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Full Length Research Article

RESISTANCE PATTERNS OF *Staphylococcus aureus* AND *Pseudomonas aeruginosa* TO SOME QUINOLONES ISOLATED IN KANO, NIGERIA.

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ABSTRACT
Two hundred (200) strains of *S. aureus* and *P. aeruginosa* were isolated from clinical samples collected from patients in Murtala Muhammad Specialist Hospital and Infectious Diseases Hospital, Kano. The confirmed isolates were tested for resistance to quinolones by the agar disk diffusion susceptibility test and the agar dilution minimum inhibitory concentration test. A resistance prevalence rate of 27% and 38% were seen in *S. aureus* and *P. aeruginosa* respectively. Eighteen *S. aureus* isolates were resistant to only one quinolone, while 9 had multiple resistances. 25 *P. aeruginosa* isolates were resistant to one quinolone, while 13 had multiple resistances. Nine resistance patterns were observed in all the isolates with *S. aureus* isolates having 8 while *P. aeruginosa* had 7. There was no significant difference (p>0.05) in the resistances observed in *S. aureus* and *P. aeruginosa* isolated from the male or female patients except in the case of 2 *S. aureus* isolates that were resistant to sparfloxacin. Quinolones could still be relied upon as effective antibiotics in treating *S. aureus* and *P. aeruginosa* infections. The need to emphasize enforcement of antibiotic use policies in developing countries is further justified by these findings.

Keywords: *Resistance, Quionolones, S. aureus, P. aeruginosa*, Kano, Nigeria

INTRODUCTION.
Quinolones have been used to treat both nosocomial and community acquired infections. The quinolones act by inhibiting topoisomerase II and DNA gyrase in Gram positive and Gram negative bacteria respectively, thereby interfering with DNA synthesis in the bacteria (Oliphant & Green, 2002). However, resistance to quinolones has been reported in various places especially in developing countries. Poverty, inappropriate prescribing methods, counterfeit and substandard drugs, poor laboratory support and surveillance, lack of antibiotic combination strategies or treatment methods and non-adherence to laid down drugs policies have been identified as the major causes of resistance to antibiotics in the developing countries (Okeke *et al.,* 1999; Okeke *et al.,* 2005).

It is known that drugs found outside hospital set-ups were often sold by hawkers and in shops (Hart & Kariuki, 1998; Okeke *et al*., 2007). These drugs are usually kept under unsuitable conditions for drugs storage and some are sold even after expiration. Another factor that has been identified as contributing to spread of antibiotic resistance in the developing countries is the use of antibiotics in fish and animal production. The drugs are used to boost the production and higher yield in meat and other animal products (WHO, 1998; WHO, 2002; Okoli *et al.,* 2005).

Staphylococcus aureus and *P. aeruginosa* have been widely incriminated as aetiologic agents causing both community acquired and nosocomial infections. In both cases, these organisms are often difficult to treat using first line antibiotics and hence require broad spectrum antibiotics. Onanuga *et al.,* (2005) tested *S. aureus* isolated from women in Abuja, Nigeria against some antibiotics and found 3.3% resistance to sparfloxacin, ciprofloxacin and ofloxacin, while 10% resistance was seen in pefloxacin. A similar study by Aibinu *et al.* (2005) in Lagos showed an increased resistance to quinolones (22.3%) compared to 2.0% obtained in 1999.

In a study by Daini *et al.* (2005) at University College Hospital, Ibadan high resistance was found to ofloxacin (44.0%), pefloxacin (30.1%) and ciprofloxacin (21.7%) from *P. aeruginosa* isolates obtained in the hospital. Olayinka *et al.,* (2004a) tested the susceptibility of some *S. aureus* isolated from Zaria Nigeria to some antibiotics and found 4.8% resistance to ciprofloxacin but no resistance to ofloxacin. A study of *P. aeruginosa* isolated from urine of students at Ahmadu Bello University, Zaria showed uniform susceptibility to ciprofloxacin (Olayinka *et al.,* 2004b). All the *S. aureus* isolates studied in Abia were resistant to nalidixic acid (Chigbu & Ezeronye, 2003).

There are no reports on the resistance patterns of *S. aureus* and *P. aeruginosa* to quinolones in Kano metropolis. In view of the significance of Kano as a centre of commerce and densely populated city, this work will provide some information on the quinolones-resistance patterns of these pathogens to aid planning and control purposes.

MATERIALS AND METHODS
Bacterial isolation and characterization: Clinical samples comprising of sputum, urine, wound swab, ear swab, urethral swab, and high vaginal swab obtained from Murtala Muhammad

specialist hospital (MMSH) and Infectious Diseases hospital (IDH), Kano were cultured on Cetrimide agar and Mannitol salt agar. Colonies that were suspected to be *S. aureus* and *P. aeruginosa* were confirmed using biochemical tests (Cheesbrough, 2002).

Antibiotic Susceptibility Tests. The 200 confirmed isolates (100 each of *S. aureus* and *P. aeruginosa*) were screened for susceptibilities to nalidixic acid, ciprofloxacin, ofloxacin, pefloxacin and sparfloxacin by the disc diffusion susceptibility test on Mueller Hinton agar (NCCLS, 2003). All resistant isolates (in the disc diffusion break points) were confirmed by the agar dilution minimum inhibitory concentration test (NCCLS, 2004).

RESULTS

The study showed that 27 of the *S. aureus* isolates were resistant to the quinolones. Eighteen were resistant to only one quinolones (i.e. 17 resistant to nalidixic acid and 1 resistant to ciprofloxacin) while 9 had multiple-resistances. Thirty eight *P. aeruginosa* isolates were resistant to the quinolones, 25 were resistant to one quinolone (23 resistant to nalidixic acid and 2 resistant to ciprofloxacin). Thirteen had multiple resistances. More resistances were found in *P. aeruginosa* isolates compared to *S. aureus* isolates especially to nalidixic acid, ciprofloxacin and ofloxacin. Sparfloxacin permitted the least number of resistant isolates (Fig. 1).

Nine resistance patterns were observed in all. 8 patterns were observed in quinolones resistant *S. aureus* isolates and 7 patterns observed in the *P. aeruginosa* resistant isolates as shown in Tables 1 and 2 respectively.

The results show that 54 of the *S. aureus* isolates were obtained from the male patients while 46 were from the female patients. There was no statistically significant difference ($p>0.05$) in the resistances of *S. aureus* isolated from male and female patients to nalidixic acid, ciprofloxacin, ofloxacin and pefloxacin (Fig. 2).

In the case of *P. aeruginosa* isolates, 59 were from the male patients while 41 were from the female patients. There were also no statistically significant differences in the resistances found in *P. aeruginosa* isolated from male and female patients (Fig. 3).

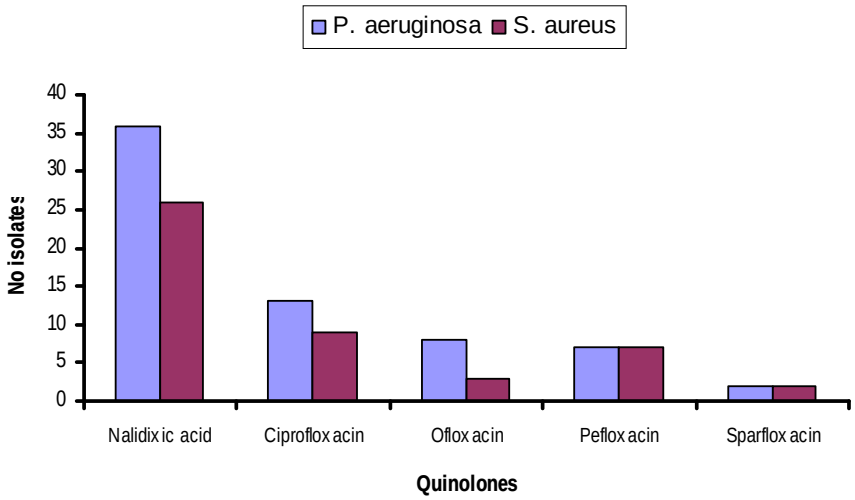

FIG. 1. DISTRIBUTION OF THE RESISTANT *S. aureus* AND *P. aeruginosa*.

TABLE 1. RESISTANCE PATTERNS OF QUINOLONE-RESISTANT *S. aureus* ISOLATES.

NUMBER OF QUINOLONES RESISTANT TO	NUMBER OF S. AUREUS ISOLATES WITH PATTERN (%)	RESISTANCE PATTERNS
1	17 (63.0%)	NAL
	1 (3.7%)	CIP
2	2 (7.4%)	NAL, CIP
	1 (3.7%)	NAL, PEF
3	2 (7.4%)	NAL, CIP, PEF
4	2 (7.4%)	NAL, CIP, OFX, PEF
	1 (3.7%)	NAL, CIP, PEF, SPX
5	1 (3.7%)	NAL, CIP, OFX, PEF, SPX.

Legend: NAL- Nalidixic acid, CIP- Ciprofloxacin, OFX- Ofloxacin, PEF- Pefloxacin, SPX- Sparfloxacin

TABLE 2. RESISTANCE PATTERNS OF QUINOLONE-RESISTANT *P. aeruginosa* ISOLATES.

NUMBER OF QUINOLONES RESISTANT TO	NUMBER OF P. AERUGINOSA WITH PATTERN (%)	RESISTANCE PATTERNS
1	23 (60.5%)	NAL
	2 (5.3%)	CIP
2	5 (13.2%)	NAL, CIP
3	1 (2.6%)	NAL, CIP, PEF
	1 (2.6%)	NAL, OFX, PEF
4	4 (10.5%)	NAL, CIP, OFX, PEF
5	2 (5.3%)	NAL, CIP, OFX, PEF, SPX.

Legend: NAL- Nalidixic acid, CIP- Ciprofloxacin, OFX- Ofloxacin, PEF- Pefloxacin, SPX- Sparfloxacin

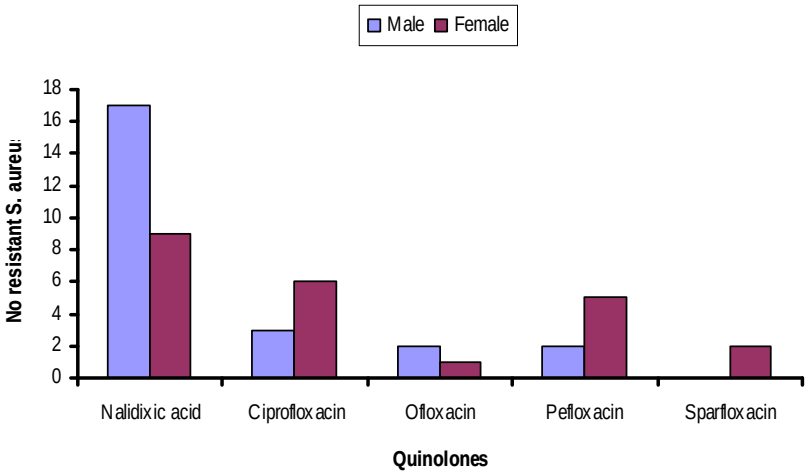

FIG. 2. DISTRIBUTION OF QUINOLONES RESISTANT *S. aureus* ISOLATES IN MALE AND FEMALE PATIENTS.

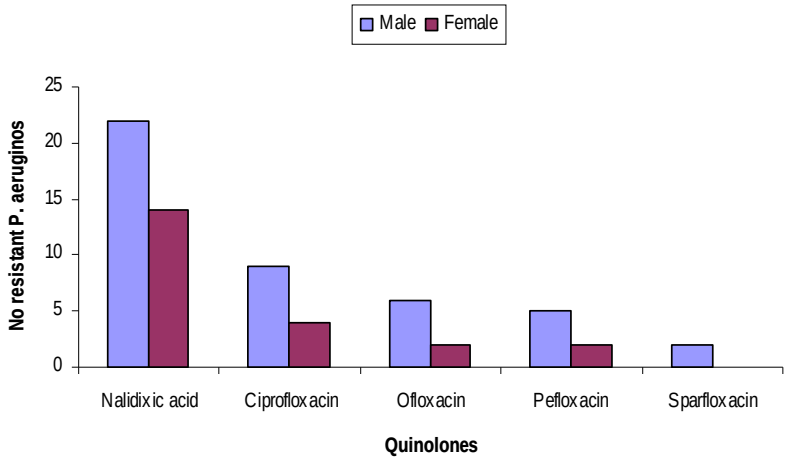

FIG. 3. DISTRIBUTION OF QUINOLONES RESISTANT *P. aeruginosa* ISOLATES IN MALE AND FEMALE PATIENTS.

DISCUSSION

An exceptionally high resistance to quinolones was reported by Enabulele *et al.,* (2006) in Benin, Edo state. This is not the case in the present study. The quinolones were found to be effective against both *S. aureus* and *P. aeruginosa* isolates tested with few resistant cases. More of the *P. aeruginosa* isolates were resistant to multiple quinolones compared to the *S. aureus* isolates. This is not unexpected since *P. aeruginosa* is a Gram negative bacterium coupled with their well known complex cell structure that has been reported to enhance their innate resistances to antimicrobial agents. In other words, Gram negative bacteria as a group are inherently resistant to a number of important antimicrobial agents that are very effective against Gram positive organisms. One logical reason for this has been found in the differences in structural and chemical compositions of the outer layers of the cells (Russell & Gould, 1988).

There was a high level of resistance to nalidixic acid by several of the isolates in this study. This may be because the antibiotic is now cheap and could be afforded by a greater number of people, a situation that could encourage abuse and misuse. However, nalidixic acid is normally not indicated in *P. aeruginosa* infections (King *et al.,* 2000) and therefore the observed high level resistance shown by *P. aeuginosa* isolates does not pose clinical difficulties.

In Respiratory Tract and Urinary Tract Infections, *P. aeruginosa* grows as aggregates of cells (microcolonies) encased in a protective alginate polysaccharide referred to as biofilm thereby resisting eradication by physical and biochemical agents (Lambert, 2002; Hogan & Kolter, 2002). The biofilm formation also assists the culprit organism to establish itself and resist drug treatments (Yi *et al.,* 2005). This implies that organisms may display clear susceptibilities to antibiotics in the *in vitro* studies and still be resistant *in vivo* especially because *P. aeruginosa* has other resistance mechanisms such as resistance to phagocytosis and to the serum bactericidal chemicals due to its mucoid capsule (Ochsner *et al*., 2002) coupled with the fact that most *P. aeruginosa* infections are both invasive and toxinogenic (Iglewski, 2004). This renders the organism difficult to treat.

Apart from the findings in Benin, high quinolones resistance have also been reported in *P. aeruginosa* isolated from other parts of Nigeria (Anupurba *et al.,* 2006; Oguntibeju & Nwobu, 2004), but reports on *S. aureus* resistance profiles have been less alarming (Olayinka *et al.,* 2004a; Onanuga *et al.,* 2005) especially in the Northern part of Nigeria where the present study is based.

The least resistance in the two groups of isolates used in this study were observed in Sparfloxacin. This is contrary to the findings by Nwanze *et al.,* (2007) when they studied UTI in Igbinedion University Teaching Hospital, Delta State. They reported that ofloxacin was the most effective followed by sparfloxacin, pefloxacin then ciprofloxacin. Idu & Odjimogho (2003) once showed that ciprofloxacin was the most effective quinolone during their study. This goes to show that regional differences probably play a role in the resistance profiles of bacteria and further justifies the need to undertake antibiotic susceptibility studies on bacterial isolates from different parts of Nigeria on a regular basis.

There was generally a low level of resistance in both *S. aureus* and *P. aeruginosa* isolated in this study. However, as it has been noted earlier, conditions do exist in Kano which favour the emergence of antibiotic resistance in pathogenic organisms. Similar conditions no doubt exist in other parts of Nigeria and in Africa as a whole. To safeguard the populace therefore, there is a need to enforce antibiotic use policies effectively in Nigeria.

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