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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 (Cullman, 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 10^8CFU McFarland standard and ATCC 25923 was used as the control strain. The antibiotics used included amoxicillin (25 µg), amoxicillin/clavulanic acid (augmentin) (30 µg), cloxacillin (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 cloxacillin, 76% to ceftriaxone, 73%, to Amoxicillin 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 cloxacillin 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 cloxacillin (83.1%), ceftriaxone (75.7%) and amoxicillin (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

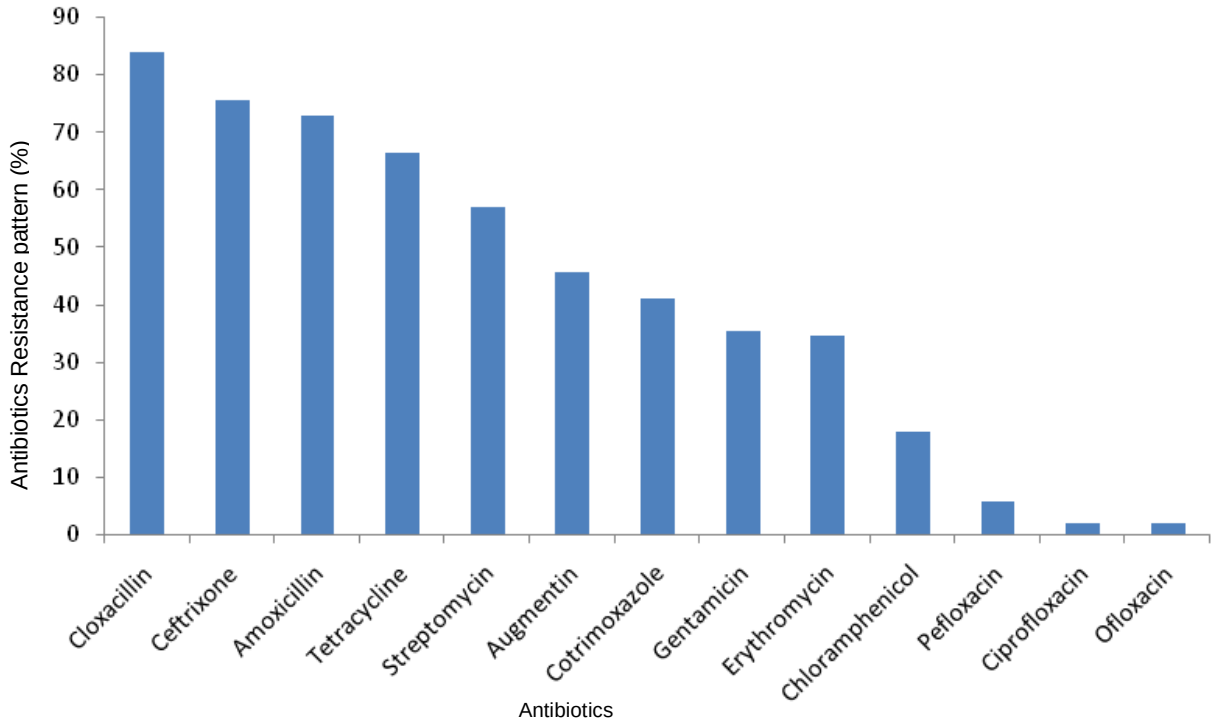

Figure 1. Antibiotic resistance pattern of *S. aureus* isolates from hospital and community sources in Ile-Ife.

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 cefriaxone. 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

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

	STR	CEF	PEF	CPX	AMX	ERY	TET	CLX	GEN	COT	CHL	AUG	β -LAC
OFX	0.12	0.08	0.27**	0.49**	-0.07	0.04	0.10	0.06	-0.10	0.02	-0.06	0.01	-0.02
STR		0.34**	0.21*	-0.02	0.23**	0.16	-0.02	0.11	0.09	0.11	0.21*	0.15	0.00
CEF			-0.15	0.08	0.39**	0.23*	-0.13	0.31**	-0.22*	-0.19*	0.04	0.26**	0.24**
PEF				0.27**	-0.13	-0.09	0.09	-0.13	0.16	0.21	0.10	-0.06	0.04
CPX					0.08	0.04	-0.05	0.06	0.04	0.02	-0.06	0.15	0.12
AMX						0.13	0.10	0.33**	-0.12	-0.09	-0.05	0.43**	0.30**
ERY							-0.11	0.19*	-0.09	-0.01	-0.03	0.24**	0.01
TET								0.09	0.12	0.35**	0.18	0.14	0.05
CLX									-0.13	-0.08	0.06	0.12	0.13
GEN										0.21**	0.06	0.14	0.10
COT											0.11	0.18	0.08
CHL												-0.08	-0.11
AUG													0.50**

* 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 = Cotrimoxazole, ERY = Erythromycin, GEN = Gentamicin, OFX = Ofloxacin, PEF = Pefloxacin, STR = Streptomycin, TET = Tetracycline, β-LAC = β-lactamase.

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

Akindele AA, Adewuyi IK, Adefioye OA, Adedokun SA, Olaolu AO (2010). Antibiogram and Beta-lactamase of *Staphylococcus aureus* isolates from different human Clinical Specimens in a Tertiary Health Institution in Ile-Ife, Nigeria. Am Eurasian J. Sci. Res. 5(4):230-233.

Ako-Nai KA, Ogunniyi AD, Lamikanra A, Torimiro SEA (1991). The characterisation of clinical isolates of *Staphylococcus aureus* in Ile-Ife, Nigeria. J. Med. Microbiol. 34:109-112.

Andersson DI (2003). Persistence of antibiotic resistant bacteria. Curr. Opin. Microbial. 6(5):452-456.

Anderson SD, Gums JG (2008). Ceftobiprole: An extended-spectrum anti- methicillin resistant *S. aureus* cephalosporin. Ann. Pharmacother. 42:806-816.

Bauer AW, Kirby WMM, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. Amer. J. Clin. Pathol. 45:493-496.

Cheesebrough M (1991). Medical Laboratory Manual for Tropical Countries Vol II: Microbiology. Educational low-priced Books Scheme, Great Britain. pp. 60-63.

Chopra I (2003). Antibiotic resistance in *Staphylococcus aureus*: concerns, causes and cures. Expert Rev. Anti-Infective Ther. 1(1):45-55.

Clinical Laboratory Standard Institute formerly NCCLS (2003). Performance standards for antimicrobial disk susceptibility tests. Approved standards 8th edn pp. 1-130.

Cullmann W (1992). The threat of resistance to new oral cephalosporins. Chemother. 2(38):10-17.

Deurenberg RH, Stobberingh EE (2008). The evolution of *Staphylococcus aureus*. Infection, Gen. Evol. 8:747-763.

Diekema DJ, Pfaller MA, Schmitz FJ, Schmitz J, Smayevsky J, Bell R, Jones N, Beach M, the Sentry Participants Group (2001). Survey of infections due to *Staphylococcus species*: Frequency of occurrence and Antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific Region for the Sentry Antimicrobial surveillance program 1997–1999. Clin. Infect. Dis. 32:114–132.

Drawz SM, Bonomo RA (2010). Three decades of β-lactamase Inhibitors. Clin. Microbiol. Rev. 23(1):160-201.

Jensen SO, Lyon BR (2009). Genetics of antimicrobial resistance in *Staphylococcus aureus.* Future Microbiol. 4(5):565-582.

Kesah CN, Ogunsola FT, Neemogha MT, Odungbemi TO (1997). An *in-vitro* Study of Methicillin and Other Antimicrobial Agent Against *Staphylococcus aureus*, 1994 - 1996. Nig Qt J. Hosp Med. 7(3):286-88

Lewis RA, Dyke KGH (2000). MecI represses synthesis from the β-lactamase operon of *Staphylococcus aureus.* J. Anti.Chem. 45:139-144.

Livermore DM (1995). β-lactamase in laboratory and clinical Resistances. Clin. Microbiol. Rev. 8(4):557-584.

Lowy FD (1998). *Staphylococcus aureus* infections. New Engl. J. Med. 339:520–532.
Lowy FD (2003). Antimicrobial resistance: the example of *Staphylococcus aureus*. J. Clin. Invest. 3(9):1265–1273.
McDougal LK, Thornsberry C (1986). The role of beta-lactamase in staphylococcal resistance to penicillinase-resistant penicillins and cephalosporins J. Clin. Microbiol. 23(5):832-839.
Noble WC (1998). Staphylococcal diseases: In Topley and Wilson's Microbiology and Microbial Infections. Leslie Collier, Alberty Balows, Max Sussman. 9th Edition. 3:231-256.
Rotimi O, Odugbemi TO, Fadahunsi O, Ogunbi O (1979). Penicillin resistance in regretted. *Staphylococcus aureus* prevalence of penicillinase In light of these findings, diagnostic medical producing strains in Lagos University Teaching microbiology laboratories should perform antibiotic Hospital. Niger. Med. J. 3:307-310.
Shittu A, Lin J, Kolawole DO (2006). Antimicrobial susceptibility patterns of *Staphylococus aureus* and Characterization of MRSA in South Western Nigeria. WOUNDS. 18(4):77-84.
Wilke MS, Lovering AL, Strynadka CJN (2005). β-lactam antibiotic resistance: A Current Structural perspective. Curr. Opinion Microbiol. 8: 525-533.
Woodford N, Livermore DM ((2009). Infection caused by Gram-positive bacteria: A review of the global challenge. J. Infection 59(S1):S4-S16.
Workman AG, Farrar WE (JNR) (1970). Activity of penicillinase in *Staphylococcus aureus* as studied by the iodometric method. J. Infect. Dis. 121:433-437.