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Full Length Research paper

Multi-drug resistant *Staphylococcus aureus* in clinical cases in Ile-Ife, Southwest Nigeria

Adegoke, Anthony Ayodeji[1*] and Komolafe, Amos Omoniyi[2]

[1]Department of Microbiology, University of Uyo, Uyo, Akwa Ibom State, Nigeria.
[2]Department of Medical Microbiology and Parasitology, Obafemi Awolowo University Hospitals Complex, Ile-Ife, Nigeria.

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A study to investigate the susceptibility pattern of *Staphylococcus aureus* to conventional antibiotics being frequently prescribed in Ile-Ife, South Western Nigeria was carried out. One hundred and seven samples from various clinical samples were collected from Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria. The isolates of *S. aureus* were characterized and identified using standard microbiological techniques. The isolates harvested were subjected to *in-vitro* antibiotic susceptibility tests using the disc diffusion method and beta-lactamase test was carried out using starch paper hydrolysis. One hundred and five isolates of *S. aureus* were harvested. The *in vitro* susceptibility of the bacterial isolates to antibiotics indicated 75.3-92.5% sensitivity to cefotaxime, ciprofloxacin and pefloxacin. However, they were 65% producers of beta lactamase while 45-72% resistant to penicillin, ampicillin, tetracycline and cotrimoxazole. Out of the 56 isolates tested with methicillin, 28 (50%) were resistant while 18 (32.1%) were sensitive. It was observed 70% of the tested isolates were beta lactamase producers. Multiple resistance was observed to 10 frequently prescribed antibiotics in the area. This suggests possible abuse of these drugs, poor hospital attendance and the need for better enlightenment campaign against the use of drug without prescription.

Key words: *Staphylococcus aureus,* ciprofloxacin, *multiple* antibiotic resistant index, beta lactamase test.

INTRODUCTION

Staphylococcus aureus is the cause of a wide range of pyogenic infections, though also a commensal of human skin and nares. The gram positive cocci in cluster are the leading cause of bloodstream, lower respiratory tract, and skin/soft-tissue infections (Lowry, 1998; Prescott et al., 2006). Staphylococcal infection leads to a worsening of some already existing superficial infections and that antibacterial treatment is beneficial when children have cases of impetigo. Infection ranges from such superficial infection to deep infection as septicaemia, making *S. aureus* an important subject of consistent studies (Komolafe and Adegoke, 2008). Each year some 500,000 patients in American hospitals contract a Staphylococcal infection (Bowersox and John, 2007).

The role of antibiotics is, however, more controversial when the skin is only colonized and not clinically infected (William, 2000; Lubbe, 2003). Infection rate from *S. aureus* is high. The recent increased recognition of community acquired infections has important clinical and pharmacological implications for the health care provider (Narinder, 2005). Urgent control measures should be taken to combat the renowned aetiology of both nosocomial and community acquired infection.

In recent years, many isolates of *S. aureus* have evolved resistance to both synthetic and traditional antimicrobial chemotherapy and their prevalence outside the hospital is of potential epidemiological threat (Daum and Seal, 2001; Kaplan et al., 2005). Obviously, beneficial retrospective studies on multi-drug resistance must put the available conventional antibiotics in the area into consideration.

In this paper, prevalence of multi-drug resistant *S. aureus* to conventional antibiotics of frequent use in Ile-Ife metropolis, south western Nigeria is presented.

*Corresponding author. E-mail: anthonyadegoke@yahoo.co.uk. Tel: 2348038398510.

Table 1. Age distribution of the subjects (patients).

Age Range	No of Patients	Percentage (%)
1 day- 1 year	25	23.4
>1year-12 years	14	13.1
>12 years-18years	6	5.6
>18	62	57.9

MATERIALS AND METHODS

Study population

The subjects comprised one hundred and seven (107) children and adults of both sexes aged between one day to 70 years having clinical features suspicious to be from Staphylococcal-related infection and were on admission at Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife. Sample collection took place before chemotherapy.

Sample analysis

Blood specimens were collected into already prepared and sterilized 10 ml thioglycolate broth while surface-based specimens like septic sores were taken with sterile cotton-tipped applicators (cotton wool swab made by Transwab Medical Wire and Equipment Co-Ltd, Cosham, United Kingdom). The swabs were also inoculated aseptically into 5 ml thioglycolate broth.

The broth cultures were incubated in the laboratory for 18-24 h. The inoculums from the broth cultures were inoculated within 24 h onto Mannitol Salt Agar (MSA) with the already heat-flamed inoculating loop. The plates were then incubated aerobically for 24 h at 37°C. After overnight incubation, the plates were examined for fermentation of mannitol indicated by colour change of the medium around each colony from red to yellow.

The organisms on the positive plates were gram stained; catalase test and coagulase test (both slide and tube test) were carried out on the Gram positive cocci in cluster isolates as described by Rigby (1986). The organism was characterized using the criteria of Cowan and Steel (Cowan and Steel, 2004).

Antimicrobial agents and *in vitro* susceptibility pattern

Our objectives to study resistance to the commonly used conventional antibiotics in this area informed the choice of antibiotics. Inhibitors of the cell wall synthesis (penicillin, ampicillin, cefotaxime, augmenting, amoxycillin, cloxacillin and ceftriazone), protein synthesis (gentamycin, streptomycin, tetracyclines, chloramphenicol and erythromycin), and nucleic acid synthesis (ciprofloxacin, cotrimoxazole and pefloxacin) were utilized for inhibition tests. The disc-diffusion method for *in-vitro* antibiotic susceptibility test described by Bauer et al. (1966) was used in this study.

Interpretation and evaluation of the antimicrobial susceptibility

The interpretation of the diameter of inhibition of the conventional multi-discs and single disc used was carried out with manufacturers' interpretation manuals. The percentage resistance was calculated as follows:

Percentage resistance = (No of resistant isolates/No of isolates tested with the antibiotic) x 100

Beta lactamase test

Based on the resistance of the *S. aureus* isolates to penicillin and other beta-lactam antibiotics used, beta-lactamase test was carried out using the method of Odugbemi et al. (1977). Strips of starch paper about 4 – 7 cm were cut and sterilized using 70% ethanol, the strips were soaked for 10 min in benzyl penicillin dissolved in phosphate buffer with 100,000 units. The cut strips were then spread evenly on Petri dishes and about 18 – 24 h old cultures grown on Nutrient Agar were inoculated on the surface of the test paper and spread over an area of 2 -3 mm. The Petri dishes were incubated at 37°C for 30 min then Gram's iodine solution was used to flood the plate and drained off immediately. The starch paper turns uniformly black within 30 s of application colonies with decolourized zones are positive for beta-lactamase but colonies with black background show beta-lactamase negative. The result was read within 5 min because if the time extended further the black background indicative of negative will start decolourising thus giving a false positive result.

RESULTS

The samples were collected from 39 females and 68 male patients on admission with cases suggestive of staphylococcal origin at Obafemi Awolowo University Teaching Hospitals, Ile-Ife, a hospital being attended by over five million people within the South-Western Nigeria and beyond. One hundred and seven isolates of *S. aureus* were recovered from various clinical samples. Some of the cases include septic wound, osteomyelitis, lung abscesses, neonatal jaundice, burkitts lymphoma, pyrexia, urethritis, septic abortion, infertility, etc. The age distribution of the patients is depicted in Table 1. All the patients had record of fever and temperature above normal body temperature.

Figures 1 to 3 show antibiotic pattern of Isolates in Ile-Ife. The isolates showed high level resistance to the frequently prescribed antibiotics in the area. Seventy-seven isolates (72.0%) and 72(67.2%) isolates were resistant to beta lactam antibiotics: penicillin and ampicillin, respecttively. About 21(24.7%) isolates were resistant to the supposedly fairly stable antibiotic to beta lactamase, cephalosporin: cefotaxime. As high as 9.7 and 7.5% isolates were resistant to beta lactam stable fluoroquinolones: ciprofloxacin and peflacine, respectively. Out of the 56 isolates tested with methicillin28 (50%) were resistant while 18 (32.1%) were sensitive (Table 2).

Multiple antibiotic resistances were observed. Twenty one (19.6%) isolates were resistant to 7 antibiotics together and another 19.6% were resistant to 5 antibiotics. A multiple resistant index per isolates of 0.2 was observed for seven frequently prescribed antibiotics in the area (Table 3). Based on the resistant pattern of the isolates against the ß-lactam antibiotics, seven of the ten isolates

Table 2. Methicillin resistance.

Antibiotics	Number (%) of resistance isolates	Number (%) of intermediate isolates	Number (%) of susceptible isolates
Methicillin	28 (50%)	10 (17.9%)	18 (32.1%)

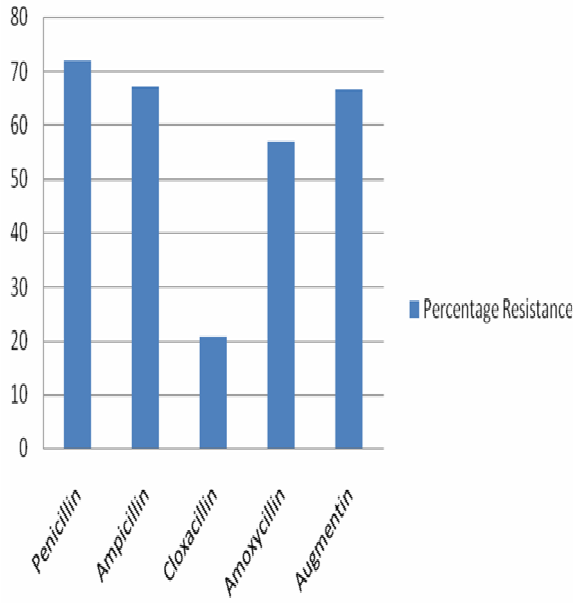

Figure 1. Percentage of the isolates to the ß- lactam antibiotics.

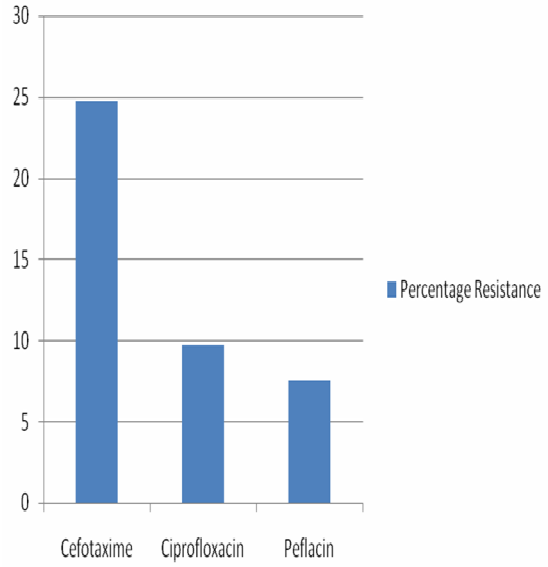

Figure 3. Percentage resistance of the isolates to third generation cephalosporin (cefotaxime) and fluoroquinolone.

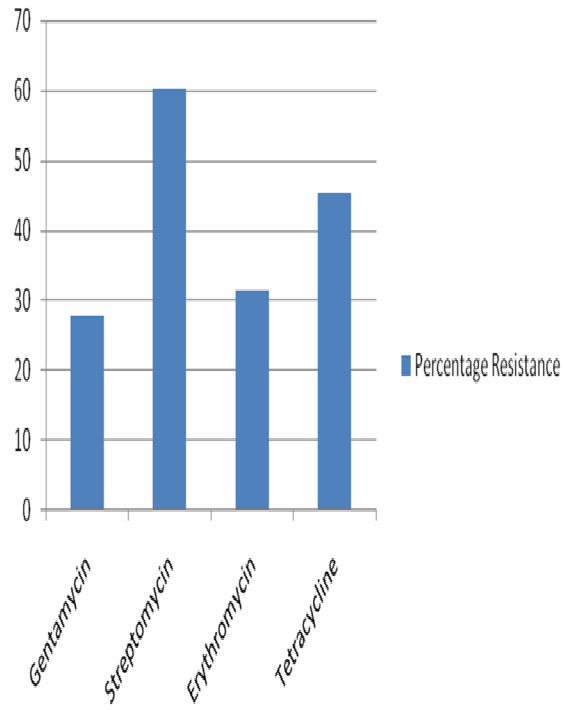

Figure 2. Percentage resistance of the isolates to aminoglycosides and tetracycline.

tested for ß-lactamase were positive for the production of the enzyme.

High percentage of multiple drug resistance quantified as index per isolates (Table 3) was illustrated in percentage. One isolate in this study was resistant to all the antibiotics used in this research work (Figure 4).

DISCUSSION

S. aureus remains the most prominent aetiology of pyogenic infections. This organism was observed in 23(21.5%) cases of clinically reported septicaemia and 80(74.8%) cases of septicaemia concomitantly occurring with other clinical symptoms make the organisms' subject of concern. Cross implication of this organism in diverse clinical cases makes it of importance to the epidemiologists. Febrile noticed among the patients could just be explained as body's immunological reaction to infection.

The trend of antibiotic resistant to large number of commonly prescribed antibiotics observed in this study confirmed the validity of earlier observation (Grisold et al., 2002). Despite the emergence of penicillin resistance in 1942, the antibiotic is still being used to treat myriads of Staphylococcal infection. The 72.0% penicillin resistance observed in this research area might have emerged from

Table 3. Multiple resistance index with respect to isolates

Number of a Antibiotics to which Resistance occurred	Number of Resistant Isolates	Multiple Resistance Index
0	2	0.18
1	4	0.38
2	1	0.09
3	12	0.11
4	20	0.19
5	21	0.20
6	14	0.13
7	21	0.20
8	9	0.10
9	2	0.02
10	1	0.01

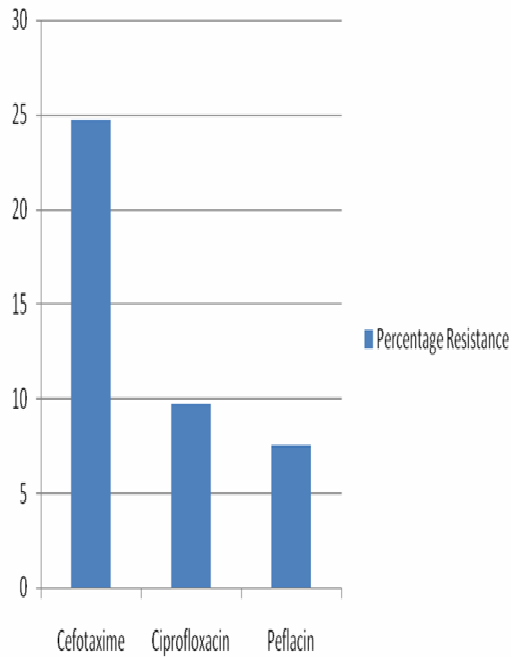

Figure 3. Percentage resistance of the isolates to third generation cephalosporin (cefotaxime) and fluoroquinolone.

the hospitals and spread to the community (Couto, et al. 2000). Even the improved form of penicillin, ampicillin had 67.2% resistance thus contributing the record of sick individuals worldwide with ampicillin resistant infection (Frikin et al., 2003). Resistance of 24.7% to the third generation cephalosporin, cefotaxime observed in this study area can be attributed to abuse of antibiotics by illegal hospital within the study area since parenteral drugs are not easily abused by individuals. Incidentally, seven of the ten isolates that produced beta lactamase were resistant to all the ß-lactam antibiotics used in this study. This revealed that the resistance is purely plasmid based since ß-lactamase production is plasmid based (Rigby, 1986). This contributed a lot to the level of multiple drug resistance as about 85.6% of the isolates show resistance to at least three or more antibiotics.

Consideration of the socio-demography of the patients revealed that the adult above 18 year dominated (57.9%). They can shift chemotherapy easily to drugs that are stable against ß-lactamase enzyme like fluoroquinolone which however cannot be used by the children in the first category (Table 1). Though higher MRSA than MSSA was observed in this study, the antibiotics could provide a better therapy than the beta lactam drugs used. The reason might not be far from the stability of the drug to beta lactamase production. The resistance however might suggest the role of mec A gene in the isolates (Murakami et al.1991). In view of the foregoing, this would be chemotherapy of choice due to the observed low resistance of 9.7 and 7.5% to Ciprofloxacin and Peflacin, respectively.

The upsurge in the antibiotic resistance noticed in this study is in agreement with an earlier report by Obseiki Ebor et al. (1987) where antibiotic abuse and high prevalence of self medication with antibiotics were identified as being responsible for the selection of antibiotic resistant bacterial strains. This piece of work has demonstrated vividly the urgent need for management strategies designed for specific groups of patients with infections in order to maximize thera-peutic benefits, cost reduction and possible reduction in the incidence of adverse drug reactions. Although the sensitivity of the organism isolated to the third generation cephalosporin (cefotaxime) and fluoroqui-nolone were generally excellent in the present study, the high cost of this group of drugs precludes their use as first choice in the treatment of septicaemia, usage policy that would be made applicable to the different tiers of our health care providers at the primary, secondary and tertiary levels. This can be done concurrently with sustained enlightenment and media publicity focusing attention on the dangers of high inci-

dence of bacterial resistance to antibacterial agents in general and the ultimate consequences

The idea of vaccine against Staphylococcal infection would be a welcomed development (Yukiko et al*.,* 2006) since vaccines are not usually available for abuse. It is time to embrace the use of local plant extract with proven therapeutic and prophylactic potency (Adebayo-tayo and Adegoke, 2008; Adegoke and Adebayo-tayo, 2009; Oloke et al., 1988).

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