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Short Communication

Antibiotic Susceptibility Pattern and Beta-lactamase Production in Isolates of *Staphylococcus aureus* from Recurrent Furunculosis in Southwestern, Nigeria

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ABSTRACT

Furuculosis is a skin infection caused by *Staphylococcus aureus*. It is characterised by honey crusted 'cropped' latent boil with potential to recur in a susceptible host. Isolates of *S.aureus* obtained from both hospitalised and non-hospitalised patients with furuncles in Southwest, Nigeria were characterised in relation to their resistance to commonly used antimicrobial agents. Exudates of 'cropped-boils' from one hundred and forty (140) individuals consisting of forty (40) hospitalised and one hundred (100) non-hospitalised cases of recurrent furunculosis were screened for *S. aureus*. One hundred and two (102) were positive for the organism by conventional biochemical tests. Detection of β-lactamase was determined by cell-suspension iodometric method. Of the 102 isolates, 30(29.4%) strains possessed β-lactamase and the minimum inhibitory concentration (MIC) of selected antibiotics was in the range of 3.95– 250µg/ml. The multiple drug resistance as evident in high MICs of the antibiotics tested could probably be due to abuse/misuse of antibiotics resulting in recurrence of furuncles in the patients.

Keywords: Antibiotic susceptibility, β-lactamase, Recurrent furunculosis, *Staphylococcus aureus*

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INTRODUCTION

Staphylococcus aureus is a recognised human pathogen responsible for a great variety of pyogenic infection in man and animals, infecting about one-third of the world population (Todar, 2008). The pathogen is also capable of living a benign lifestyle in the nasal passage and skin (Highet *et al*., 1992). It is the causative agent of many suppuration processes ranging from localised abscess which can occur in any part of the body, to fatal septicaemia and pneumonia. Furunculosis is a primary skin infection characterised by latent honey crusted 'cropped' boil and suppuration in susceptible host. People with diabetes and suppressed immune system or acne are at great risk (Zimakoff *et al*., 1988).

Staphylococcus aureus isolated from furunculosis are drug resistant (El-Gilany and Hanan, 2009). The emergence of drug resistant strains isolated from pathogenic processes has been attributed to the increasing introduction of various antibiotics into general use (Hanan *et al*., 2005). Penicillin was the first antibiotic used for *Staphylococcus* infections and penicillin resistance appeared shortly after its introduction. This was followed by resistance to methicillin, amoxillin, tetracycline and to a lesser extent, erythromycin, gentamycin and other antibiotics (Mostafizur *et al*., 2005). *Staphylococcus aureus* developed intrinsic resistance to penicillins because of its remarkable ability to hydrolyse beta-lactam antibiotics. The organism also has great ability to degrade skin lipid barrier and spread within skin loci (Laube and Farrell, 2002; Hoegr, 2004). In this study, isolates of *S.aureus* from cases of furunculosis were screened for ß-lactamase production and the antimicrobial susceptibility of the isolates was investigated.

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MATERIALS AND METHODS

Sample Collection and Bacterial Isolation

A total of one hundred and forty (140) exudates of 'cropped' boil from hospitalised and non-hospitalised patients with recurrent cases of furunculosis were screened for the isolation of *S.aureus* on mannitol salt agar (Biotech). Suspected staphylococcal isolates were confirmed by catalase test, coagulase test, DNAse test and haemolytic test (Harold, 2007). *Staphylococcus aureus* strain ATCC 29213 was used as reference strain.

Antimicrobial Susceptibility Test

The antimicrobial susceptibility pattern of the isolates was determined using method of Kirby-Bauer (Cheesebrough, 2000). All the strains were tested to the following antibiotics: Cloxacilln (5µg/ml), Gentamicin (10µg/ml), Cotrimozaxole (25µg/ml), Chloramphenicol (30µg/ml), Augmentin (30µg/ml), Amoxicillin (25µg/ml), Erythromycin (5µg/ml), and Tetracycline (10µg/ml). The zones of inhibitions were measured with a meter rule and interpreted as recommended by NCCL (1998) (now Clinical and Laboratory Standards Institute, CLSI). *Staphylococcus aureus* ATCC 29213 was used as reference strain.

Determination of Minimum Inhibitory Concentration (MIC)

Graded decreasing double-fold concentrations of each antibiotic was prepared in nutrient broth and to each dilution was added 0.lml of a 10^{-2} diluted culture of each strain, including the standard strain. The tubes were incubated at 37^0C for 24hrs to determine the MIC of each antibiotic (Cheesebrough, 2002).

β-lactamase Detection

Overnight pure culture of each isolate of *S. aureus* was harvested and homogenised in phosphate buffered penicillin G. The bacterial suspension measuring $x10^7$ cells/ml using MacFarland turbidity standard was tested for β-lactamase production by the cell suspension iodometric method (Catling, 1975).

RESULTS

One hundred and two (102) haemolytic isolates positive for catalase test, coagulase test, mannitol sugar fermentation and DNAse test were selected for further analyses. In the antibiotic susceptibility testing, the percentage resistance of the isolates to the antibiotics used was found to be highest for tetracycline (55.88%) and erythromycin (43.13%), while resistance to Augmentin (12%) was found to be the lowest. Cloxacillin elicited (18.62%) resistance and amoxicillin, an amino acid penicillin was (35.29%). Cotrimoxazole, a double blocker antibiotics elicited 30.39% resistance while the organisms showed 21.56% resistance to chloramphenicol (Table 1).

In the determination of minimum inhibitory concentration of selected antibiotics, the values obtained showed resistance of the *S. aureus* isolates to Augmentin (3.95 -250 µg/ml), Cefotaxime (15.63-250µg/ml), Ceftriaxone (31.25-250µg/ml), Penicillin (62.5-250µg/ml) and Cloxacillin (15.63-250 µg/ml) when *p*-value of <0.05 was considered statistically significant (Table 2).

Table 1: Antimicrobial Susceptibility Pattern of the *S.aureus* Isolates

ANTIBIOTICS	RESISTANCE	SUSCEPTIBLE		
	R 0≤5	**S1 5 ≤ 15**	**S2 15 ≤ 25**	**S3 25 ≤ 35**
Amoxicillin	36 35.29%	44 43.13%	18 17.65%	4 3.92%
Augmentin	12 11.75%	84 82.35%	6 5.88%	0 0%
Cloxacillin	19 18.62%	60 58.82%	14 13.72%	9 8.82%
Cotrimoxazole	31 30.39%	42 41.17%	28 27.45%	1 0.9%
Chloramphenicol	22 21.56%	48 47.05%	26 25.43%	6 5.88%
Gentamicin	14 13.72%	48 47.05%	26 25.43%	14 13.72%
Erythromycin	44 43.13%	14 13.72%	37 36.27%	7 6.86%
Tetracycline	57 55.88%	15 14.70%	18 17.64%	12 11.76%

DISCUSSION

Staphylococcus aureus is a common cause of skin infection and it has been isolated from 89.2-100% of recurrent and non-recurrent furunculosis patients ((Wiese-Posselt et al., 2007; El-Gilany and Hanan, 2009). The frequency of the pathological distribution of *S. aureus* obtained in this study reflects a typical prevalence pattern of the organism in furunculosis and corroborated the findings of Dahl (1987) in the strategies for management of recurrent furunculosis. The susceptibility of the organism to augmentin (amoxicillin and clavulanic acid) suggests the efficacy this antibiotic in the management of the infection.

Table 2: Minimum Inhibitory Concentration of the Antibiotics on Selected Strains of *Staphylococcus aureus*

Strain Number	β - lactamase	Pathological source	MIC (µg/ml)				
			Augmentin	Cloxacillin	Cefotaxime	Ceftriaxone	Penicillin G
SA01	+	Neck	250	62.5	62.5	125	125
SA02	+	Ear	7.8	250	250	250	62..5
SA03	+	Armpit	250	15.63	31.25	125	62.5
SA04	+	Buttock	250	125	250	250	125
SA05	+	Ear	3.95	31.25	62.5	62.5	62.5
SA06	+	Breast	3.95	250	125	62.5	62.5
SA07	+	Thigh	250	125	250	125	62.5
SA08	+	Ear	15.63	62.5	62.5	62.5	125
SA10	+	Elbow	15.63	125	62.5	62.5	125
SA11	+	Ear	250	250	125	62.5	62.5
SA13	+	Armpit	7.8	125	15.63	31.25	62.5
SA16	+	Eye	15.63	62.5	62.5	31.25	62.5
SA18	+	Cheek	1.93	250	62.5	125	125
SA23	+	Nose	7.8	250	31.25	31.25	125
SA24	+	Head	15.63	250	15.63	62.5	62.5
SA25	+	Armpit	3.95	125	125	62.5	250
SA33	+	Chin	62.5	250	15.63	250	125
SA40	+	Head	15.63	62.5	62.5	62.5	125
SA45	+	Breast	31.63	250	125	125	62.5
SA46	+	Buttock	3.95	250	31.25	125	62.5
SA50	+	Head	15.63	125	15.63	125	62.5
SA51	+	Ear	15.63	125	31.25	62.5	62.5
SA52	+	Lip	31.25	250	125	31.25	62.5
SA53	+	Ear	250	125	31.25	62.5	62.5
SA62	+	Head	125	125	125	31.25	125
SA63	+	Nose	62.5	125	125	125	62.5
SA64	+	Armpit	125	250	250	250	125
SA91	+	Head	125	62.5	62.5	125	125
SA94	+	Nose	125	31.25	250	125	125
SA97	+	Head	31.25	250	31.25	31.25	62.5
***S. aureus*29213**		Reference strain	7.50	NT	10.5	10.8	12.0

***SA: Staphylococcus aureus; NT-Not tested;* MIC: Minimum Inhibitory Concentration**

Unlike the report of Akindele and others who documented that ß-lactamase producing *S. aureus* had the highest percentage susceptibility to erythromycin (Akindele *et al*., 2010), most of the *S. aureus* examined in this study were resistant to tetracycline and erythromycin. This may be attributed to the nature of the infection and misuse of the antibiotics in our setting. It is not usual to purchase antibiotics over the counter and tetracycline and erythromycin are relatively inexpensive. Therefore, *in vitro* susceptibility of antibiotics should be ascertained before usage.

One of the major mechanisms of resistance to β-lactams was the expression of the enzymes, beta-lactamases such as penicillinase and cephalosporinase (Liang *et al*., 2003). High level beta-lactamase production was observed among the *S.aureus* tested in this study. This is consistent with the results of beta-lactamase production in *S.aureus* from various clinical sources in Nigeria (Kesah *et al*., 1997; Akindele *et al*., 2010).

The range of MICs obtained for the selected antibiotics against the isolates of *S. aureus*, perhaps accounts for the observed recurrent furunculosis in this study population. The MICs obtained for penicillin and cefotaxime (an extended β-lactamase spectrum) among the resistant isolates is in agreement with the result of Oyelese and Oyewo (1995) on the menace of β –lactamase production on antibiotic prescription in community-acquired infection. Also, our report supports the analysis of Clewell (2008) on the threat to chemotherapeutic application of β-lactam antibiotics. Ninety per cent (90%) of the *S. aureus* strains gave the MICs for the antibiotic tested, beyond the modal MICs for the reference strain. Therefore, there is a need to institute strategies for antibiotic resistance surveillance to form the basis for developing and

implementing measures that can reduce the burden of infections in our setting.

In conclusion, this study offers primary evidence of the involvement of *S.aureus* in the epidemiology of furunculosis in Nigeria. Noticeably, *Staphylococcus aureus* remains an agent of recurrent furunculosis and the treatment of the infection requires careful evaluation of the commonly available antibiotics especially the beta-lactams. Lack of information on the biodata of the patients, previous antibiotic usage, underlying cause of the infection and genetic profiling studies present limitations to this study and form the basis for further work.

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