African Journal of Biotechnology Vol. 11(94), pp. 16071-16076, 22 November, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2397
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Antibiotic resistance profile in community-associated *Staphylococcus aureus* strains isolated from a Nigerian peri-urban community

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Accepted 5 October, 2012

The present study was undertaken to determine the antibiotic resistance profile and the prevalence of community-associated methicillin resistant *Staphylococcus aureus* (CAMRSA) in Ile-Ife, south-Western Nigeria. Ear, eye, skin and soft tissue swabs were collected from 50 subjects at the Primary Health Centre of a university teaching hospital at Ile-Ife. Isolation, identification and susceptibility pattern of the isolates was determined. The methicillin resistant strains were confirmed by the polymerase chain reaction (PCR) detection of the *mecA* gene. The entire tests were done according to standard protocols. Forty *S. aureus* strains were identified and were observed to be 85% multidrug resistant but were all susceptible to ciprofloxacin. Phenotypic resistance to oxacillin was observed in 5 (12.5%) of the *S. aureus* strains, of which 4 were multi-drug resistant. However, the *mecA* gene was detected in only one of the MRSA strains. The study reveals that the community *S. aureus* strains were multidrug resistant. The study suggests public health education and adequate drug monitoring measures to curtail an increase in antibiotics resistance in the community.

Key words: *Staphylococcus aureus,* community, antibiotics, resistance, methicillin.

INTRODUCTION

Staphylococcus aureus is an ubiquitous Gram positive bacterial pathogen that is specifically responsible for various pyogenic and toxin-mediated infections in humans (Lowy, 1998). The organism possesses the ability to acquire antibiotic resistance genes which favours the emergence of antimicrobial resistance (Lowy, 2003; Deurenberg and Stobberingh, 2008). The introduction of methicillin was rapidly followed by methicillin-resistant *S. aureus* isolates (MRSA) (Jevon, 1961) and ever since, the prevalence of MRSA is continuously rising worldwide. It has been reported that over 50% of *S. aureus* isolates in the USA and most countries in Latin America are resistant to methicillin (Moran et al., 2006; Miller et al., 2007; Rodríguez et al., 2010). MRSA initially confined to the hospital settings have been reported to be the cause of death of healthy individuals without risk factors for hospital-acquired infections in the community settings (Lowy, 2003; Shukla, 2005).

Methicillin resistance is often linked to several other unrelated groups of antibiotics so that treatment choices are limited (Lowy, 2003; Woodford and Livermore, 2009), with a serious implication for therapy. While there is ample data on prevalence of antibiotic resistance in the hospital in Ile-Ife in south-Western Nigeria, there is a paucity of information of such in the community. It is on this premise therefore, the antibiotyping and prevalence of methicillin resistance in community isolates of *S. aureus* at the Primary Health Centre of the University

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Teaching Hospital in Ile-Ife, Nigeria was carried out.

MATERIALS AND METHODS

Study population

The study centre included the Primary Health Centre of the Obafemi Awolowo University Teaching Hospital complex (OAUTHC) Ile-Ife, Nigeria. Based on Institutional protocol and the patients or guardian consent for inclusion in the study, swab samples were collected from ear, eye, skin and soft tissue infections from non-hospitalised subjects that presented themselves for consultation. Samples were collected from infants and adults irrespective of the sex. A patient was classified as non-hospitalised when the period of hospitalisation was less than 48 h or have not been hospitalised within the last 12 months (Wylie and Nowicki, 2005). Based on questionnaire response, patients that do not fall within the criteria of non-hospitalisation status were excluded from the study.

Identification of *S. aureus*

A total of 50 samples were collected. Specimen from foci of infection were collected with sterile cotton-tipped applicators (Evepon, Nigeria), inoculated into nutrient broth (Oxoid, UK) within 3 to 4 h of collection and incubated at 37°C for 18 to 24 h. Thereafter, the cultures were streaked on nutrient and mannitol salt agar (MSA) (Oxoid, UK) and incubated at 37°C for 24 h. When Gram-stained films were examined, the isolates were Gram-positive cocci appearing in clusters and were identified as *S. aureus* by the tube coagulase test (Cheesebrough, 1991).

Antibiotic susceptibility typing

The antibiotic susceptibility typing was determined as described for disk diffusion (Bauer and Kirby, 1966; NCCLS, 2000a) on Mueller Hinton agar. The isolates were tested against commercially prepared antibiotic disks (AbtekBiologicals Limited, UK) namely amoxicillin (25 µg), cloxacillin (5 µg), chloramphenicol (30 µg), cotrimoxazole (25 µg), gentamicin (10 µg), erythromycin (5 µg), tetracycline (10 µg), the following antibiotics ceftriaxone (30 µg), ciprofloxacin (10 µg), pefloxacin (5 µg), ofloxacin (5 µg), streptomycin (10 µg) by (Fondoz Laboratories, Nigeria) and oxacillin (1 µg) (Oxoid Limited, UK). The diameters of the zone of inhibition were measured and interpreted. A two-fold dilution of Oxacillin monohydrate salt and Vancomycin hydrochloride (Sigma, Germany) that started with an antimicrobial stock concentration of 5,120 µg/ml were used to test for minimal inhibitory concentration on all the oxacillin resistant strains. The medium for the susceptibility testing was Mueller Hinton agar supplemented with 2% NaCl for the agar dilution for oxacillin test (NCCLS, 2000b). All the isolates including the reference strains were adjusted to (10^7 cfu/ml) McFarland standard. ATCC 25923 and ATCC 4330 were used as the control *S. aureus* strains. Isolates that were resistant to more than two classes of antibiotic were classified as multidrug resistant.

DNA analysis

The detection of the *mec*A gene was carried out on all the isolates resistant to oxacillin. The strains examined for polymerase chain reaction (PCR) were grown in nutrient agar plates at 37°C overnight. DNA was extracted from the test organisms by re-suspending one bacterial colony in 50 µl of deionised water followed by the boiling of the suspension (Aranda et al., 2004). The suspension was placed on ice for at least 2 min and then pulse centrifuged at 4000 revolution per minute for 30 s. The test for *nuc* gene was carried out (Brakstad et al., 1992) and the *mec* gene test was carried out (Strommenger et al., 2003). The *nuc* and *mec*A primer sequence were forward 5'- GCG ATT GAT GGT GAT ACG GTT-3', reverse 3'- AGC CAA GCC TTG ACG AAC TAA-5' and forward 5'- AAA ATC GAT GGT AAA GGT TGG C 3', reverse 3'- AGT TCT GCA GTA CCG GAT TTG C-5' (MWG- Biotech AG, Ireland), respectively. The conditions for PCR for the *mec*A in a thermocycler (Bio-Rad, USA) were 94°C for 3 min 30 cycles of amplification, with denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s and final extension for 4 min. Twenty microlitre (20 µl) of the PCR products obtained were used for the gel electrophoresis with a 100 base pair molecular weight marker (Roche, Germany) in 1.5% (w/v) Tris- acetate- EDTA (TAE) agarose gel by horizontal electrophoresis (Sigma, Germany). The gel was run at 80 V and 400 mA constant current for 50 min.

RESULTS

A total of 40 (80%) *S. aureus* strains were cultured from the 50 samples obtained. The distribution of the *S. aureus* isolates showed that 6 were isolated from the ear swab, 2 from the eye, 32 from skin and soft tissue infections (Table 1). The susceptibility profile revealed that 30 (75%) of the isolates were resistant to amoxicillin; ceftriaxone 29 (72.5%), 12 (30%) to cloxacillin and oxacillin 5 (12.5%) of the β-lactam class of antibiotics. Between the aminoglycosides, 26(65%) of the isolates were resistant to streptomycin as against 7 (17.5%) to gentamicin. The least resistance was observed in the quinolones group of antibiotics. All the isolates were susceptible to ciprofloxacin (Table 2). The minimum inhibitory concentrations of the 5 oxacillin resistant strains included four MRSA strains with MIC 4 µg/ml and one with MIC 8 µg/ml.

The frequency of resistance revealed that of the 40 isolates tested, 85% of the isolates were multi-drug resistant to the representative classes of antibiotics. Of these, 17 (50%) were resistant to 3 classes of antibiotics, 8 (23.5%) to 4 classes, 7 (20.6%) to 5 and 2 (5.9%) to 6 classes (Table 3). The antibiotypes of the 5 community-associated methicillin resistant *S. aureus* (CAMRSA) strains revealed they were all resistant to the β-lactam group of antibiotics but represented by ceftriaxone, 4 to aminoglycosides, 2 to erythromycin, tetracycline, chloramphenicol, respectively, and 1 to cotrimoxazole (Table 4). The 5 strains were susceptible to ciprofloxacin (quinolone) and vancomycin. The PCR result indicated that the five MRSA strains possessed the *nuc* gene but the *mec* gene was detected in only one of the strains (CAMRSA1) at 532 base pair (bp) as shown in Figure 1.

DISCUSSION

The characterization of pathogenic organisms for epidemiological purposes is important in combating outbreaks of infection caused by these organisms. The

Table 1. The distribution of *S. aureus* isolates and age categorization of the subjects.

Source	Age in years	Number of strain
Ear	<1	2
	1-5	3
	6-12	NC
	13-19	1
	20-35	NC
	36-60	NC
	>60	NC
Eye	<1	1
	1-5	NC
	6-12	NC
	13-19	1
	20-35	NC
	36-60	NC
	>60	NC
Skin and soft tissue infection	<1	7
	1-5	3
	6-12	2
	13-19	3
	20-35	9
	36-60	7
	>60	1
Total		40

*NC = *S. aureus* isolates were not cultured.

Table 2. Antibiotic susceptibility pattern of *S. aureus* isolates.

Antibiotic	Number (%) of susceptible isolate	Number (%) of resistant isolate
Amoxicillin	11 (27.5)	29 (72.5)
Ceftriaxone	10 (25)	30 (75)
Cloxacillin	28 (70)	12 (30)
Oxacillin	35 (87.5)	5 (12.5)
Chloramphenicol	30 (75)	10 (25)
Cotrimoxazole	25 (62.5)	15 (37.5)
Erythromycin	26 (65)	14 (35)
Gentamicin	33 (82.5)	7 (17.5)
Streptomycin	14 (35)	26 (65)
Tetracycline	16 (40)	24 (60)
Ciprofloxacin	40 (100)	0 (0)
Ofloxacin	39 (95.5)	1 (2.5)
Pefloxacin	38 (95)	2 (5)

study considered the antibiotic susceptibility pattern of community-associated *S. aureus* strains obtained from a primary health centre in Ile-Ife, south western Nigeria. Out of a total of 50 samples examined, *S. aureus* accounted for 40 (80%) of the organisms cultured, indicating that *S. aureus* is not the only etiologic agent responsible for infections observed in the subjects. The antibiotic susceptibility profile of the *S. aureus* isolates revealed that resistance to the β-lactams is high and comparable to the resistance rate in nosocomial strains

Table 3. Frequency of multiple antibiotic resistance of *S. aureus* strains.

Number of antibiotic	Frequency of resistance n (%)
3	17 (50)
4	8 (23.5)
5	7 (20.6)
6	2 (5.9)
Total	34 (85)

Table 4. Antibiotype of the MRSA strains.

Isolates	Antibiotype
CAMRSA1	Ceftriaxone, Streptomycin, Erythromycin, Tetracycline, Chloramphenicol
CAMRSA2	Ceftriaxone, Tetracycline
CAMRSA3	Ceftriaxone, Streptomycin, Tetracycline, Chloramphenicol
CAMRSA4	Ceftriaxone, Streptomycin, Cotrimoxazole
CAMRSA5	Ceftriaxone Streptomycin, Erythromycin

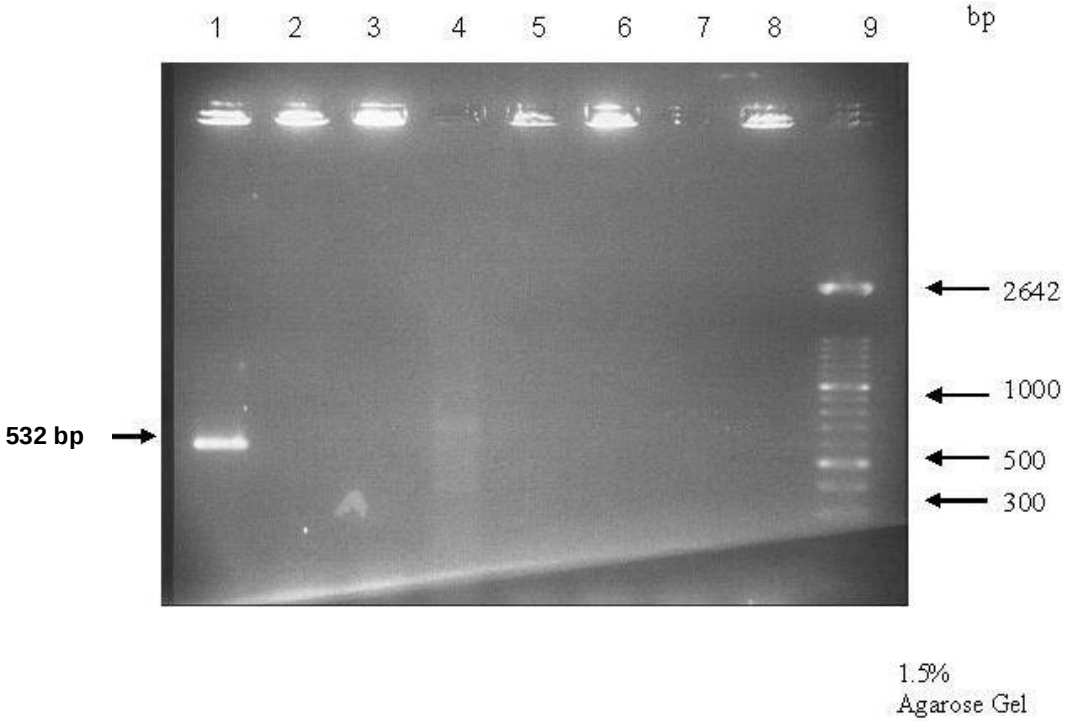

Figure 1. PCR detection of the *mecA* gene in CAMRSA strains shown by the arrow on the left. Lane 1, CAMRSA1; lane 2, CAMRSA2; lane 3, CAMRSA3; lane 4, CAMRSA4; lane 5, CAMRSA5; lanes 6 to 8, negative control; lane 9, 100 bp molecular weight ladder.

(Ako-Nai et al., 1991; Shittu et al., 2006; Torimiro et al., 2005). The β-lactam antibiotics are relatively available and cheap and therefore, prone to abuse. Besides, substandard ampicillin, ampicloxacillin and tetracycline have been reported in Nigeria (Agom et al., 1990) and this may have contributed to the high prevalence of resistance observed in this group of antibiotics. Apart from this, many hospitals in developing countries have been observed to have compromised infection practices resulting in nosocomial pathogens disseminated to the

community (Meers, 1988; Okello et al., 1997).

A total of 65% of the *S. aureus* isolates obtained were resistant to streptomycin as compared with 17.5% gentamicin resistance. The low proportion of *S. aureus* isolates resistant to gentamicin could be due to complexity of the drug as well as its (injectable) method of administration (Umolu, 2002), thereby making it less prone to abuse by users. All the isolates were susceptible to ciprofloxacin. The susceptibility of *S. aureus* isolates to ciprofloxacin has been reported to be a marker of CAMRSA strains (Woodford and Livermore, 2009).

The emergence of multi-drug resistance in bacteria has global health implications and has featured prominently as the cause of morbidity and mortality (WHO, 2001). In this present study, it was observed that 85% of the isolates were multi-drug resistant. The reason for the relatively high multi-drug resistance among community strains could be associated with self-medication, adulterated drugs, misuse and abuse in the community due to accessibility and across-the-counter purchase of these antibiotics (Agom et al., 1990; Okeke and Lamikanra, 1995) thereby enhancing the population of resistant *S. aureus* strains. In addition, it had been reported that acquired resistance in the community is common in developing countries where the need for antibiotics is driven by the high incidence of infectious diseases (Kunin, 1993; Okeke et al., 1999).

Although, the detection of the *mec*A gene is purported to be a gold standard for methicillin resistance, but the *mec*A gene was not detected in all the phenotypic methicillin resistance strains except in one strain with MIC 8 µg/ml in this present study. Also, the MRSA strains showed different antibio-types from each other, indicating differences among the strains. Galdiero et al. (2003) have reported that differences between strains analysed, low level methicillin resistance or borderline isolates could be a factor for the discrepancy in the detection of the *mecA* gene in MRSA strains. MRSA strains low in *mec*A gene detection had been reported in South Western Nigeria (Adesida et al., 2005; Shittu et al., 2006). The discrepancy observed for non-detection of *mec*A gene suggest a possible subject for further investigation. All the MRSA strains were susceptible to vancomycin. The efficacy of vancomycin and the quinolones suggest they can readily be employed in the treatment of MRSA infections in the study community.

The study shows the prevalence of multi-drug resistance amongst the community-associated *S. aureus* strains is high, which gives cause for concern and suggest the need to reduce the trend. In addition, it was observed from the questionnaire response that 75% of the subjects had taken antibiotic before hospital visit, indicating that the MRSA strains cultured had risk factor for hospital infection. It had been reported that the dangerous consequences of staphylococcal infection have become more serious when evidences from all over the world indicated that the antibiotic patterns in the hospitals were also seen in the community (Jessen et al., 1969; Chambers, 2001; Bassetti, 2009).

The study suggests adequate public health education and promotion of appropriate use of antibiotics in the community. In addition, effective infection control practices in the hospitals in order to reduce the trend of multi-drug resistance in the community are important.

ACKNOWLEDGEMENTS

The Authors are grateful to Professor (Mrs) O. G. Omitogun of the Obafemi Awolowo University (OAU), Ile-Ife, Nigeria for reading through the manuscript and making useful suggestions and comments. We are also grateful to the Central Science Laboratory Obafemi Awolowo University Ile-Ife, Nigeria for the provision of some of the facilities for the DNA analysis.

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