Full Length Research Paper

Analysis of Beta-lactamase production and Antibiotics resistance in *Staphylococcus aureus* strains

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The antibiotic susceptibility profile and the association of β-lactamase production to antibiotic resistance in *Staphylococcus aureus* strains were analysed. The disk diffusion antibiotic susceptibility pattern was conducted on *S. aureus* strains cultured from clinical specimens using standard bacteriological methods. The β-lactamase production was assayed using the modified Perret's iodometric assay and data were statistically analysed. Of the 107 *S. aureus* isolates identified, 77 (72%) of the isolates were multi-drug resistant and 75 (70.1%) produced β-lactamase. β-lactamase production and resistance to amoxicillin, amoxicillin/clavulanic acid (augmentin) and ceftriaxone resistance was significant (p-value< 0.05). The study suggests strict infection control measures and encouragement of prudent antibiotic use.

**Key words:** *Staphylococcus aureus*, Antibiotics, Beta-lactamase, Resistance

INTRODUCTION

*S. aureus* is a major pathogen that can cause various forms of diseases varying from simple to life-threatening infection in human population (Lowy, 1998; Diekema et al., 2001). The invasion of the host tissues by *S. aureus* apparently involves the production of a formidable array of extracellular enzymes (invasins) which facilitate the actual invasive process. Some may occur also as cell-associated proteins by breaking down primary or secondary defenses of the host which can facilitate the growth and spread of the pathogen. The damage of the host as a result of this invasive activity may become part of the pathology of an infection (Noble, 1998).

β-lactam antibiotics are among the most frequently prescribed antibiotics worldwide in the control of *S. aureus* infection. They act on peptidoglycan synthesis by molecularly acting on transpeptidases and carboxypeptidases thereby disrupting cell wall formation of the pathogen. However, the efficacy of antibiotics for therapy have suffered a setback due to the growing trend of multiply resistant strains observed in the organism to β-lactam and other antibiotics (Lowy et al., 2003; Deurenberg and Stobberingh, 2008; Jensen and Lyon, 2009).

Resistance to β-lactam group of antibiotics in *S. aureus* is mediated through a variety of β-lactamases or the expression of low-affinity penicillin binding protein PBP2a. The chromosomally mediated penicillin binding protein 2a initiates resistance to methicillin which confers a low affinity for all β-lactams and other unrelated group of antibiotics, thereby limiting choice for treatment (Lowy, 2003; Woodford and Livermore, 2009). β-lactamase is the predominant extracellular enzyme synthesized after exposure of *S. aureus* to β-lactam antibiotics (McDougal and Thornsberry, 1986; Livermore, 1995; Chopra, 2003). The enzyme is encoded in the plasmid or chromosome and its expression can either be constitutive or inductive. It deactivates the drug by cleaving the β-lactam ring. The hydrolytic ability of β-lactamase in conferring resistance

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in *S. aureus* largely depends on its location, kinetics, quantity Physiochemical conditions and interplay of determinants (Livermore, 1995). In addition, selective pressure from excess antibiotic use accelerates the emergence of resistance. β-lactamase has been observed to be responsible for resistance in β-lactam, β-lactamase inhibitors and extended spectrum cephalosporins (Gullman, 1992; Anderson and Gums, 2008).

Antibiotic resistance in *S. aureus* have an adverse effect on healthcare management of infections. In response to the increasing rate of antibiotic resistance in *S. aureus*, this study aims to analyze the antibiotics susceptibility patterns, the production of β-lactamase and its association to antibiotic resistance in clinical *S. aureus* strains.

MATERIALS AND METHOD

Identification of *S. aureus*

Samples were collected with sterile cotton-tipped applicators from infected wounds, burns, eyes, septic spots and otitis media of subjects according to institutional protocol and patients consent at the Obafemi Awolowo University Teaching Hospital complex (OAUTHC). The samples were each inoculated on to nutrient broth and incubated at 37°C for 24 h. Thereafter, the cultures were streaked on mannitol salt agar (MSA) (Oxoid) and incubated at 37°C for 18 to 24 h. The *S. aureus* isolates were identified on the basis of Gram staining, colony morphology on mannitol salt agar, positive catalase and coagulase (in tubes) results (Cheesebrough, 1991).

Antimicrobial agents and susceptibility testing

The antibiotic susceptibility testing was determined as described for disk diffusion (Bauer and Kirby, 1966; Clinical Laboratory Standard Institute (CLSI), 2003) on Mueller Hinton agar. All the inoculums were standardized to 10^6 CFU McFarland standard and ATCC 25923 was used as the control strain. The antibiotics used included amoxicillin (25 μg), amoxicillin/clavulanic acid (augmentin) (30 μg), cloxacinil (5 μg), chloramphenicol (30 μg), Trimethoprim/sulfamethoxazole (cotrimoxazole) (25 μg), gentamicin (10 μg), erythromycin (5 μg) and tetracycline (10 μg) (Abtek Biologicals- UK) while ceftriaxone (30 μg), ciprofloxacin (10 μg), ofloxacin (5 μg), pefloxacin (5 μg) and streptomycin (10 μg) were products of Fondoz Laboratories, Nigeria. The susceptibility of each antibiotic was determined from measurement of the zone of inhibition of growth. Multi- antibiotic resistance was analysed based on resistance to a β-lactam and two classes of antibiotics (Shittu et al., 2006).

β-Lactamase Starch Test Assay:

All the isolates were tested for their ability to produce β-lactamase using the Perret’s iodometric assay as modified by Workman and Farrar (1970). Nutrient agar plates containing 0.2 % starch were prepared and pure cultures of *S. aureus* strains were streaked on the agar surface and incubated at 37°C overnight. Each plate was flooded with 3ml of freshly prepared phosphate buffered saline (pH 6.4) containing iodine (3 mg/ml), potassium iodide (15 mg/ml) and penicillin G (50 mg/ml). The solution was poured away and the plates were left for 10 min. The decolourisation of the starch-iodine complex indicates β-lactamase production.

Data Analysis

The statistical analysis was conducted using SAS package version 8.0. The statistical analysis was run at 95% confidence limit, two tailed test and P-values <0.05 were considered as significant. Pearson product moment correlation was used to test for the association between variables (Antibiotics and β-lactamase production).

RESULTS

Figure 1 shows the resistance pattern in approximate percent values in descending order of the 107 *S. aureus* isolates tested; 83% of the isolates were resistant to cloxacinil, 76% to ceftriaxone, 73%, to Amoxcillin and the least resistance was observed in both ciprofloxacin and ofloxacin at 2% value each.

The frequency of the multiple antibiotic resistance showed that 28 (26.2%) of the isolates were resistant to 3 classes of antibiotics, 28 (26.2%) to 4, 15 (14.02%) to 5, 5 (4.7%) to 6 and 1 (0.94%) to 7 classes. Overall, 77 out of the 107 isolates tested, indicating 72% of the isolates were multiply resistant.

β-lactamase production was observed in 75 (70.1%) of the isolates. The Pearson correlation analysis showed there was a positive association in resistance observed in amoxillin, amoxicillin/clavulanic acid (augmentin) and ceftriaxone resistance to β-lactamase production with values 0.30, 0.50 and 0.24 respectively. Resistance observed in these antibiotics were significant to β-lactamase production at p-values <0.05 at 95% confidence limit. Resistance in *S. aureus* to cloxacinil and other non β-lactam antibiotics to β-lactamase production were not significant at p-value >0.05 (Table 1).

DISCUSSION

Antibiotic resistance poses a threat to patient and public health. Reports describing MRSA resistant to 20 different antimicrobial compounds representing most of the available classes (Jensen and Lyon, 2009) and multiple resistances observed in other bacteria pathogens has brought to light the fact that there are now bacteria which are resistant to all antibiotics available to clinical practitioners.

The antibiogram typing of the isolates in this study revealed the isolates showed a high level of resistance to the antibiotics screened. Resistance observed was high in 3 of the β-lactam group of antibiotics comprising cloxacinil (83.1%), ceftriaxone (75.7%) and amoxcillin (72.9%) while it was low in the 3 quinolone antibiotics tested, thereby reflecting a relative susceptibility to the quinolones (Figure 1). The trend showed that antibiotic susceptibility profile of the isolates reflect the predominance of relatively resistant strains in the study setting.
which agrees with previous reports of other investigators (Ako-Nai et al., 1991; Shittu et al., 2006; Akindele et al., 2010). However, Akindele et al. (2010) reported that there was a low resistance of S. aureus against ceftriaxone. On the contrary, a high resistance to ceftriaxone was observed in this present study as shown in Figure 1.

Multi-drug resistance indicates a serious future threat to public health. It was observed in this study that, 72% of the isolates were multi-drug resistant. It has been reported that horizontal gene transfer is a factor in the occurrence of antibiotic resistance in clinical isolates. Jensen and Lyon (2009) in their review on the genetics of antimicrobial resistance in S. aureus reiterated that the evolution of multi-resistance is driven by chromosomal mutation and the acquisition of discrete preformed antimicrobial resistance genes that are exchanged between organisms. In addition to possessing more diverse genetic accessory elements such as plasmids and transposons, compensatory mutations in S. aureus isolates with larger genomes may block reversion to the sensitive phenotype even in the absence of selection and may therefore contribute to the persistence in the resistant strains (Andersson, 2003).

The production of β-lactamase in S. aureus appears to be consistently high in Nigeria. Overall, 75 (70.1%) of the S. aureus isolates in this study produced β-lactamase which agrees with 70-80% β-lactamase prevalence in other previous reports (Rotimi et al., 1979; Kesah et al., 1997; Akindele et al. 2010). The spread of β-lactamase genes had been enhanced by their integration within mobile genetic elements such as plasmids and transposon which facilitate the rapid transfer of genetic materials between microbes (Wilke et al., 2005).

The study revealed that the resistance of S. aureus to amoxicillin, amoxicillin/clavulanic acid and ceftriaxone, were significantly linked to the production of β-lactamase (p<0.05). Amoxicillin/clavulanic acid and ceftriaxone as an extended spectrum cephalosporins are both meant to extend the spectrum of activity of the β-lactam drugs by inhibiting the activity of β-lactamase (Livermore, 1995). However, a significant association of β-lactamase production to ceftriaxone and amoxicillin/clavulanic acid resistance may indicate the presence of metalloenzyme and or over production of chromosomally encoded beta-lactam and extended spectrum β-lactamases (Cullman, 1992; Drawz and Bonomo, 2010). It is regrettable that the various classes of β -lactamase produced by S. aureus isolates in this study were not determined. Future studies on the classes of β-lactamase and its induction in S. aureus isolates should provide insight into the nature of β-lactam resistance.

Cloxacillin and other non β-lactam antibiotics, resistance is not significantly linked to β-lactamase production. Cloxacillin exhibit a similar mechanism of resistance to methicillin and this may not be far from the discrepancy observed to resistance that is not linked to β-lactamase. It has been reported that the MecI, methicillin resistance repressor represses the synthesis of β-lactamase by reducing the specific activity of plasmid carrying MecI in...


**S. aureus** (Lewis and Dyke 2000).

### Conclusion

A high resistance to the β-lactam antibiotics as compared to the other group of antibiotics were observed. The study suggests strict infection measures and efforts to promote appropriate and prudent use of antibiotic should be encouraged. This is important in order to curtail the role of selective pressure that tends to favour the emergence of drug resistance. In addition, the study provides valuable information to clinicians for better management of diseases caused by *S. aureus* strains.

### REFERENCES


### Table 1. The Pearson's correlation of the relationship of β-lactamase production to antibiotic resistance in *S. aureus* strains.

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* Significant at 0.05 level of probability. ** Significant at 0.01 level of probability.

Legend: AMX= Amoxycillin, AUG =Augmentin, CEF = Cetriaxone, CHL = Chloramphenicol, CIP = Ciprofloxacin, CLX = Cloxacin, COT = Cotrimoxazol, ERY = Erythromycin, GEN = Gentamicin, OFX = Ofloxacin, PEF = Pefloxacin, STR = Streptomycin, TET = Tetracycline, β-LAC = β-lactamase.