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Full Length Research Paper

Antibiotic susceptibility patterns and penicillinase activity of *Staphylococci* spp from wound specimens in Lagos

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Forty-five *Staphylococci* spp comprising 33 (73.3%) *Staphylococcus aureus* and 12 (26.7%) *Staphylococcus epidermidis* isolated from both non-surgical and post-surgical septic wounds were evaluated for penicillinase activity. Thirty (90.9%) of the *S. aureus* isolates produced beta-lactamases. Beta-lactamase production accounted for the high level resistance (70-90%) to the penicillins. Five (16.7%) of the penicillinase-producing *S. aureus* (PPSA) were resistant to methicillin, while 25 (83.3%) PPSA were sensitive. Resistance to the cephalosporins by the PPSA was between 7 and 30% indicating that the beta-lactamase produced was mostly penicillinase. Ciprofloxacin, vancomycin and rifampicin were the most active of the antibiotics against methicillin-resistant *S. aureus* (MRSA) with activity of between 90 and 100%. They also gave low minimum inhibitory concentration (MIC) values (0.125-8 µg/ml for ciprofloxacin, 0.125-2 µg/ml for vancomycin and 2-32 µg/ml for rifampicin). Methicillin activity of 83.3% was comparable with those of erythromycin, ofloxacin and pefloxacin (range 73.3-86.7%). Azithromycin activity against the PPSA was 90%. *S. aureus* is the specie of *Staphylococci* most frequently isolated from septic wound and have shown high rate of resistance to beta-lactam antibiotics which could be attributed to their production of beta–lactamase enzyme especially, penicillinase.

Key words: Antibiotic, susceptibility patterns, penicillinase activity, *Staphylococci*.

INTRODUCTION

Staphylococcus aureus causes a variety of superficial and deep pyogenic infections. Superficial infections of *S. aureus* include skin abscesses (furuncles and carbuncles), folliculitis, wound sepsis, conjunctivitis, cellulitis, impetigo and scalded skin syndrome. Deep seated infections include endocarditis, osteomyelitis, pyelomelytis, septic arthritis, skin, brain and lung abscesses (Daum, 2007).

Ninety percent of staphylococci strains are penicillin resistant (Baorto, 2004), and this has been attributed to the production of the enzyme, β-lactamase, which has the ability to cleaves the β-lactam ring of the antibiotic, producing penicilloic acid in the process. β-lactamase activity and by extension, the presence of penicilloic acid can be detected by measuring the resulting change in pH of the culture medium with an indicator dye, or by its property of reducing iodine; and in the process, reversing the blue-black colour formed when starch complexes with iodine (Guignard et al., 2005; Rotimi, 1988). Newer generations penicillins such as methicillin and nafcillin are however, stable to beta-lactamase activity (Guignard et al., 2005). The iodometric method was adopted in this study.

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Bete-lactamase producing *S. aureus* are often recovered from patients that had been hospitalised hence, are designated hospital-acquired (nosocomial) infection (Johnson et al., 2001). Recent studies have identified *S. aureus* as the main etiological agent of many infections in sub-Saharan Africa (Olatunji et al., 2007; Feleke et al., 2007; Mulu et al., 2006), and a number of investigations have reported that it is among the most frequently encountered bacterial species in microbiology laboratories in Nigeria (Obidike et al., 2009; Odetoyin et al., 2008; Ambe et al., 2007). Development of resistance to methicillin is also very common among this specie and had over the years, posed serious therapeutic problems because of its multi-drug resistance patterns. A community-associated MRSA clone with a unique resistance profile has also been reported from South-West Nigeria (Ghebremedhin et al., 2009). To understand and potentially predict trends in antibiotic-resistance patterns and to establish adequate infection control programs, it is crucial to understand the local epidemiology of *S. aureus* in Nigeria. Knowledge of the local antimicrobial resistance patterns of bacterial pathogens is essential to guide empirical and pathogen specific therapy. The objectives of this study are therefore, to investigate penicillinase activity among clinical isolates of staphylococci and to evaluate the susceptibility patterns of these isolates to β-lactamase-stable β-lactam antibiotics and non-β-lactam antibiotics.

MATERIALS AND METHODS

Specimen collection

Wound specimens were collected from out-and-in-patients of General hospital Gbagada and R-Jolad hospital, both in Lagos, Nigeria. A total of 98 samples; 30 from R-Jolad hospital and 68 from General hospital were collected. Ten of the samples from R-Jolad were from surgical wounds and the remaining 20 were from non-surgical wounds. Twenty eight of the 68 samples from General Hospital were from surgical wounds while the remaining 40 were from non-surgical wounds (Table 1).

Samples were collected early in the morning before the wounds were dressed. Before collection of samples, the wounds surfaces were cleaned with cotton swab moistened in normal saline. This step was necessary in order to remove surface contaminants that are not really pathogens associated with the infection. Thereafter, sterile swabs were used to collect samples from deep portion of the wound. These include head and neck region, the arm, abdomen, the limb and pelvic region.

Processing of samples

The specimens were inoculated onto Mannitol salt agar plates by using the swab to prepare a butt on a corner of the plates. The inoculum was spread over the entire surface by streaking in quadrant, the loop being flamed between each streaking to obtain discrete colonies. A smear of each sample was also prepared on a slide, Gram stained and viewed under the microscope. The inoculated plates were then incubated aerobically at 37℃ overnight. The cultural characteristics of the isolates were studied and they were Gram stained and cultivated on nutrient agar slope for further identification.

Identification of isolates

Preliminary identification of the isolates was done by carrying out biochemical tests which include; test for catalase production, ability to ferment glucose and coagulase production. Novobiocin susceptibility test was performed on isolates that are coagulase negative to differentiate suspected *S. epidermidis* from *S. saprophyticus*. The isolates were confirmed with Analytical Profile Index (API STAPH V4.0) kits.

Beta-lactamase production test

The iodometric method was used to assay for β-lactamase production among the isolates. A dilute solution of penicillin (1:10,000 dilutions) was prepared and 1ml each was dispensed into test tubes labelled according to the number of the isolates to be tested. A 10% (w/v) of starch solution was prepared and 1ml each was added to the tubes containing the penicillin solution. One millilitre of 18 h broth culture of the isolates was added to each of the tubes containing the starch solution and penicillin. Three controls were set up thus: a tube containing only 1 ml of starch solution (A); a tube containing starch solution and penicillin only (B); and a tube containing starch solution and culture without penicillin solution (C). The tubes containing the test organisms and the controls were incubated at 37℃ for 1 h. After incubation, a few drops of Lugol's iodine were added to all the tubes including controls A, B and C and results read within 10 sec. The absence of a blue-black colouration in the tubes containing the test organisms confirms β-lactamase production. Expectedly, the control tubes; A, B and C showed blue-black colouration.

Antibiotics susceptibility test

The Kirby-Bauer (1959) method of disc diffusion was used in this study. Four colonies of a pure culture of each of the staphylococcal isolates on nutrient agar plate were inoculated into 5 ml of brain heart infusion broth in a test tube and incubated at 37℃ for 6 h. The turbidity of the broth culture was adjusted with Phosphate Buffered solution (PBS) to match the turbidity of No. 0.5 Mcfarland Standard. A sterile cotton swab was dipped into the broth culture and excess fluid drained off the side of the tube. The charged swab was used to evenly inoculate the entire surface of Mueller Hinton agar plate (Bauer et al., 1959).

The following antimicrobial agents these respective concentrations were used in this study: ampicloxacin (15 µg), amoxicillin (10 µg), ampicillin (10 µg), benzyl penicillin (penicillin G) (10 units), methicillin (1 µg), ceftriazone (30 µg), ceftazidime (30 µg), cefaclor (30 µg) and cefuroxime (30 µg). Others are ciprofloxacin (5 µg), pefloxacin (10 µg), norfloxacin (10 µg), ofloxacin (5 µg), gentamicin (10 µg), rifampicin (5 µg), azithromycin (15 µg), and erythromycin (15 µg) and vancomycin (30 µg) (NCCLS, 2001). The antibiotics were Oxoid products in single disk packed in cartridges of fifty units each. The antibiotics-impregnated disks were placed firmly on the surface of the agar with sterile forceps. The plates were incubated at 37℃ for 18 h. The diameter of the zone of inhibition was measured in millimetre with a ruler under reflected light. Isolates were classified as susceptible, intermediate or resistant according to National Committee for Clinical Laboratory Standards (NCCLS, 2001).

The Minimum Inhibitory Concentrations (MICs) of the antimicrobial agents were determined by agar dilution testing according to guidelines of the NCCLS (NCCLS, 2001). The lowest dilution of the antibiotics that inhibits visible growth of the organism was recorded

Table 1. Distribution of wound swab by hospital location and sex.

Type of wound	Gbagada Hospital	R-Jolad Hospital	Total
Non-surgical	40-Male=25 -Female=15	20-Male=12 -Female=8	60
Post-surgical	28-Male=18 -Female=10	10-Male=6 -Female=4	38
Total	68	30	98

Table 2. Occurrence of *Staphyloccus* spp. in non-surgical and post-surgical wound infections and their distribution by anatomical sites.

Anatomical site/ nature of wound	*S. aureus*	*S. epidermidis*	Total % in parentheses
Head and neck	5	4	9
The arm	8	2	10
Abdomen	10	2	12
Limb and pelvic	10	4	14
Total no. of isolates	33	12	45
Non-surgical wound	29 (74.4)	10 (25.6)	39 (100)
Post-surgical	4 (66.7)	2 (33.3)	6 (100)
Total	33	12	45 (100)

as the MIC for that antibiotic.

Screening for methicillin resistance was done by disk diffusion testing with 1 µg of oxacillin per disk placed on inoculated Mueller-Hinton agar with 2% NaCl supplementation. The zones of inhibition were determined after an incubation of 24 h at 30℃. Methicillin resistance was defined according to NCCLS breakpoints (NCCLS, 2001).

The Statistical Package for the Social Sciences (SPSS) was used for statistical analyses. Pearson chi-square test and analysis of variance (ANOVA) was used to analyze characteristics associated with bacterial isolates. P value <0.05 was considered statistically significant and a trend toward association was established for p value >0.05 and <0.15 at 95% confidence interval (CI).

RESULTS

Staphylococcus spp were isolated from 45 (45.9%) of the 98 septic wounds swab samples. Thirty three (73.3%) of the isolates were *S. aureus* while the remaining 12 (26.7%) isolates were *S. epidermidis.* Four (66.7%) of the six isolates from post-surgical wound sepsis were *S. aureus* while the remaining 2 (33.3%) isolates were *S. epidermidis. S. aureus* accounted for 29 (74.4%) of the 39 isolates from non-surgical wounds (Table 1).

The distribution of the isolates by anatomical sites is shown in Table 2. The head and neck region (Five of *S. aureus* and four of *S. epidermidis*), arm (eight of *S. aureus* and two of *S. epidermidis*), abdominal region (ten isolates of *S. aureus* and two of *S. epidermidis*), limb and pelvic region (Ten of *S. aureus* and four isolates of *S. epidermidis*).

The susceptibilities of the staphylococci to the β-lactam antibiotics are shown in Table 3. Higher activities were recorded against *S. aureus* by ceftriazone (93.9%), cefaclor (69.7%), cefuroxime (75.8%) and ceftazidine (87.9%). Against *S. epidermidis* the antibiotics activities were ceftriazone (91.7%), cefaclor (33.3%), cefuroxime (50%) and ceftazidime (66.7%). Among the penicillins, the activity of ampicloxacillin, amoxicillin, ampicillin and benzyl penicillin against *S. aureus* were 30.3, 27.3, 19.1 and 9.1%, respectively. Against *S. epidermidis* activities were ampicloxacillin (25%), amoxicillin (25%), ampicillin (16.7%) and benzyl penicillin (8.3%).

The MIC range for the β-lactam antibiotics against *S. aureus* were ampicloxacillin (2-128 µg/ml), amoxicillin (2-128 µg/ml), ampicillin (4-512 µg/ml), benzyl penicillin (4-1024 µg/ml), ceftazidime and cefuroxime (1-128 µg/ml). Against *S. epidermidis* the MIC range were ampicloxacillin and amoxicillin (2-128 µg/ml), ampicillin (4-256 µg/ml), benzyl penicillin (4-1024 µg/ml), ceftazidime and cefaclor (16-128 µg/ml), cefuroxime (2-128 µg/ml) and ceftriazone (1-64 µg/ml) (Table 4).

Beta-lactamase production was observed amongst 30 (90.9%) of the *S. aureus* isolates and 10 (83.3%) of the *S. epidermidis* isolate. Five (16.7%) of the β-lactamase producing *S. aureus* were resistant to methicillin while 25 (83.3%) were sensitive. Two (20%) of the *S. epidermidis* isolates were resistant to methicillin while 8 (80%) were sensitive.

The susceptibilities of PPSA to other antibiotics are

Table 3. Susceptibility patterns of the staphylococci to β-Lactam antibiotics.

Antibiotics	*S. aureus* No. tested=33	*S. epidermidis* No. tested=12
Ampicloxacin	10 (30.3)	3 (25)
Amoxicillin	9 (27.3)	3 (25)
Ampicillin	6 (18.1)	2 (16.7)
Benzyl Penicillin	3 (9.1)	1 (8.3)
Ceftazidime	29 (87.9)	8 (66.7)
Cefuroxime	25 (75.8)	6 (50)
Cefaclor	23 (69.7)	4 (33.3)
Ceftrizone	31 (93.9)	11 (91.7)

Thirty three *S. aureus* and 12 *S. epidermidis* isolates were tested.
Percentages of isolates sensitive to antibiotics are given in parentheses

Table 4. MICs (µg/ml) of the staphylococci to the β-Lactam antibiotics.

Antibiotics	*S. aureus*			*S. epidermidis*		
	MIC range	MIC_{50}	MIC_{75}	MIC range	MIC_{50}	MIC_{75}
Ampicloxacin	2-128	8	32	2-128	16	32
Amoxicillin	2-128	16	64	2-128	16	64
Ampicillin	4-512	32	128	4-256	16	64
Benzyl Penicillin	4-1024	128	512	4-1024	64	125
Ceftazidime	2-128	8	16	16-128	8	32
Cefuroxime	2-128	4	32	2-128	4	64
Cefaclor	1-128	4	32	16-128	8	64
Ceftriazone	1-128	4	16	1-64	4	16

Table 5. Susceptibility of penicillinase producing *Staphylococcus aureus* (PPSA) to other antibiotics.

Antibiotics	No. of Isolates	No. (%) sensitive	No. (%) resistant
Ciprofloxacin	30	30 (100)	0 (0)
Pefloxacin	30	26 (86.7)	4 (13.3)
Ofloxacin	30	26 (86.7)	4 (13.3)
Norfloxacin	30	15 (50.0)	15 (50.0)
Gentamicin	30	16 (53.3)	14 (46.7)
Erythromycin	30	22 (73.3)	8 (26.7)
Rifampicin	30	27 (90.0)	3 (10.0)
Azithromycin	30	27 (90.0)	3 (10.0)
Vancomycin	30	30 (100)	0 (100)

given in Table 5. Highest activity was observed with ciprofloxacin and vancomycin (100%), followed by azithromycin and rifampicin (90%) against *S. aureus*. Moderate activity (73.3-86.7%) was obtained with pefloxacin, ofloxacin and erythromycin. MIC ranges of these antibiotics to the PPSA were methicillin (0.125-16 µg/ml), ciprofloxacin, ofloxacin and pefloxacin (0.125-8 µg/ml). Others were norfloxacin (4-64 µg/ml), gentamicin (2-64 µg/ml), rifampicin (2-32 µg/ml), erythromycin (4-128 µg/ml), azithromycin (0.25-16 µg/ml) and vancomycin (0.125-2 µg/ml) (Table 6).

DISCUSSION

The result of this study showed that *S. aureus* is the predominant *Staphylococci* isolated from clinical specimens justifying earlier reports by Lowry (1998) and Baorto (2004). While *S. aureus* accounted for 73.3% of the isolates in this present study, *S. epidermidis*

Table 6. MICs of the penicillinase producing *Staphylococcus aureus* (PPSA) to other antibiotics.

Antibiotics	No. of Isolates (β-lactam +ve)	MIC range (µg/ml)	MIC_{75} (µg/ml)	MIC_{90} (µg/ml)
Methicillin	30	0.125-32	0.25	8
Ciprofloxacin	30	0.125-8	0.25	4
Pefloxacin	30	0.125-8	0.25	4
Ofloxacin	30	0.125-8	0.25	4
Norfloxacin	30	4-64	8	32
Gentamicin	30	2-64	4	32
Erythromycin	30	4-128	16	32
Rifampicin	30	2-32	8	16
Azithromycin	30	0.25-16	0.5	8
Vancomycin	30	0.125-2	0.25	2

accounted for the remaining 26.7%. Colonization of post surgical septic wound by staphylococci is not very common except however, in few cases of immune-compromization in individual (Baorto, 2004; Johnson, 2001). Post surgical dressing with Eusol lotion and Savlon are intended to reduce the incidence of bacterial colonization at these sites and this is done every day at the patient's bed side. This could have reduced the rate of colonization of post surgical wounds with *Staphylococci* in contrast to the high incidence reported in non-surgical wounds. It was more difficult to monitor hygienic and aseptic practices in out-patients to which most of the non-surgical wounds fall within.

Susceptibility of the staphylococci to the penicillins and cephalosporins showed variation in patterns. The isolates exhibited low susceptibility to the penicillins compared to the cephalosporins and this is not out of place considering the fact that majority of the *S. aureus* isolates (90.9%) produced β-lactamase. Percentage susceptibility as low as 30.0, 27.3, 18.1 and 9.1% were recorded with *S. aureus* for ampicloxacillin, amoxicillin, ampicillin and benzyl penicillin respectively. A similar study on penicillins conducted in the Lagos University Teaching Hospital (LUTH), Idi-Araba by Egwari and Nwachukwu (1994), showed moderate (50.3%) susceptibility of *S. aureus* to ampicloxacillin and a relatively higher susceptibility (72.3%) for coagulase-negative staphylococci. In that same study, methicillin resistance to *S. aureus* was 58.9 and 30.6% for coagulase-negative staphylococci; a sharp contrast to the reduction in resistance (16.7% for *S. aureus* and 20% for *S. epidermidis*) recorded in this study. Incidence of methicillin-resistant staphylococci (MRS) seems to be on the decline and this may be associated with the discontinuation of penicillin based therapy for staphylococcal infections in many centers. However, MRSA remains a major cause of nosocomial infections worldwide (Menichetti, 2005; Chambers, 2001; Johnson, 2001).

Resistance to penicillins has been attributed to β-lactamase production (Chambers, 2001; Lowy, 1998; Egwari and Nwachukwu, 1994). Egwari and Nwachukwu (1994), reported 88.9% penicillinase activity in their staphylococcal isolates, however, in this study, 100% penicillinase activity in *S. aureus* isolates was recorded. This result also is reflected in the values of MIC_{75} (0.25 µg/ml) and MIC_{90} (8µg/ml) obtained against PPSA. Ciprofloxacin had been reported by Ruiz (2003), as the most effective of all the second generation quinolones against Gram-negative bacteria. These MIC values are in agreement with the NCCLS standard values (0.12-8 µg/ml) for ciprofloxacin against *Staphylococci* (NCCLS, 2001). Ofloxacin recorded a fairly good activity of 86.7% for *S. aureus.* Earlier reports by Ruiz (2003) on the activity of norfloxacin against Gram-positive organisms suggested that norfloxacin is not very active against staphylococci hence the 50% activity recorded for this antibiotic is justified. This is partly due to the presence of Nor A efflux pump system in the staphylococci. Nor A is an ATP-dependent efflux pump capable of pumping out hydrophilic quinolones like enoxacin and norfloxacin, but not affecting the hydrophobic quinolones like ciprofloxacin and sparsfloxacin (Ruiz, 2003). Reduced susceptibility to norfloxacin may therefore, be ascribed to decreased accumulation of the drug with the organisms cytoplasm. There are no reports of resistance to quinolones by PPSA ascribed to β-lactamase production.

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