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IN VITRO EFFECT OF SOME QUINOLONE ANTIBIOTICS ON STRAINS OF STAPHYLOCOCCUS AUREUS ISOLATED FROM A HOSPITAL ENVIRONMENT.

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ABSTRACT

A total of 30 different strains of *Staphylococcus aureus* were isolated from some selected wards of Madonna University Teaching Hospital (MUTH), Elele, Nigeria, using blood agar and nutrient agar. All the isolates were subjected to some selected quinolones (ciprofloxacin, pefloxacin, ofloxacin, norfloxcin and sparfloxacin) to determine their antibiotic susceptibility pattern using the disk diffusion method. Ofloxacin had the highest percentage susceptibility of 93.3%, followed by ciprofloxacin with 73.3%; pefloxacin was next with 70%, sparfloxacin 63.3%, while norfloxacin recorded the lowest percentage of 50%. The minimum inhibitory concentration (MIC) of the quinolones to the isolates was also determined. The results show that all the tested quinolones had an MIC ranging from 2.5-10μg/ml.

Key words: Staphylococcus aureus, quinolones, hospital environment

INTRODUCTION

Staphylococcus aureus, the most common cause of staphylococcal infections, is a Gram positive, coagulase and catalase positive, spherical bacterium frequently living on the skin or in the nose of a person, that can cause a range of illnesses from minor skin infections (such as folliculitis, impetigo, cellulitis and abscesses), to life threatening diseases such as pneumonia, meningitis endocarditis, toxic shock syndrome (TSS) and septicemia [1]. In addition, 40% of all cases of urinary tract infections (UTI) are due to *Staphylococcus aureus* [2], while in infants the organism causes a severe disease known as staphylococcal scalded skin syndrome (SSSS) [3].

Staphylococcus aureus is one of the few organisms associated with nosocomial infections in health care institutions. In actual fact, they account for as much as one-third of all cases of nosocomial infections in many hospitals. The infections are mostly asymptomatic with human carriers presenting a much more stable problem, being a frequent source of confusion to infection controllers and healthcare providers. This is because most patients serve as formidable reservoirs of antibiotic – resistant pathogens that are responsible for cross-infections in health care institutions [4, 5, 6].

Staphylococcus aureus infection can be spread through contact with discharge from an infected wound, skin-to-skin contact with an infected person, and contact with objects such as towels, sheets, clothing or athletic equipment used by an infected person [7].

Treatment of *Staphylococcus aureus* infection is normally carried out with antimicrobial agents. However, the organism has been known to develop resistance to many of the commonly used antibiotics. This resistance, especially to penicillin, is mediated by penicillinase (β – lactamase) production, which is an enzyme that breaks down the β -lactam ring of the penicillin molecule. To overcome this problem, penicillinase resistant penicillins such as methicillin, oxacillin,

cloxacillin, dicloxacillin and flucloxacillin were developed to treat penicillin resistant *Staphylococcus aureus* infections. Methicillin was the first antibiotic in this class to be used, having been introduced in 1959; but two years later, the first case of methicillin–resistant *Staphylococcus aureus* (MRSA) was reported, reaching its peak in the 1980s when there was an explosion in MRSA prevalence in hospitals where it is now endemic [8, 7, 9].

After the destruction of the efficacy of methicillin by the microorganism, vancomycin became the drug of choice for treating MRSA infections. However, treatment failures, adverse side effects and emergence of vancomycin-resistant MRSA led to urgent requirements for alternative anti-MRSA therapies. In view of this, linezolid (a new agent) was recently developed for Gram-positive bacterial infections, including MRSA. However, resistance to this drug is already developing, thus necessitating the need for the development of more superior anti-MRSA drugs [10, 11, 12].

It is important in this fight to overcome the menace of MRSA, to develop/test for drugs that will target specifically, and inhibit the more aggressive virulent factors of the organism. This need has led to the manufacture of a variety of antimicrobial agents and antibiotics, one of which is a group of new drugs known as quinolones.

Quinolones are antimicrobial agents effective in the treatment of selected community-acquired and nosocomial infections. They are usually administered orally, but some can be given intravenously for treatment of serious infections. They are bactericidal and exhibit concentration-dependent killing. The mode of action of all quinolones involves inhibition of bacterial DNA synthesis by blocking of the DNA gyrase and topoisomerase iv enzymes.

Early quinolones such as nalidixic acid, oxolinic acid and cinoxacin had poor systemic distribution and limited antibacterial activity and were only used primarily for treatment of Gram-negative urinary tract infections. The fluorinated derivatives (e.g. ciprofloxacin, ofloxacin, norfloxacin, enofloxacin, pefloxacin, lomefloxacin etc) have greater antibacterial activity with low toxicity and achieve clinically useful levels in blood and tissues [13, 14].

Due to the ability of *Staphylococcus aureus* to resist treatment with the more common antibiotics, search for more efficacious alternatives by medical and allied scientist has continued unabated. In this study therefore, five (5) quinolones, namely, ciprofloxacin, pefloxacin, ofloxacin norfloxacin and sparfloxacin were tested for antibacterial activity against *S. aureus* isolated from a hospital environment, with a view to making appropriate recommendations to infection controllers and healthcare providers.

MATERIALS AND METHODS

Sources and collection of specimen: A total of 30 swab samples were collected at different times from floors, sink taps, tables and toilets in selected wards, at Madonna University Teaching Hospital, Elele, Rivers State, Nigeria using sterile swab sticks (Evapon sterile swab stick). Each collected sample was immediately taken to the laboratory for culture on blood agar and nutrient agar. After 18-24hrs incubation period at 37^0C, *Staphylococcus aureus* isolates were initially identified based on their cultural characteristics on blood agar and nutrient agar. Confirmation of the isolates was carried out through microscopy, catalase, coagulase and motility tests. The confirmed isolates were sub-cultured using peptone water and after another 18hrs incubation at 37^0C, preserved in the refrigerator at 4^0C as a stock culture that will be used for assay of antibacterial activity.

Antibacterial assay: pure cultures of bacterial isolates were subjected to antimicrobial susceptibility using the disk diffusion (or Kirby Bauer) method as applied by [15] Bruner *et al*., (1995). A volume of 0.1ml of purified stock culture of *S. aureus* was transferred to each nutrient agar plates and spread over the surface of the medium using a bent glass rod (or Hockey stick) in duplicates. The surface of the agar plate was allowed to dry. Single discs, each impregnated with standard concentrations of one of the five test quinolones, (ciprofloxacin (CIP), ofloxacin (OFX), pefloxacin (PEF), sparfloxacin (SPAR) and norfloxacin (NORF)) were carefully and asceptically placed on the inoculated agar medium. The plates were then inverted and incubated at 37^0C for 24hrs after which observations were made for emergence of zones of inhibition. Zones measuring 18mm diameter and above were regarded as indicative of susceptibility while those between 13-17mm were regarded as intermediate and the ones less than 12mm resistant.

Determination of Minimum inhibitory Concentration (MIC): The minimum inhibitory concentration (MIC) of the antimicrobial agents was determined by the agar dilution method as adopted by [15] Brauner *et al*., (1995). Serial dilutions of standard concentrations of each of the test drug were carried out and incorporated into 9ml volumes of Diagnostic Sensitivity Test agar broth (oxoid) in test tubes to give final concentrations of 2.5µg/ml, 5.0µg/ml, 7.5µg/ml, 10µg/ml, 20µg/ml and 30µg/ml. As reported by Jawetz et al., (2001), 1ml of standard concentrations of *S. aureus* was added into each tube and incubated for 24hrs at 37^oC. The MIC for each drug was recorded as the lowest concentration of the drug that inhibited visible growth. Microbial growth however was indicated by turbidity presence while clearance indicated 'no growth' or bactericidal activity.

RESULTS

Results of the susceptibility test show that Ofloxacin (OFX) exhibited a greater antibacterial activity than the rest of the drugs. As shown in Table 1, Ofloxacin inhibited 28 of the isolates while only 2 were resistant. It is followed by ciprofloxacin (CIP) which inhibited 22 of the isolates with only 8 showing resistance.

The least susceptibility was achieved with norfloxacin (NORF) which inhibited 15 of the isolates, while the rest of the 15 isolates were resistant.

Table 1: Antibiotic susceptibility pattern of *S. aureus* isolated from MUTH

Isolate	OFX		CIP		PEF		SPAR		NORF	
	S	R	S.	R.	S.	R.	S.	R.	S.	R
S. aureus	28	02	22	08	21	07	19	11	15	15

Key

CIP	**Ciprofloxacin**
OFX	Ofloxacin
PEF	Pefloxacin
SPAR	Sparfloxacin
NORF	Norfloxacin
S.	Susceptible

RESISTANT

Table 2 shows the percentage susceptibility pattern of *Staphylococcus aureus* to the quinolones tested. With ofloxacin 93.3% of the isolates were susceptible, followed by ciprofloxacin, which inhibited 73.3% of the isolates. Pefloxacin was next with 70% susceptible, while sparfloxacin achieved 63.3% susceptibility with the organism. Norfloxacin achieved the least susceptibility of 50% with the isolates.

Table 2: Percentage (%) susceptibility of *Staphylococcus aureus* strains to some selected quinolones

Number of *S. aureus*	OFX	CIP	PEF	SPAR	NORF
	S 28	S 22	S 21	S 19	S 15
30	93.3	73.3	70	63.3	50

Key

S. Sensitive

The results of the tube dilution (Table 3) show that all the strains of Staphylococcus aureus were killed by these drugs at MIC ranging from 2.5-10µg/ml. At MIC of 2.5µglml OFX was active against most Staphylococcus aureus isolated from the sites. At the same concentration, PEF was bactericidal to a lower number of the isolates but showed greater inhibition at MIC 5µg/ml-7.5µg/ml, while SPAR was able to exhibit its antibacterial activity against the isolates at MIC 7.5µg/ml-10µg/ml. The ability to inhibit most of the strains of this organism at MIC of 2.5µg/ml still shows that ofloxacin (Tarivid) is the drug of choice in the treatment of hospital acquired Staphylococcus aureus infections.

Table 3: MIC ranges of some quinolones on some strains of *S. aureus* (µg/ml)

CIP	OFX	PEF	SPAR
2.5-5.0	2.5-5.0	5.0-7.5	7.5-10.0

DISCUSSION

From the results, (Table 1), it was observed that ofloxacin (Travid) exhibited greater inhibitory effect against the organism than the rest of the quinolones studied. The drug inhibited 28 (93.3%) of the isolates showing that if this drug is not abused, it could provide succor to the prevalence of MRSA in hospitals and in the community. This fact was further established by the finding that the drug was able to achieve this nearly 100% inhibition rate at MIC of 2.5µg/ml (Table 2).

Even though, Tarivid was observed as the best of the quinolones from the results, the other members of the group studied were no less effective in exerting their antibacterial effect against isolates of the organism. Remotely following Tarivid and inhibiting 22 (73.3%) of the isolates at MIC 2.5 – 5.0µg/ml was ciprofloxacin while pefloxacin and sparfloxacin inhibited 21 (70.0%) at MIC 5.0-7.5 µg/ml and 19(63.3%) at MIC of 7.5-10.0 respectively. These results show that if properly used and the right does administered, these drugs could also be effectively utilized to treat infections due to *S.aureus* in addition to Tarivid. However, the last member of the group studied, norfloxacin, was not as effective as it was able to inhibit only 15 (50%) of the isolates. Since 50% of the isolates are still resistant, it follows that if abuse of this drug is continued through indiscriminate usage, efficacy of the drug could still reduce further, the organism could then develop complete resistant to it thereby rendering the drug ineffective in the treatment of *S.aureus* infections.

Over time, some bacteria, including *Staphylococcus aureus* have been known to develop ways to circumvent effects of antibiotics. This is especially possible due to the widespread use of antibiotics, which spurred evolutionary adaptation that enabled bacteria to survive these powerful drugs. Drug abuse is the bane of our society and an enabling factor to antibiotic resistance. To save the quinolones, and indeed other newly developed antibiotics, from suffering from ineffectiveness as some of those before them, we recommend that none of these quinolones must be taken without proper prescription from a qualified medical practitioner. Such medical practitioners on their part, should be guided by results of sensitivity tests from reputable medical laboratories. In localities where antibiotics can be purchased without prescription like buses, provision stores, street hawkers etc as is commonly

observed in some cities in Nigeria, laws could be enacted that will ban the sale of such drugs in those unauthorized and unapproved places.

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