ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY JANUARY2013 ISBN 1595-689X VOL 14(1) 2013
AJCEM/21301 -http://www.ajol.info/journals/ajcem

http://dx.doi.org/10.4314/ajcem.v14i1.1
AFR. J. CLN. EXPER. MICROBIOL 14(1): 1-4

MOLECULAR IDENTIFICATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN BENIN-CITY NIGERIA

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ABSTRACT

We use the molecular techniques of PCR and PFGE to identify MRSA from clinical isolates of *Staphylococcus aureus* causing infections among hospitalized patients in Benin-City, Nigeria. A total of 36 isolates were obtained from the University of Benin Teaching Hospital between July-September, 2007. The MRSA strains were selected according to their phenotypic characteristics (antibiotic resistant profiles), susceptibility to oxacillin by E-test, and detection of β-lactamase. This was verified by a latex agglutination test for PBP2a production combined with PCR for *mecA* gene carriage. Four isolates representing 11% were confirmed as MRSA according to the molecular techniques used with two PFGE types (H and L) and one agr type (1). Multi resistance to the various antibiotics used was observed in one of the clones. The isolation of MRSA in health institution indicates that adequate steps in limiting spread are urgently needed. Also, for the first time two MRSA clones according to the PFGE classifications have been identified in Nigeria.

Keywords: methicillin-resistant *Staphylococcus aureus*, MRSA, PFGE, PCR, molecular techniques.

INTRODUCTION

Since 1961, Methicillin-resistant *Staphylococcus aureus* (MRSA) strains have assumed increasing importance internationally as a cause of both nosocomial and community-acquired infections (1). A recent United States study found that Infections caused by MRSA result in increased lengths of hospital stay, health care costs, morbidity and mortality when compared to those caused by methicillin-sensitive *Staphylococcus aurues* (MSSA) (2). Although the presence of MRSA in the Asia-Pacific region has been well recorded, data on the true prevalence of MRSA in the region are limited. Data from Australia have been published and shown that in hospital isolates from the Eastern Seaboard, the percentage of *S. aureus* that were MRSA remained relatively constant at approximately 30% from 1986 to 1994.

Soon after the first reports of methicillin resistance in *S. aureus* in 1961, the unusual behaviour of the strains in susceptibility tests were noted. Early reports indicated that the MRSA were heterogenous in their expressions of resistance to β-lactam agents, in that large differences in the degree of resistance were seen among the individual cells in a population (3). Infection control measures, such as placing hospitalized patients colonized or infected with MRSA in contact precautions (the use of gowns and gloves), have been demonstrated to limit the spread of this pathogen (4). The use of surveillance cultures (e.g anterior nares, axillae and perineum) greatly improves the detection of MRSA colonization compared to clinical cultures alone.

The anterior nares are the most frequent site of MRSA colonization, with a single culture of this site having a sensitivity of approximately 85% (5).

Antimicrobial resistance of *S. aureus* especially MRSA continues to be a problem for clinicians worldwide. However, few data on the antibiotic susceptibility patterns of *S. aureus* isolates in South Africa have been reported and the prevalence of MRSA in the KwaZulu-Natal (KZN) province (6). Methicillin resistance in *Staphylococcus* spp. is primarily mediated by the *mecA* gene, encoding penicillin-binding protein 2a (PBP2a). This protein has reduced affinity for β-lactam antibiotics. Because the *mecA* gene is heterogeneously expressed *in vitro* selective media are necessary to facilitate recovery of MRSA in cultures (7).

The time from culture inoculation to identification of MRSA is typically 48 h, with some methods taking as long as 96 h (8) with sensitivity of any single selective medium ranging between 65 and 100%. Several techniques to shorten the time of identification of MRSA in the laboratory have been developed in the last decade, including slide latex agglutination assays to detect PBP2a, a colorimetric cycling probe assay to directly detect the *mecA* gene (9), a real-time PCR method to detect the *mecA* gene (10) in conjunction with *S. aureus*-specific genome fragments, such as *nuc* and *sa442*.

Although data on the prevalence of MRSA infections in Africa are limited, one of the earliest reports in the continent was in South Africa (11). Studies in the 1980s and 1990s on MRSA in South Africa have also been described (12). The recent reports of *S. aureus* intermediately resistant to

vancomycin and teicoplanin in Africa (13) also indicate that information on antibiotic resistance is critical for optimal decisions regarding hospital formulary and infection control policies. In addition, characterization of strains is important in understanding the epidemiology of *S.aureus* and evaluating the effectiveness of infection control and antimicrobial prescribing measures (14).

The main objective of this study was to use the molecular techniques of Polymerase Chain Reaction (PCR) and Pulsed Field Gel Electrophoresis (PFGE) to identify MRSA from clinical isolates of *S.aureus* causing infections among hospitalized patients in Benin-City, Nigeria.

MATERIALS AND METHODS

Study area – a total of 36 clinical isolates of *Staphylococcus aureus* were obtained from hospitalized patients at the University of Benin Teaching Hospital, Benin City Nigeria between July-September, 2007. Identification and confirmation of isolates were conducted based on growth and fermentation on mannitol salt agar (MSA), colonial morphology, Gram staining and positive results to both catalase and coagulase tests.

Identification of MRSA

The MRSA strains were selected according to their phenotypic characteristics (antibiotic resistant profiles), susceptibility to oxacillin by E-test (AB Biodisk), and detection of β-lactamase. This was verified by a latex agglutination test (bioMerieux) for PBP2a production combined with PCR for *mecA* gene carriage.

Extraction of chromosomal DNA

DNA was extracted by suspending portions of four to five colonies in 1ml dH_2O equivalent to 2MF, centrifuged for 10mins at 13000rpm. The supernatant was rejected and the sediment resolved with 100ml lysis buffer (50mM Tris-HCl pH=7.5, 1% Triton X-100, 1mM EDTA pH=8.0) + 0.2 µl proteinase K (1µl/ml from stock 20mg/ml). Initial incubation was done at 56°C for 1 hour followed by 95°C for 10mins. The DNA extracts were stored at -20°C prior to use. PCR for the presence of the gene *lin* was used to test for the quality of the DNA extracts (15).

Classification of MRSA strains was performed by PCRs according to agr groups (16). The presence of toxin genes (*tst*, *PVL*, *egc*) was investigated by PCRs using specific primers and programmes (17). Chromosomal DNA extraction of MRSA was performed into agarose disks and clonal types was defined by PFGE of SmaI digests (18, 19).

Latex agglutination test for PBP2a production

The test was performed according to the manufacturer's instructions. Briefly, the isolates were subcultured onto blood agar at 37°C for 18 hours to obtain fresh growth. A loopful of cells was suspended in four drops of extraction reagent 1 and was placed in a boiling water bath for 3 minutes. After allowing the suspension to cool to room temperature (approx.10 minutes), one drop of extraction reagent 2 was added and the mixture was vortexed thoroughly. The suspension was then centrifuged at 4500 rpm for 5 minutes. The latex agglutination test was performed with the supernatant, and 50µl of the supernatant was mixed with a drop of sensitized latex. For the negative control, 50 µl of the supernatant was mixed with 1 drop of negative control latex. The samples were mixed for 3 minutes on a shaker and the results were evaluated according to the following plan:

Sensitized latex	Control latex	Results
+	-	MRSA
-	-	MSSA
-	+	Undetermined

RESULTS

A total of thirty-six *S. aureus* isolates collected from hospitalized patients in Benin-City, Nigeria between July and September 2007 were used in this study. Four isolates representing 11% were confirmed as MRSA (Table 1) according to the molecular techniques used with two PFGE types (H and L) (Figure 1) and one agr type (1). Multi resistance to the various antibiotics used was observed in one of the clones. There was no detection of any toxin genes from the strains (Table 2).

TABLE 1: PREVALENCE OF MRSA AMONG THE CLINICAL ISOLATES OF *S.AUREUS*

Number of *S. aureus* isolates	MRSA	%
36	**4**	**11.1**

TABLE 2: RESULTS OF GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF THE 4 MRSA STRAINS

PFGE type	agr	Antibiotic resistance	Toxin gene (PVL, *tst*, *sem*, *seg*)	No. of strains
H	1	K, ER, SXT	-	2
L	1	Multiresistant	-	2
TOTAL				4

K- kanamycin, ER-erythromycin, SXT-cotrimoxazole; multiresistance- resistance to more than three antibiotic groups.

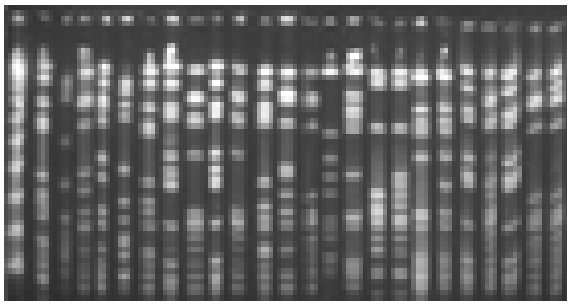

FIGURE 1: PFGE showing large genomic DNA fragments after digestion with restriction enzymes (Standard controls and test strains). Since the bacterial chromosome is typically a circular molecule, this digestion yields several linear molecules of DNA. After digestion of the DNA and electrophoresis through an agarose gel, if the DNA banding pattern between any two isolates is identical, then these isolates are considered the same strain. Conversely, if two isolates are not the same strain, then the sites at which the restriction enzymes act on the DNA and the length between these sites would be different; thus their DNA banding patterns will be different.

DISCUSSION

Antimicrobial resistance has been noticed as one of the paramount microbial threats in the twenty-first century (20). *S. aureus* has always been a stumbling block for antimicrobial chemotherapy and the introduction of new classes of antimicrobial agents is usually followed by the emergence of resistant forms of this pathogen (21). Surveillance on the antimicrobial susceptibility patterns of *S. aureus* is important in understanding new and emerging resistance trends and in the management of both hospital and community-acquired infections.

Methicillin resistance of *S.aureus* remains to be a significant problem. Rapid and accurate determination of methicillin resistance is important for initiation of appropriate antimicrobial therapy. Misdiagnosing this resistance leads to treatment failures and spread of infections with these resistant strains. The increasing reports about vancomycin resistance among *S.aureus* isolates mandate the use of glycopeptide antibiotics which yet appear to be the only choice, sparingly. Therefore, striving for the identification of methicillin resistance as soon as bacterial growth is observed is the only way to limit the superfluous use of glycopeptide class on sensitive isolates.

A major problem in the treatment of *S. aureus* infections is the multidrug resistance pattern of the pathogen to a number of antibiotics. In the last few years, understanding of the genetic basis for methicillin resistance has advanced significantly. Multi-resistant MRSA have been reported to be relatively high in African countries including Morocco, Kenya and Cameroun (22), but their antibiotypes were not determined.

This study has provided baseline information for physicians, clinical microbiologists and public health officials on critical issues regarding pathogen specific therapy. The isolation of MRSA in health institution indicates that adequate steps in limiting spread are urgently needed. Also, for the first time two clones (H and L) according to the PFGE classifications have been identified in Nigeria with one agr type (1) but no detection of any of the toxin genes tested. Continuous surveillance on resistant pattern and molecular characterization or epidemiological typing in order to generate isolate-specific genotypic or phenotypic characters that can be used to elucidate the source and route of spread of MRSA infection in Benin City, Nigeria is relevant and of utmost importance.

ACKNOWLEDGEMENT

The assistance of the Laboratory of Professor Iris Spiliopoulou , Department of Microbiology
School of Medicine, University of Patras, Greece is greatly appreciated.

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