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Full Length Research Paper

Plasmid-mediated antibiotic resistance in *Staphylococcus aureus* from patients and non patients

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Out of 156 samples collected from non-patients, nose and ear, 32% were found to harbor *Staphylococcus aureus*. Also 26 hospitals strains of *S. aureus* were isolated from clinical specimens. 62% of the total isolates including the clinical isolates were found to be multiple resistant, being resistant to 4 or more types of antibiotics. Resistance to methicillin and co-trimoxazole were the highest (76%) while resistance to gentamycin and erythromycin were the lowest (32%). Of the 74 strains, 62% were found to exhibit multiple resistant to 4 or more antimicrobial agents tested. 47% of the antibiotic resistant strains harboured plasmids ranging in molecular sizes from 0.282 kb and grouped into 6 plasmid profiles. Transformation experiment revealed that 41.2% of the resistant strains carried resistant plasmids of sizes 1.26, 23.13 and 25.12 kb. Plasmid-mediated resistance to amoxycillin, tetracycline and chloramphenicol was found.

Key words: Plasmids, *Staphylococcus aureus*, antibiotics, resistance.

INTRODUCTION

Staphylococcus aureus is one of the most successful and adaptable human pathogens. It is also a common colonizer of the skin and the nose. Its remarkable ability to acquire antibiotic resistance has contributed to its emergence as an important pathogen in a variety of settings (David et al., 2006; Sampathukumar, 2007; Hotu et al., 2007). Methicillin-resistant *S. aureus* (MRSA) has remained a major cause of nosocomial disease world-wide (Emori et al., 1993; Edmund et al., 1999; Larsen et al., 2008) causing 50% or more of hospital-acquired *S. aureus* infections in several countries (Aires de Saisa and de Lencastre, 2004; Tietz et al., 2005; Wolter et al., 2008). The emergence of community-acquired MRSA that is capable of causing infections in otherwise healthy people has been reported (Gorak et al., 1999; Coronado et al., 2007; Diep et al., 2008).

Different patterns of anti-biotic resistance and plasmid profiles among strains of *S. aureus* have been reported (Akinyemi et al., 1997; Bhaktar et al., 2003; Diep et al., 2006).

Staphylococcal antibiotic resistance has been associated with resistant plasmids that have the ability to mediate the production of drug inactivating enzymes such as β-lactamases (Adeleke et al., 2002) and other functions (King et al., 2006; Diep et al., 2008). This paper investigate the nature of plasmids that determine antibiotic resistance in *S. aureus* isolates from both patients and non patients in Nigeria.

MATERIALS AND METHODS

Bacteriology

A total of 156 human nasal and ear swabs from non-patients residing in two Local Government Areas of Lagos State of Nigeria, were screened for *S. aureus* colonization over a five month period. Also 26 clinical samples from urinary tract infections, wound infections, ear infection and eye infection respectively, were collected from Lagos State University Teaching Hospital, Ikeja and Olabisi Onabanjo Teaching Hospital Sagamu, during the above period. The swab samples were collected using commercially prepared sterile swab sticks (Oxoid, U. K.), consisting of neat swab of absorbent cotton wool on wooden applicator sticks. After collection, the wooden applicator stick was broken to an appropriate short length for

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Table 1. *Staphylococcus aureus* colonization in non-patients.

Sources of Isolate	Number of samples	Number of positive	Percentage (%) positive
Non-Patient Nose	90	36	40%
Non-Patient Ear	66	12	18%
Total	156	48	31%

Table 2. *Staphylococcus aureus* from clinical samples

Sample	LASUTH (Ikeja)	OOUTH (Sagamu)	Total
Wound Swab	8	6	14
Urine	2	2	4
Ear Swab	2	2	4
Ear Swab	-	4	4
Total	12	14	26

LASUTH = Lagos State University Teaching Hospital; OOUTH = Olabisi Onabanjo University Teaching Hospital.

insertion into Stuart's Transport medium in a screw capped bijou bottle. The isolates were characterized using established microbiological methods which included colonial morphology, Gram-stain characteristics, ability to produce enzyme peroxidase and coagulase to separate the *S. aureus* strains form the coagulase-negative staphylococci (Cowan, 1985). Informed consent of the non-patients was obtained as well as approval of the ethical committee of National Institute of Medical Research, Yaba, Lagos.

Antimicrobial susceptibility testing

The antimicrobial susceptibility pattern of the isolates was determined using the Kirby-Bauer-National Committee for Clinical Laboratory Standard (NCCLS) modified disc diffusion technique (Cheesebrough, 2000). All the strains were tested for their sensitivity to the following antibiotics: Methicillin (5 ug), Augmentin (30 ug), Amoxycillin (25 ug), Erythromycin (15 ug), Tetracycline (30 ug), Cloxacillin (5 ug), Gentamycin (10 ug), Cotrimoxazole (25 ug), and Chloramphenicol (30 ug) (All from Abtek U.K.).

The zones of inhibition were recorded and the isolated classified as "resistant", intermediate" or "sensitive" based on the interpretative chart updated according to the current NCCLS Standards. *S. aureus* NCTC 6571 was used a control.

Plasmid isolation

Plasmid DNA was isolated, separated and stained as previously described (Zuccarelli et al., 1989). Plasmid profile groups were constructed by grouping strains possessing the same profile (containing the same number and molecular mass or part of a profile) constituting a core profile. Bacterial strains that carried the plasmid were regarded as constituting one plasmid group.

Genetic transfer

Transformation was done as described by Honahan (1983), using *Escherichia coli* K-12 HB101 (ara-14, galk2, hsd 520, lacyl, leu, nt 101, proA2, recA13, rps 120, supE44, thiixyl-5) as recipient and pBR322 as the positive control. Co-transformation of resistant character was determined by testing all transformants against all antibiotics to which the donor strains were resistant. Extracts from transformants were obtained as described above and subjected to agarose gel electrophoresis. Transformation was confirmed as positive only when resistant transformants were shown to contain a plasmid(s) of a size to that found in the original isolate.

Plasmid curing

The curing of the resistant plasmids of the bacterial isolated was done as described by Daini et al. (2005).

RESULTS

Out of 156 samples collected from non-patients, 36 isolates from the nose were found positive by the tube coagulase test, for *S. aureus,* while 12 isolates from the ear were positive for *S. aureus*, also by the tube coagulase test. In general, most of the organisms were found to be coagulase bound (Table 1). The distribution of *S. aureus* isolates from various clinical specimens is shown in Table 2. The resistance pattern of the isolates to other antimicrobial agents is shown in Table 3. This study revealed that clinical strains have the highest resistance rate of 78% on the average, while the non-patient nasal isolates had the lowest resistance rates of 42% on the average. The antibiotic susceptibility test further revealed that resistance to methicillin and contrimoxazole were the highest (76%), while the least resistance was to gent-amycin and erythromycin (32%). The antibiotic resistant pattern shared by 3 or more isolates (Table 4) showed that no particular resistance pattern could be ascribed to any source as the pattern cuts across all the sources. Sixty-two percent of the total number of isolates was found to exhibit multiple resistance as determined by the resistance of such isolates to 4 or more antibiotics (Table 5).

Table 3. Antibiotic resistance pattern of isolates.

Antibiotic	HN (n = 36)	HE (n = 12)	PS (n = 26)	Total (n = 74)
Augmentin (AU)	16 (44)	10 (83)	22 (85)	48 (65)
Amoxycillin (Am)	18 (50)	8 (67)	26 (100)	52 (70)
Erythromycin (Er)	12 (33)	0 (0)	12 (46)	24 (32)
Tetracycline (Tc)	18 (50)	6 (50)	26 (100)	50 (68)
Methicillin	22 (61)	10 (83)	24 (92)	56 (76)
Gentamicin (Ge)	6 (17)	2 (17)	16 (62)	24 (32)
Cotrimoxazole (Co)	28 (78)	6 (50)	22 (85)	56 (76)
Chloramphebicol (Cl)	5 (28)	2 (17)	14 (54)	26 (35)
Mean (%)	(42)	(46)	(78)	(56)

HN = Human nose, HE = human ear, PS = pathological samples.

Table 4. Major antibiotic resistance pattern of isolates (pattern shared by 3 or more Isolates).

Resistance pattern	No (%) of isolates	Isolates source
Au, Am, Er, Tc, Me, Ge, Co, Ci.	6 (7.5%)	HN, WK
Au, Am, Er, Tc, Me, Ge, Co, Ci.	9 (12.1%)	HN, US
Au, Am, Tc, Me, Co.	3 (4.5%)	Hn, He, Ws
Au, Am, Me	5 (6.1%)	HE, ES

HN, HE = Human samples; WK, US, Ws, ES = clinical samples.

Table 5. Number of (%) of multiple resistant *Staphylococcus aureus*.

Sources isolate	No. (%) of isolates resistant to 4 or more antibiotics	No. (%) of isolate resistant to methicillin plus 3 or more other antibiotics
HN (n = 36)	16 (44)	14 (39)
HE (n = 12)	6 (50)	6 (50)
PS (n = 26)	24 (92)	22 (85)
Total n = 74	46 (62)	42 (57)

The clinical isolates had the highest rate of multiple resistance (92%). Furthermore, out of the 46 isolates that had multiple resistance, 57% of them had multiple resistance that include methicillin (Table 5). A total number of 34 isolates of multiple resistant *S. aureus* were screened for plasmids and 16 isolates harboured plasmid with molecular mass ranging from 0.28 to 25.12 kb. Plasmids were not detected in 18 of the resistant strains indicating that their resistance was probably chromosomal. Six different plasmid profile groups for the antibiotic resistant strains could be defined. The number of strain per plasmid profile group vary from 1 – 6 (Table 6) Transformation experiment showed that 41.2% of the resistant strains that harboured plasmids were able to transfer their resistance plasmids to *Escherichia coli* K 12HB101. Plasmid–determined resistance to amoxycillin, tetracycline, and chloramphenicol was found. All the resistant plasmids isolated have molecular sizes of 1.26, 23.13 and 25.12 kb (Table 7). All the strains harbouring resistant plasmids were cured of their plasmids upon treatment with sodium dodecyl sulphate (SDS), with resultant loss of their plasmid-associated properties. This indicates that the antibiotic resistant genes of the *S. aureus* isolates used in this study were plasmid mediated.

DISCUSSION

The detection of *S. aureus* in the non-patient nasal cavity, in 40% of the samples screened, is similar to that obtained by Hidron et al. (2005). There is a remarkably high prevalence of resistance to commonly used antimi- crobial agents by *S. aureus* isolates in this study. 62% of the total isolates have multiple antibiotic resistance as indicated by resistance to 4 or more of the eight anti-biotics used. Also 75% of the isolates had multiple resistance that include methicillin. This is in agreement with the findings of Lowy (2003) and Amorin et al. (2007). Previous studies have shown that methicillin resistant *S. aureus*

Table 6. Plasmid profile groups of antibiotic resistant *Staphylococcus aureus* isolates.

Plasmid profile	No. of isolates	Molecular mass (kb) range of plasmids
0	18 (52%)	Nil
1	3 (9%)	0.282 – 1.259
2	5 (15%)	0.708 – 25.12
3	6 (18%)	3.548 – 23.13
4	1 (3%)	3.548 – 23.13
5	1 (3%)	2.027 – 23.13

Table 7. Characteristics of some of the *Staphylococcus aureus* resistant plasmids.

Isolate	Plasmids molecular Size (kb)	Antibiotic gene transferred to *E. coli* HB IOI	Transformant resistant plasmid size (kb)
PS1	23.13, 6.57	Au, Am, Tc, Er, Cl	23.13
PS 2	23.13, 6.57	Am, Tc, Er, Cl	23.13
HN 32	25.12, 9.42	Au, Am, Tc	25.12
HN 33	25.12, 9.42	Au, Am, Tc, Cl	25.12
HN 8	23.13, 1.26, 0.564	Am, Tc, Cl	23.13, 1.26
HN 9	23.13, 1.26, 0.564	Am, Tc, Cl	23.13

(MRSA) of hospital origin have multiple resistance, while MRSA from the community tends to be sensitive to other antibiotics apart from the β-lactam antibiotics (Adcock et al., 1998; Taiwo et al., 2003; Olayinka and Olayinka, 2003; Chen et al., 2006). This study has shown that 85% of clinical isolates were methicillin and multiple resistant, while only 39% of isolates from non-patients nasal colonization were methicillin and multiple antibiotic resistant. This is an agreement with the earlier studies of Adcock et al. (1998), Beilman et al. (2005) and Faria et al. (2005).

Resistance to high levels of antibiotics has been ascribed in most instances to the presence of plasmids (Adeleke and Odelola, 1997; Bhakta et al., 2003; Daini et al., 2006; Diep et al., 2006). The most common plasmids encountered were 23.13 kb in size. This is similar to that of Olukoya et al. (1995), Adeleke and Odelola (1997) and Diep et al. (2006). 41.2% of the drug-resistant strains carried resistant plasmids. Plasmid-determined resistance to amoxycillin, tetracycline, and chloramphenicol was found. This is in agreement with the findings of Diep et al. (2006) and Han et al. (2007). The emergence of resistant plasmids in this study could be due to over-zealous desire to treat every infection, diagnosed or undiagnosed and to the over the counter availability of antibiotics (Okeke et al., 1999; Ibeachi and Mbata, 2002; Daini et al., 2006). Plasmid profiling analysis distinguished more strains than the antimicrobial susceptibility patterns in agreement with the findings of Olukoya et al. (1995), Adeleke and Odelola (1997) and Daini et al. (2005). Plasmid profiling analysis has been shown to be a good epidemiological tool in investigating epidemics or outbreaks of bacterial diseases (Pasisi and Hecty, 1980; Mayer, 1988).

The transformation experiment enabled us to detect non-self transmissible plasmids, while curing of the resistant strains of the resistant plasmids with SDS showed showed that their antimicrobial resistant genes were plasmid mediated (Adeleke et al., 2002; Daini et al., 1995; Daini et al., 2005; Diep et al., 2006). The high prevalence of multiple antibiotic resistant methicillin resis-tant *S. aureus* among the patients in this study raises the important issues for infection control in this environment. There is also the need for consistent on-going antimicrobial resistance surveillance for important and commonly isolated clinically significant pathogens of staphylococcal species to form the basis for developing and implementing measures that can reduce the burden of antimicrobial resistance and prevent a probable impending public health problem.

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