat the increasing level of microbial resistance.

Comparing the different ciprofloxacin brands, there were variations in antimicrobial potency in terms of percentage susceptibility of the clinical isolates and MIC of the drugs, but the difference was not statistically significant ($p > 0.05$). Since there was no significant difference in the antimicrobial activity of the different ciprofloxacin infusions sampled, cheaper brands of this drug can be purchased by consumers who cannot afford to buy the more expensive branded drug and the same effect would be obtained.

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