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Evaluation of Susceptibility Profiles of Some Bacteria to Flouroquinolones in Kano Metropolis, Nigeria

^{1*}Mohammed, B. and ²Ahmad, R. H.

Department of Microbiology, Bayero University, Kano. P. M. B. 3011

Correspondence Author: bshchemiron@yahoo.com; 08126620363

Abstract

Resistance to broad spectrum antibiotics such as flouroquinolones by bacteria is becoming a major threats towards the chemotherapy of some common pathogenic bacterial infections in the world especially the developing nations. In view of that, susceptibility profiles of some pathogenic bacteria to some flouroquinolones were evaluated using modified Kirby-Bauer Disc Diffusion Technique. Four flouroquinolones antibiotics; ofloxacin and ciprofloxacin (second generation), levofloxacin (third generation), moxifloxacin (fourth generation) were tested on five bacterial species (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Klebsiella pneumoniae*) sourced from Aminu Kano Teaching Hospital, Kano, Nigeria, and re-confirmed at Microbiology Laboratory in Bayero University, Kano. The results of the study revealed that 8(26.7%) of *E. faecalis* isolates were sensitive levofloxacin, 6(20.0%) were sensitive to ciprofloxacin and 1(3.33%) were sensitive to ofloxacin, while 0(0.00%) was sensitive to moxifloxacin. Seven isolates (23.3%) of *S. aureus* were sensitive to levofloxacin, 15(50.0%) were sensitive to ciprofloxacin and 4(13.3%) were sensitive to ofloxacin, while none (100%) were sensitive to moxifloxacin. *E. coli* isolates were 96.6% resistant to levofloxacin, *Klebsiella pneumoniae* and *P. aeruginosa* exhibited 76.6 and 50.0% resistance to the antibiotic respectively. There was significant difference in the performance of the four antibiotics used, ciprofloxacin better than others (*p*-value 0.00604). There was no significant difference in the percentages of sensitive bacterial species to the antibiotics used in the research (*p*-value 0.614), however, *P. aeruginosa* showed relatively higher number of sensitive isolates 30(40%). It can be concluded that 58% of the isolates were moderately sensitive to all the antibiotics in this study.

Key words: flouroquinolones, clinical bacterial isolates, sensitivity and resistance profiles.

INTRODUCTION

Flouroquinolones are broad spectrum antibiotics capable of exerting their antibacterial activity on wide range of bacteria (both Gram-positive and Gram-negative); they are highly effective for the treatment of a variety of clinical and veterinary infections (Valery *et al.*, 2014). They are derivatives of quinolones group of antibiotics having fluorine atom attached to the central ring system, typically at the 6-position or C-7 position. They are synthetic bactericidal agents which prevent bacterial DNA from unwinding and duplicating; the antibiotics bind with enzymes DNA gyrase and topoisomerase IV which brings about the unwinding of DNA (Willey *et al.*, 2008), a necessary step for its replication. United State doctors in 2015 doled out 32 million prescription for the drugs, making them the country's fourth most popular class of antibiotic (Jo, 2018). In African countries, widespread use of the antibiotics came into being in the early 2000s, after patents for the first generation expired. By that time, quinolone antibacterial agents

had been used intensively worldwide and resistant lineages of many bacterial species had evolved (Jo, 2018).

Researchers divided quinolones into generations based on their antibacterial spectrum; the earlier generation agents are in general more narrow-spectrum than the later ones (Jo, 2018). Flouroquinolone antibiotics most frequently prescribed today consist of ciprofloxacin, levofloxacin, moxifloxacin and to some extent their generic equivalents (Oliphant and Eray, 2002). Flouroquinolones have proved effective in many infections, including uncomplicated or complicated urinary tract infections (Jo, 2018), respiratory tract infections, gonorrhoea, bacterial gastroenteritis and soft tissue infections due to Gram-negative organisms. In Kano State, flouroquinolones are among the most widely prescribed antibiotics for the treatment of bacterial infections. Treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents including flouroquinolones (Fred, 2006).

According to Alkali *et al.* (2019) more than 80% of nosocomial infection in Kano are caused by the negative bacteria predominantly *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *E. coli*.

Resistance to fluoroquinolones by bacteria has been reported in many parts of the world by Hooper (2001) therefore, it is important to periodically evaluate the susceptibility profile of clinical bacterial isolates to these antibiotics so as to ascertain their relevance or otherwise in clinical applications. The study aimed at evaluating the susceptibility profiles of some bacteria to fluoroquinolones in Kano Metropolis.

MATERIALS AND METHODS

Sourcing of Bacterial Clinical Isolates

One hundred and fifty (150) bacterial isolates (thirty isolates each of *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) were randomly collected from Microbiology Laboratory at Aminu Kano Teaching Hospital, Kano, Nigeria from September to December, 2015.

Confirmation of the Test Isolates

Gram Staining Test

The isolates were subjected to Gram staining reaction to ascertain their identity as Gram positive or Gram negative as reported by Cheesbrough (2006).

Biochemical Test for Identification of the Isolates

The tests carried out include; coagulase test, catalase test, indole, Methyl red, Voges-Proskauer, Triple sugar Iron and citrate as described by (Cheesbrough 2006; Shamsuddeen *et al.*, 2013).

Preparation of Standardized Inoculum

Standardization of the inocula was performed using the protocols of CLSI (2014). The turbidity of each inoculum suspension was matched with that of 0.5 McFarland turbidity standards 1.5×10^8 CFU/ML.

Antibiotic susceptibility test

The antibiotic susceptibility profiles of the isolates were determined using the Kirby-Bauer modified disc diffusion technique as described by CLSI (2014). Disc potency of 5µg was used for each antibiotic against the test organisms as reported by Cheesbrough (2006).

Fifteen milliliters of warm and sterile Muller Hinton agar (MHA) was dispensed into separate Petri dishes and allowed to solidify. Using sterile swab sticks, each standardized inoculum was smeared evenly on the surface of the MHA plates and allowed to dry. Then, using sterile forceps, disks of the fluoroquinolone antibiotics with potencies of 5µg were placed at the distance of 5cm from each other and 3cm from the edge of the plates. Then plates were inverted and incubated at 37°C for 24hrs. Zone diameters of inhibition were measured and recorded in millimeter (Cheesbrough, 2006).

Statistical Analysis

The results were analyzed by two-way analyses of variance (ANOVA), using Microsoft Excel.

RESULTS

The results of the susceptibility profiles of the bacterial isolates revealed that 22(73.3%), 23(76.6%) and 15(50%) of *E. faecalis*, *S. aureus* and *Pseudomonas aeruginosa* were moderately sensitive to levofloxacin (16 to 20mm zone diameter of inhibition), while 23(76.6%), 29(96.6%) and none of the *E. faecalis* and *S. aureus* were resistant to the antibiotic (≤ 15 mm zone diameter of inhibition) (Table 1). Most of the isolates were moderately resistant to ofloxacin (Table 2), similarly, the isolates were mostly moderately sensitive to ciprofloxacin (Table 3). Between 18 (60.0%), 20(66.6%), 22(73.3%), 25(83.3%) and 27(90.0%) of the isolates were moderately sensitive to moxifloxacin (Table 4).

Table 1. Susceptibility Profiles of some Bacteria to Levofloxacin Antibiotics

| Organisms | No of isolates tested | Sensitive (%) | Mod. Sensitive (%) | Resistant (%) |
|-------------------------------|-----------------------|---------------|--------------------|---------------|
| <i>E. faecalis</i> | 30 | 8(26.7) | 22(73.3) | 0(0.0) |
| <i>S. aureus</i> | 30 | 7(23.3) | 23(76.6) | 0(0.0) |
| <i>E. coli</i> | 30 | 0(0.0) | 1(3.33) | 29(96.6) |
| <i>Klebsiella pneumoniae</i> | 30 | 0(0.0) | 7(23.3) | 23(76.6) |
| <i>Pseudomonas aeruginosa</i> | 30 | 0(0.0) | 15(50.0) | 15(50.0) |

p-value 0.184

Key: Sensitive = ≥ 21 mm, Moderately sensitive = 16 to 20mm, Resistant ≤ 15 mm Source: CLSI (2014)

Table 2. Susceptibility Profiles of some Bacteria to Ofloxacin Antibiotics

| Organisms | No of isolates tested | Sensitive (%) | Mod. Sensitive (%) | Resistant (%) |
|----------------------|-----------------------|---------------|--------------------|---------------|
| <i>E. faecalis</i> | 30 | 1(3.3) | 14(46.6) | 15(50.0) |
| <i>S. aureus</i> | 30 | 4(13.3) | 24(80.0) | 2(6.66) |
| <i>E. coli</i> | 30 | 10(33.3) | 18(60.0) | 2(6.66) |
| <i>K. pneumoniae</i> | 30 | 4(13.3) | 23(76.6) | 3(10.0) |
| <i>P. aeruginosa</i> | 30 | 9(30) | 21(70.0) | 0(0.0) |

p-value 0.00476

Key: Mod. Sensitive= Moderately sensitive, Sensitive = ≥ 16 mm, Moderately sensitive = 13 to 15mm, Resistant ≤ 12 mm Source: CLSI (2014)

Table 3. Susceptibility Profiles of some Bacteria to Ciprofloxacin Antibiotics

| Organisms | No of isolates tested | Sensitive (%) | Mod. Sensitive (%) | Resistant (%) |
|----------------------|-----------------------|---------------|--------------------|---------------|
| <i>E. faecalis</i> | 30 | 6(20.0) | 17(56.6) | 7(23.3) |
| <i>S. aureus</i> | 30 | 15(50.0) | 15(50.0) | 0(0.0) |
| <i>E. coli</i> | 30 | 14(46.6) | 16(53.3) | 0(0.0) |
| <i>K. pneumoniae</i> | 30 | 13(43.3) | 17(56.6) | 0(0.0) |
| <i>P. aeruginosa</i> | 30 | 13(43.3) | 17(56.6) | 0(0.0) |

p-value 0.00033

Key: Sensitive = ≥ 21 mm, Moderately sensitive = 16 to 20mm, Resistant ≤ 15 mm Source: CLSI (2014)

Table 4. Susceptibility Profiles of some Bacteria to Moxifloxacin Antibiotics

| Organisms | No of isolates tested | Sensitive (%) | Mod. Sensitive (%) | Resistant (%) |
|----------------------|-----------------------|---------------|--------------------|---------------|
| <i>E. faecalis</i> | 30 | 0(0.0) | 22(73.3) | 8(26.6) |
| <i>S. aureus</i> | 30 | 0(0.0) | 27(90.0) | 3(10.0) |
| <i>E. coli</i> | 30 | 1(3.33) | 20(66.6) | 9(30.0) |
| <i>K. pneumoniae</i> | 30 | 1(3.33) | 25(83.3) | 4(13.3) |
| <i>P. aeruginosa</i> | 30 | 8(26.6) | 18(60.0) | 4(13.3) |

P-value 0.00009

Key: Sensitive = ≥ 21 mm (zone diameter of inhibition), Moderately sensitive = 16 to 20mm, Resistant ≤ 15 mm, Source: CLSI (2014)

DISCUSSION

The results of the study had revealed that there was wide spread of resistance to levofloxacin by the *E. coli*, *K. pneumoniae* and *P. aeruginosa* isolates; 75% of the isolates were not 100% sensitive to the antibiotic. This finding was contrary to that of Siegrist *et al.* (1999), who obtained 100%, 94%, 97%, 98% and 87% sensitivity to levofloxacin by *Enterococcus faecalis*, *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* respectively in a susceptibility study on clinical isolates of bacteria conducted in Switzerland.

Generally, over 80% of the isolates used in this study were moderately sensitive to ofloxacin, this was not in conformity with the findings of Griedrys-Kalemba and Bilska (1993), who reported that *Enterococcus faecalis*, *Staphylococcus aureus*, *E. coli* and *Klebsiella pneumonia* were frequently susceptible to ofloxacin with less than 10-20% resistance to the antibiotic in a susceptibility study of bacteria to ofloxacin in Poland. Similarly, the results of the study have shown that *Pseudomonas aeruginosa* isolates were not

resistant to the antibiotic, but significant percentage (70%) were moderately resistant; this is contrary to the findings of the above mentioned researchers who obtained up to 40% resistance to ofloxacin by *Pseudomonas aeruginosa* isolates.

Most of the isolates were moderately resistant to ciprofloxacin; however, there was no significant difference (p-value 0.08625) between the resistant and the moderately resistant isolates. No resistance against ciprofloxacin was observed among the isolates except some isolates of *Enterococcus faecalis*. This finding is not in conformity with that of Reis *et al.* (2016) who reported some level of resistance against ciprofloxacin by most the species used in their study.

Most of the isolates used in this study were found to be moderately sensitive to moxifloxacin, this finding is not in conformity with that of Gita *et al.* (2007) who recorded 97.5% susceptibility to moxifloxacin by some clinical isolates of bacteria (*Staphylococcus albus*, *Pseudomonas aeruginosa*, *Streptococcus viridans*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* in

India. Similarly, there was increase in resistance to moxifloxacin by the isolates used in this study (18.4%) when compared with 2.75% recorded by Gita *et al.* (2007).

Resistance to fluoroquinolones by bacteria such as *S. aureus*, *E. coli*, *P. aeruginosa*, *N. gonorrhoeae*, *S. pneumoniae* were reported to be the same for all the generations of the antibiotics. This could be due to chromosomal mutation, acquisition of extra-chromosomal materials (plasmids, integrons, transposons) through conjugation, all these results in the alteration of binding sites (DNA gyrase and topoisomerase IV enzymes); other causes of resistance include activation of efflux pump and over expression of porins which brings about reduction in the uptake or accumulation of the antibiotics in the bacteria as reported by

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CONCLUSION

In conclusion, there was high resistance to fluoroquinolones among the isolates used in this study, however, ciprofloxacin was found to be the best among the fluoroquinolones tested. Similarly, *E. faecalis* is the most resistant specie observed in this study.

RECOMMENDATION

It is recommended that susceptibility profile of bacteria be determined before patients are placed on fluoroquinolone antibiotics. Then patients should adhere strictly to the dosage of antibiotic recommended by physicians.

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