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Full Length Research Article

Comparative analysis of antibacterial potency of six fluoroquinolone antibiotics commonly prescribed in Uyo, Akwa Ibom State, Nigeria in the treatment of *Salmonela typhi* infections

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Comparative analysis of antimicrobial potency of six common fluoroquinolone antibiotics was done. They were chosen based on their availability and affordability which includes: Ciprofloxacin, Pefloxacin, Levofloxacin, Norfloxacin, Ofloxacin and Sparfloxacin, in the treatment of *Salmonela typhi* infections in Uyo, Akwa Ibom state of Nigeria. Sensitivity test of different concentrations of the six fluoroquinolones were carried out on the test organism (*Salmonella tyhpi*) using agar well diffusion method. The Minimum Inhibitory Concentration (MIC) which indicates the strength of each of the fluoroquinolones used on the test organism were determined. The result obtained showed that Pefloxacin had the highest antibacterial strength against the test organism with MIC of 0.0003, followed by Norfloxacin (0.0006), Ciprofloxacin (0.0010), Levofloxacin (0.0016), Ofloxacin (0.0042) and Sparfloxacin having the lowest with MIC of 0.0398. The analysis of variance (ANOVA) showed a significant difference (P<0.05) among the MIC of the six fluoroquinolones used. Post Hoc Test result indicates a significant of 1.000 among the MIC of the six fluoroquinolones.

Keywords: Antimicrobial, Antibiotics, fluoroquinolones and *Salmonela typhi*

INTRODUCTION

Typhoid fever is a systemic infection, caused mainly by *Salmonella typhi* found only in man (Kalra, *et al*., 2003). It is characterized by the sudden onset of a sustained and systemic fever, severe headache, nausea, and loss of appetite. Other symptoms include constipation or diarrhea, enlargement of the spleen, possible development of meningitis, and/or general malaise (Wain *et al*., 2015). In 2010 there were 27 million cases reported, while in 2003 it resulted in about 161,000 deaths. The risk of death may be as high as 25% without treatment, while with treatment it is between 1% and 4% (Crump *et al*., 2010). The prevalence of *Salmonella typhi* in relation to age group shows that pregnant women between ages of 35-44 had the highest rate of infection (71.8%), those within the age groups of 25-26, 15-24 also had a prevalence of 68.1% and 66.7% respectively. (World Health Organisation [WHO], 2015).

Typhoid fever is treated with antibiotics which kill the bacterium. With appropriate antibiotic therapy, there is usually improvement within one to two days and recovery within seven to ten days. Several antibiotics are effective for the treatment of typhoid fever. Chloramphenicol was

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the original drug of choice for many years but due to rare serious side effects, chloramphenicol has been replaced by other effective antibiotics such as the macrolides, penicillin, and the fluoroquinolones. The choice of antibiotics is guided by identifying the geographic region where the infection was contracted (WHO, 2014). The fluoroquinolones are widely regarded as optimal for the treatment of typhoid fever in adults (Chinh *et al*., 2000). Fluoroquinolones are readily absorbed but their complete absorption is not always achieved following oral administration (Sharma *et al*., 2009). Distribution of fluoroquinolones to tissues is superior to that of most other drugs because there is little binding to plasma proteins (Abraham *et al*., 2003). Their metabolism is inactivating and is primarily by glucuronides conjugation at the 3-carboxylic group. The primary route of elimination of most of fluoroquinolones is through kidney by glomerular filtration and tubular secretion (Sharma *et al*., 2009). The fluoroquinolones attain excellent tissue penetration, kill *S. typhi* in its intracellular stationary stage in monocytes/macrophages and achieve higher active drug levels in the gall bladder than other drugs. They produce a rapid therapeutic response, i.e. clearance of fever and symptoms in three to five days, and very low rates of post-treatment carriage (Arnold *et al*., 1993; Cristiano *et al*., 1995).

The fluoroquinolones are in the family of synthetic broad-spectrum antibiotic drugs called Quinolones. They exert their antibacterial effect by preventing bacterial DNA from unwinding and duplicating. The majority of quinolones in clinical use are fluoroquinolones, which have a fluorine atom attached to the central ring system, typically at the 6-position or C-7 position. Most of them are named with the -oxacin suffix (Anderson and MacGowan, 2003; Heeb *et al*., 2011). Naldixic acid, oxolinic acid and pipemidic acid are classified as first generation fluoroquinolones and are active against some Gram negative bacteria, highly protein bound drugs and short half life. Norfloxacin, enoxacin, ciprofloxacin, ofloxacin and lomefloxacin are second generation fluoroquinolones and are protein binding (50%), longer half life than previous agents and improved activity against Gram negative bacteria. Temafloxacin, sparafloxacin and grepafloxacin are third generation fluoroquinolones and are active against Gram negative bacteria as well as Gram positive bacteria. Clinafloxacin, trovafloxacin, moxifloxacin and gatifloxacin are fourth generation fluoroquinolones and show extended activity against both strains of bacteria, active against anaerobes and atypical bacteria (Sharma *et al*., 2009).

MATERIALS AND METHODS

Drugs procurement

Six fluoroquinolones antibiotics were procured in a retail registered pharmacy outlet. The information concerning the authenticity of the drugs were confirmed by looking at the manufacturing date, expiry date, batch number, NAFDAC registration number and manufacturing industry. The drugs were properly stored at the temperature of 25^{o}C and was used the following day.

The six fluoroquinolones are:

Ciprofloxacin tablet USP 500mg (CIPROTAB®): NAFDAC Registration Number 04-0723; Batch Number VG1423; Manufacturing Date 10/2014; Expiry Date 09/2017; Manufactured by GeltecPvt.Ltd. India.

Pefloxacin tablets 400mg (PEFLOTAB®): NAFDAC Registration Number 04-1946; Batch Number V511; Manufacturing Date 3/2015; Expiry Date 2/2018; Manufactured by Medibios laboratories Pvt. Ltd. India.

Levofloxacin 500mg tablets (LEVOKIN®): NAFDAC Registration Number 04-5423; Batch Number PB45039; Manufacturing Date 10/2014; Expiry Date 09/2017; Manufactured by CIPLA LTD. India.

Norfloxacin tablets Bp 400mg (NARACIN®): NAFDAC Registration Number A4-3913, Batch Number RA4001; Manufacturing Date 01/2014; Expiry Date 12/2016; Manufactured by Rajatpharmachem Ltd. India.

Ofloxacin Extended release tablets 400mg (GFLOX®): NAFDAC Registration Number A4-1072; Batch Number 13488; Manufacturing Date 08/2013; Expiry Date 7/2016; Manufactured by OSAKA pharmaceuticals Pvt.Ltd. India.

Sparfloxacin TABLETS 200mg (SPARFON®): NAFDAC Registration Number 04-7903; Batch Number 04S30011401; Manufacturing Date 05/2014; Expiry Date 04/2017; Manufactured by Saga laboratories. India.

Collection and screening of the test organism

Stock culture of the test organism, *Salmonella typhi* was obtained from microbiology Department, University of Uyo, Akwa Ibom State, Nigeria. Initial identification was based on the morphological behaviour of the isolate on various differential media. All media were prepared according to the manufacturer's specification and sterilized at 121 °C for 15 minutes at 15 l°°b pressure. The species level identification was then carried out by

Table 1. Susceptibility tests of *S. typhi* against the six fluoroquinolones

CIPROFLOXACIN		PEFLOXACIN		LEVOFLOXACIN		NORFLOXACIN		OFLOXACIN		SPARFLOXACIN	
CONC. (mg/ml)	DZI (mm)	CONC. (mg/ml)	DZI (mm)	CONC. (mg/ml)	DZI (mm)	CONC. (mg/ml)	DZI (mm)	CONC. (mg/ml)	DZI (mm)	CONC. (mg/ml)	DZI (mm)
0.5000	34	0.4000	32	0.5000	28	0.2000	30	0.2000	24	0.1000	6
0.2500	32	0.2000	30	0.2500	26	0.1000	28	0.1000	22	0.0500	4
0.1250	30	0.1000	28	0.1250	24	0.0500	26	0.0500	18	0.0250	-
0.0625	28	0.0500	26	0.0625	22	0.0250	24	0.0250	16	0.0125	-
0.0313	26	0.0250	24	0.0313	20	0.0125	22	0.0125	10	0.0063	-
0.0156	24	0.0125	22	0.0156	18	0.0063	20	0.0063	8	0.0031	-
0.0078	22	0.0063	20	0.0078	12	0.0031	16	0.0031	-	0.0016	-
0.0039	12	0.0031	18	0.0039	8	0.0016	12	0.0016	-	0.0008	-
0.0020	8	0.0016	16	0.0020	6	0.0008	8	0.0008	-	0.0004	-
0.0010	-	0.0008	12	0.0010	-	0.0004	-	0.0004	-	0.0002	-

Key:
DZI = Diameter of the zone of inhibition (distance traverse)
CONC = Concentrations

standard biochemical tests and by comparing their characteristics with those of known taxa, as described by Jolt *et al*. (1994), Cheesbrough (2006) and Oyeleke and Manga (2008). The test organism was sub cultured in Nutrient agar and stored in slant before use.

Antibiotic susceptibility testing by agar well diffusion method

A tenfold serial dilution was carried out using distilled water on the selected fluoroquinolones using a 24 hours dose of the drugs to get a stock solution of 1mg/ml for ciprofloxacin, pefloxacin 0.8mg/ml,levofloxacin 1mg/ml, norfloxacin 0.4mg/ml, ofloxacin 0.4mg/ml and sparfloxacin 0.2mg/ml. A two-fold serial dilution of the stock solution of each of the fluoroquinolones was carried out. 60 plates and were inoculated with the test organism (*Salmonella typhi*) and to each of the labelled plates, a hole was bored on the seeded agar plates using the sterile-flamed cork borer of 4mm×4mm in size, and the solution of the antibiotics was filled starting from the least concentration. The plates were allowed to stand for 1 hour on the sterilized bench in order to allow for diffusion of the antibiotics into the media before the growth of micro-organism and were incubated for 24 hours at 37^{o} C. The plates were observed and the diameter of the zone of inhibition around each well was measured

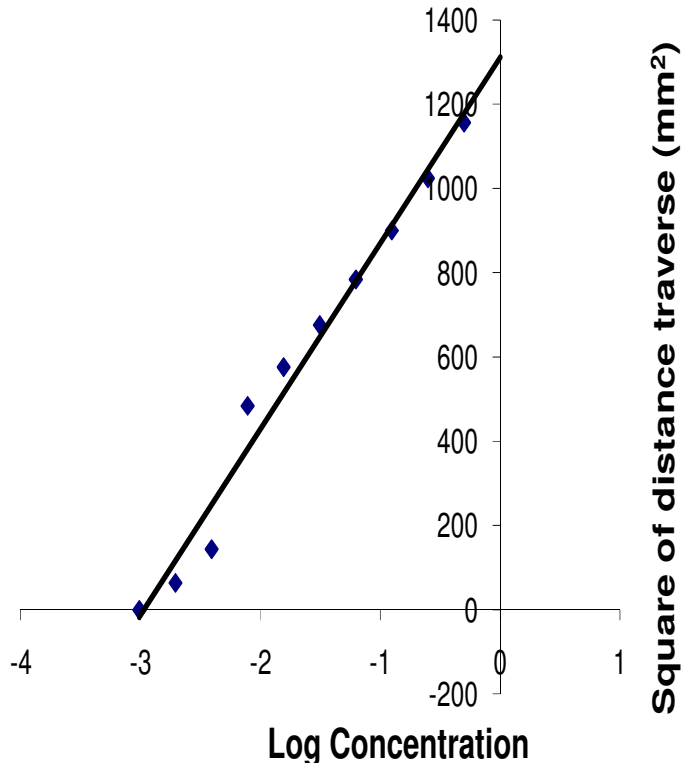

Figure 1. Plot of square of distance traverse (mm^2) against log concentration for ciprofloxacin
MIC = log^{-1} -3.0 = 0.0010

RESULTS AND DISCUSSIONS

Results

Determination of minimum inhibitory concentration (mic) using the agar-diffusion technique

A graph of square of distance traverse (mm^2) was plotted against logarithm of the corresponding concentration of the fluoroquinolones, to get a straight line graph. The extrapolation of the graph cuts the logarithm of concentration axis at an intercept, in which the antilog gives the relative MIC of the agent against the test organism. (Andrews, 2001; Turnidge *et al*., 2003).

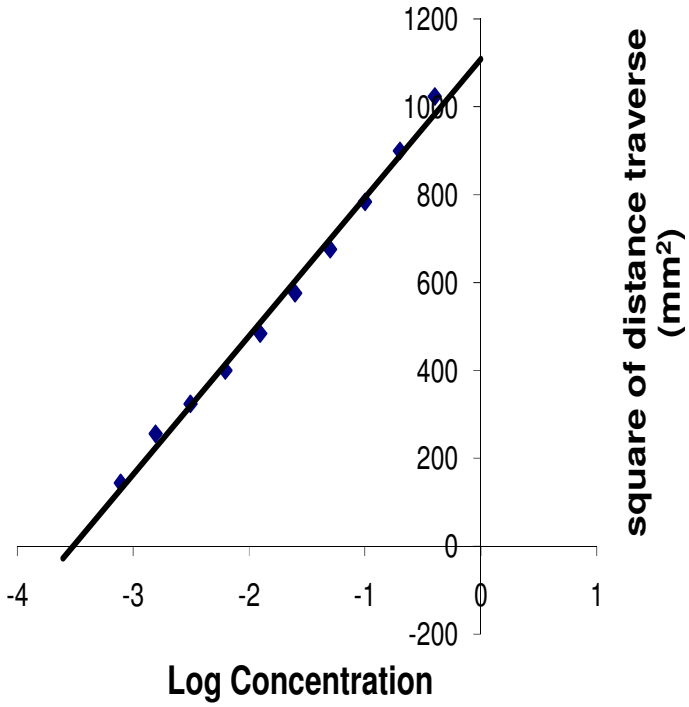

Figure 2. Plot of square of distance traverse (mm^2) against log concentration for pefloxacin
MIC = log^{-1} -3.55= 0.0003

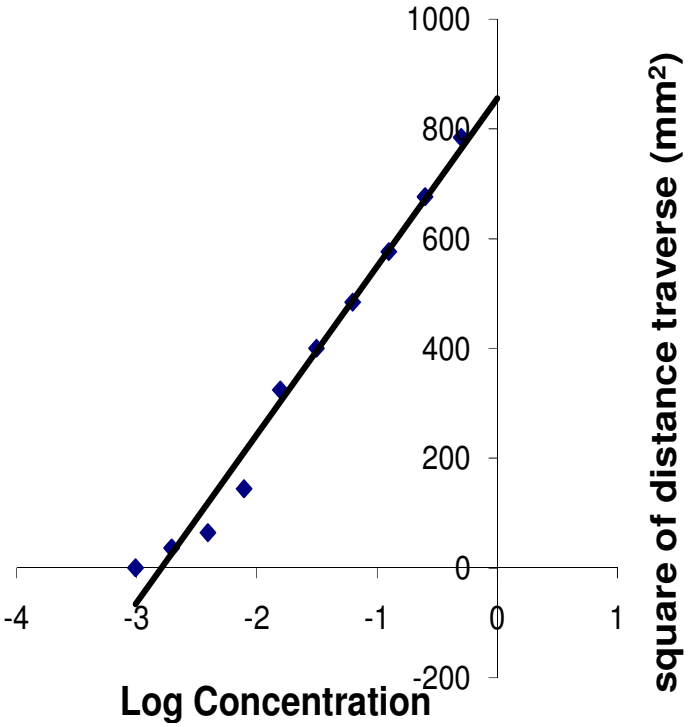

Figure 3. Plot of square of distance traverse (mm^2) against log concentration for levofloxacin
MIC = log^{-1} -2.8= 0.0016

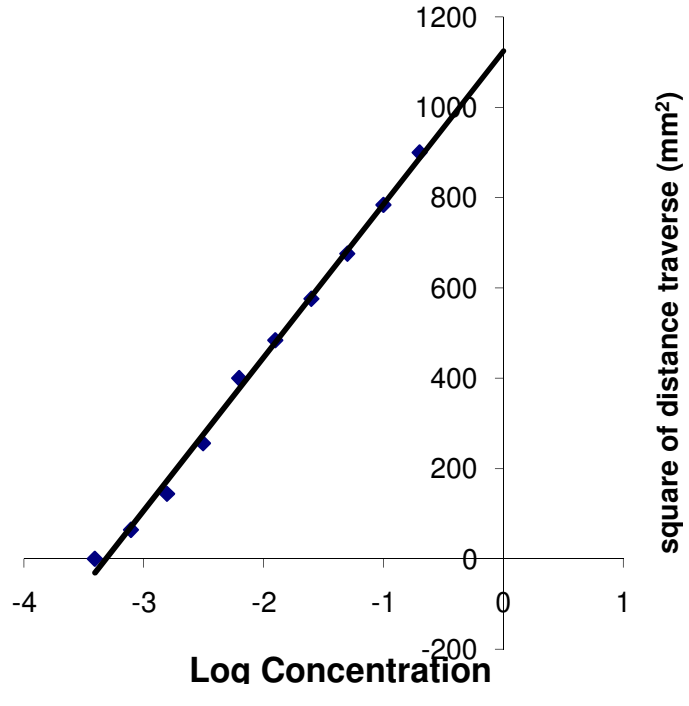

Figure 4. Plot of square of distance traverse (mm^2) against log concentration for Norfloxacin
MIC = log^{-1} -3.25= 0.0006

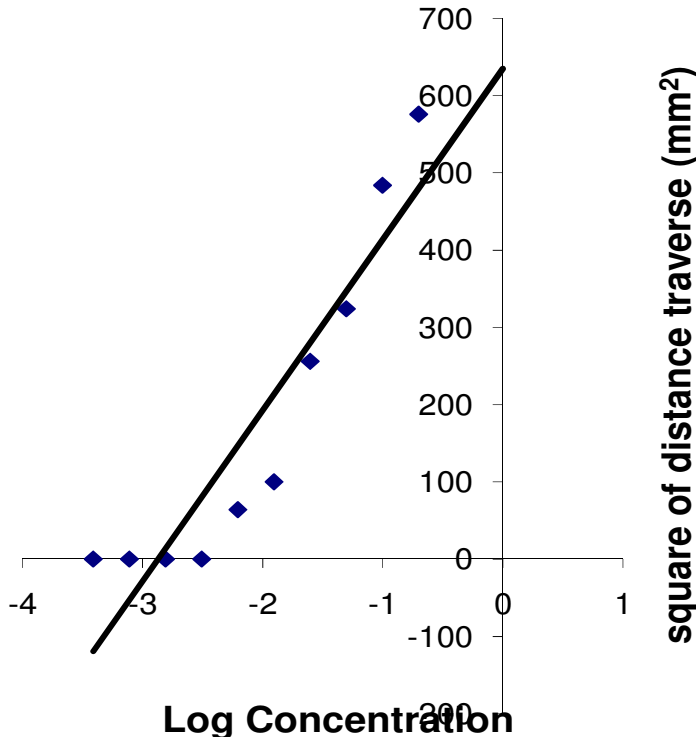

Figure 5. Plot of square of distance traverse (mm^2) against log concentration for Ofloxacin
MIC = log^{-1} -2.38= 0.0042

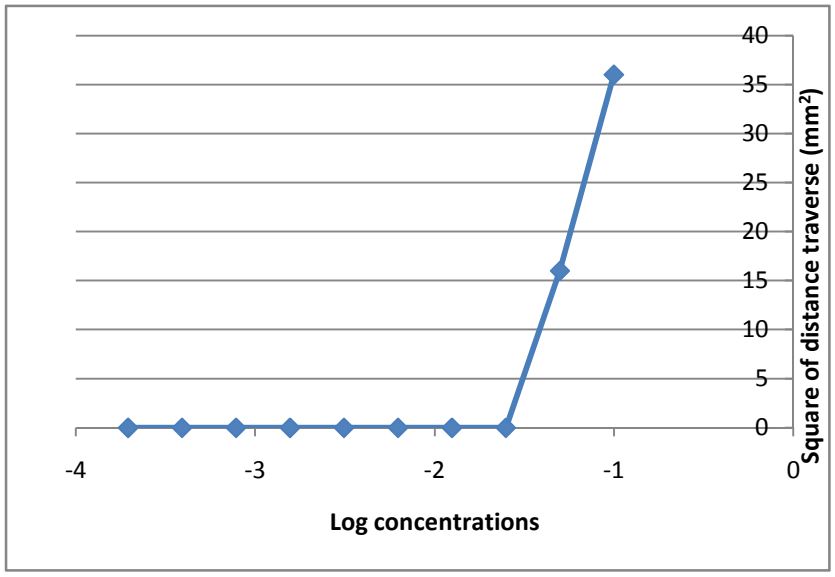

Figure 6. Plot of square of distance traverse (mm^2) against log concentration for Sparfloxacin
MIC = log^{-1} -1.4= 0.0398

Table 2. Summary of the minimum inhibitory concentration (MIC) of the six fluoroquinolones

FLUOROQUINOLONES	MIC (mg/ml)
Pefloxacin	0.00028±0.00001
Norfloxacin	0.00056±0.00001
Ciprofloxacin	0.00100±0.00010
Levofloxacin	0.00158±0.00001
Ofloxacin	0.00417±0.00001
Sparfloxacin	0.03980±0.00010

Results were expressed as mean ± SD and differences between sets obtained were determined using ANOVA followed by Duncan Post Hoc Test with the use of SPSS v 17 software. Differences were considered significant at $P<0.05$.

Data analysis was carried out on the mean MIC of the six fluoroquinolones and the table below summarize the analytical test result starting from the fluoroquinolone with the highest antimicrobial strength to the lowest antimicrobial strength.

Discussion

The mean inhibitory concentrations (MICs) of the six fluoroquinolones were determined and the result showed that pefloxacin had the highest antibacterial strength against the test organism (0.0003), followed by norfloxacin (0.0006), ciprofloxacin (0.0010), levofloxacin (0.0016), ofloxacin (0.0042) and sparfloxacin being the lowest (0.0042) (Figures 1-6 and table 2).

From the literature, by World Health Organism (WHO-2015), ciprofloxacin is effective in the treatment of typhoid fever and the test result confirms that. The result also confirms the effectiveness of pefloxacin in the treatment of typhoid fever (EMDEX, 2014/2015). Levofloxacin has moderate activity against Gram-negative bacteria and the result confirms the reason why it is not recommended as a first-line treatment for typhoid fever (Wispelwey and Schafer, 2010). Norfloxacin showed a weak antibacterial strength against the test from the result and this confirms it usefulness as an alternative therapy in the treatment of typhoid fever when first-line therapy is not available (Naber, 1991.) Ofloxacin showed a weak antibacterial strength against the test organism and this confirms it ineffectiveness in the treatment of typhoid fever. (WHO, 2015). Sparfloxacin showed a very weak antibacterial strength against the test organism and is ineffective for the treatment of typhoid fever (Turnidge *et al*., 2003).

The MIC results were subjected to data analysis using one-way analysis of variance (ANOVA) with a significant level $P<0.05$. The results were expressed as mean ±SD

and differences between sets obtained were determined using ANOVA followed by Duncan Post Hoc Test. The ANOVA result show a significant difference (P=0.000) among the fluoroquinolones in the treatment of typhoid fever. The Post Hoc Test result indicates a significant of 1.000 among the MIC of the six fluoroquinolones.

CONCLUSION

The six fluoroquinolone antibiotics exhibited varying activities against *Salmonella typhi.* The antibacterial strength of the antimicrobial agents as indicated by the Minimum Inhibitory Concentration (MIC) showed that Pefloxacin had the highest antibacterial strength against the test organism, followed by Norfloxacin, Ciprofloxacin, Levofloxacin, Ofloxacin and lastly, Sparfloxacin showing a very weak antibacterial strength against *Salmonella typhi.*

RECOMENDATION

In the prescription of flouroquinolone antibiotics in the treatment of Salmonella *typhi,* it is recommended that the order of potency as concluded above should be considered.

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