Int J Pharm Bio Sci 2014 July ; 5 (3) : (B) 185 - 193

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PREVALENCE AND SUSCEPTIBILITY PROFILES OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN THE UNIVERSITY OF GHANA HOSPITAL, LEGON, ACCRA, GHANA

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the important causal agents of nosocomial infections worldwide. The prevalence and susceptibility profiles of MRSA in the University of Ghana Hospital, Legon, Accra were investigated from 65 swab samples. Samples were inoculated onto blood agar plates and incubated at 37°C aerobically for 24 h. After overnight incubation, isolates were tested biochemically and a total of 11 (16.9%) isolates of *Staphylococcus aureus* were identified from the swab samples. The *S. aureus* isolates were later confirmed as MRSA by growth on Oxacillin-Resistant Screening Agar Base (ORSAB) and detection of the *mec*A gene using polymerase chain reaction (PCR) technique. Kirby-Baeur disc diffusion method was also used to determine the susceptibility of the isolates. There was only 1 (9.1%) MRSA isolate detected from the samples. The MRSA isolate was susceptible to co-trimoxazole, tetracycline, and gentamicin but resistant to ampicillin, cephalexin, cefotaxime, ciprofloxacin, prulifloxacin, ofloxacin, cloxacillin, roxithromycin, lincomycin, and cefoxitin antibiotics used in this investigation. Despite the low prevalence rate in this study, there is still the need for strong continuous surveillance programs to monitor MRSA and its antibiotic profiles.

KEY WORDS: **Staphylococci, Methicillin-sensitive *Staphylococcus aureus,* Methicillin-resistant *Staphylococcus aureus,* Polymerase Chain Reaction**

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INTRODUCTION

Antibiotic resistance has increased greatly in recent years and is posing ever-increasing therapeutic problems worldwide. The ways that bacteria overcome drug actions are varied, ranging from intrinsic to acquired resistance properties[1]. *Staphylococcus aureus* is a Gram-positive bacterium that grows both in aerobic and anaerobic conditions. The organism is most common in nasal membranes and skin of warm-blooded animals as a commensal but it is able to cause many kinds of infections, such as skin infections, food poisoning, pneumonia, sepsis, and many others [2]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a biotype of *S. aureus* that has gained resistance to β-lactams which include penicillins (methicillin, dicloxacillin, natcillin, oxacillin, etc.), cephalosporins, and carbapenems. The development of such resistance does not make the organism to be more intrinsically virulent than other *S. aureus* strains that are susceptible to these drugs, except that the resistance makes treatment with standard antibiotics more difficult and thus more dangerous. Infections with MRSA are associated with greater morbidity and mortality than similar infections with methicillin-sensitive strains. The transmission of MRSA commonly occurs by direct contact with colonized or infected health personnel and patients [3]. It can also be transmitted through the ingestion of food containing enterotoxins. Poor sanitary conditions and overcrowding in communities increase the risk of MRSA. This makes MRSA infections more prone in settings such as hospitals, prisons, schools, and nursing homes. Nasal colonization can also cause self-infection [4]. Currently the methods available for the diagnosis of MRSA infections include the traditional culture, isolation, sensitivity testing, and molecular analysis of specific genes using polymerase chain reaction (PCR) method. The traditional culture method aims to isolate *S. aureus* that is resistant to methicillin (oxacillin-resistant) while the molecular methods (PCR), aim to detect specific genes (*mec*A) that code for proteins and molecules that provide resistance to the bacteria. Resistance of *S. aureus* to natural penicillin is associated with the production of an enzyme called β-lactamase or penicillinase [5]. This enzyme produced by the β-lactamase gene, makes the bacteria resistant to penicillin by cleaving the β-lactam ring of the antibiotic. However, these β-lactamase producing bacteria are susceptible to methicillin and other semi-synthetic penicillin-based drugs. MRSA on the other hand, is resistant to methicillin and the other semi-synthetic β-lactams. This resistance is due the presence of a gene, the *mec*A, which confers resistance to the bacteria through the production of penicillin-binding proteins (PBP2′ or PBP2a) of lower affinity for the drug, a reduction in the expression of high affinity or a change in conformation of the PBP2′ or PBP2a which leads to complete resistance to all β-lactams [6]. The *mec*A gene is located on a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*).

The treatment of choice for serious infections due to MRSA is glycopeptide antibiotics such as vancomycin [7]. However, appropriate management of MRSA colonization remains unclear due to the fact that it has been reported that, vancomycin is not effective for eradicating MRSA carriage as reported by the Centre for Diseases Control and Prevention (CDC) [8]. An important reason for concern of MRSA is the development of glycopeptide resistance in *Staphylococcus* species. In the past years clinical infections with strains of *S. aureus* with reduced susceptibility (intermediate resistance) to vancomycin and other glycopeptides has been reported in the United States of America, Japan, and several European countries [9]. Reduced susceptibility to vancomycin has occurred in MRSA strains because infections are associated with significant morbidity which often requires prolonged antimicrobial therapy [5]. Modification of bacterial cell wall proteins in response to prolonged vancomycin exposure is likely responsible for the emergence of glycopeptide resistance in MRSA isolates. MRSA is a problem of the world for the bacteria have been isolated from different continents including India. In Ghana the

children's block of the Korle-Bu Teaching Hospital (KBTH), Accra was closed down on January, 2012 as a result of an MRSA outbreak in the ward as reported by the Ghana News Agency (GNA)[10].The prevention and control of MRSA is a challenge in hospitals and communities all over the world as well as in Ghana. Regular screening of MRSA has been shown to be an effective measure in the control of hospital-acquired infections [11]. Hence it is necessary to determine the prevalence of MRSA in the University of Ghana, Hospital, Legon, Accra to better understand the dynamics of MRSA to prevent future outbreaks in the hospital. Also a comparison of phenotypic microbiological techniques and PCR detection of the *mec*A gene in MRSA will give information on the best method for the diagnosis of MRSA infections which have become a medical problem worldwide. Therefore this study was designed to determine the prevalence and susceptibility profiles of MRSA in the University of Ghana Hospital, Legon, Accra.

MATERIALS AND METHODS

(i) Sample collection and processing

Samples were collected between March and August, 2013, from the University of Ghana Hospital, Legon, Accra. The samples were collected aseptically by swabbing the nostrils, wounds of the study participants, and other sites of the hospital environment. In all, a total of 65 samples were collected including nasal swabs (35) and wounds (6). Also other sites in the hospital environment including, bed sheets (8), treatment room materials (5), sinks (6), and taps (5) were also sampled. All specimens were sampled by using sterile cotton swabs. For all the human subjects, the sampling procedure was approved by the Ethics and Protocol Review Committee, School of Allied Health Sciences (SAHS), College of Health Sciences, University of Ghana, and participants provided their informed consent before participating in the study. Participants were also provided on voluntary basis and coded to protect anonymity.

(ii) Bacterial isolation

The swabs were transported to the laboratory using Stuart transport media, inoculated onto blood agar plates and incubated at 37°C aerobically. The plates were examined after 24 h for growth. *S. aureus* colonies were identified based on their colonial characteristics exhibited on the media. For instance, colonial forms that appeared raised and relatively larger with slightly translucent creamy to yellow pigmentation on blood agar plates were preliminarily regarded as *S. aureus*. Also presumptive identification of staphylococcal strains was done in the present investigation based on the Gram staining reactions, catalase, and agglutination tests [12]. The later was performed using human plasma (1:10 dilution of human plasma) obtained from PAA Laboratories (GmbH, Pasching, Austria). *S. aureus* (25923) from the American Type Culture Collection (ATCC) was also used as the control bacterial strain in the agglutination test. A further test to confirm the identity of the isolates were done by sub-culturing onto prepared selective differential medium, Oxacillin-Resistance Screening Agar Base (ORSAB: CM 1008, Oxoid Limited, Basingstoke, UK) with the selective supplement SR 0195 (polymyxin B and oxacillin) and incubated for 24 h at 37°C in the presence of air. After overnight incubation MRSA colonies appear as intense blue colouration on the ORSAB agar surface. Positive growth control was *S. aureus* (MRSA strain) ATCC 43300 whiles negative growth controls were methicillin-sensitive *Staphylococcus aureus* (MSSA ATCC 25923) and *Escherichia coli* ATCC 25922.

(iii) Antibacterial susceptibility testing

The pattern of resistance of the isolates was determined using modifications of the disc diffusion method [13]. For this assay, 3-5 pure discrete colonies of *S. aureus* isolates from an 18-24 h blood agar plate were transferred into 5 ml sterile physiological saline (0.85%). The absorbance of the bacterial suspension was measured using a spectrophotometer and adjusted to a density of 10^6 colony forming units per millilitre (CFU/ml) according to the British Society for Antimicrobial Chemotherapy (BSAC) standards [14]. A sterile

cotton swab was dipped into the adjusted inoculum suspension, rotated several times and pressed firmly on the inside wall of the tube above the fluid level to get rid of excess inoculum on the swab. A dried surface of Mueller-Hinton agar (MHA: Oxoid Limited, Basingstoke, UK) plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60% each time to ensure an even distribution of inoculum. Standard reference antimicrobial discs including, ampicillin (20 µg), co-trimoxazole (25 µg), cephalexin (30 µg), tetracycline (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), prulifloxacin (5 µg), ofloxacin (5 µg), cloxacillin (5 µg), roxithromycin (15 µg), lincomycin (2 µg), and gentamicin (10 µg), were applied firmly to the surface of the inoculated agar plate with the aid of a sterile forceps. Also cefoxitin (30 µg) was included in the test to determine methicillin resistance. The discs were obtained from Axiom Laboratories, India. Within 30 min after the discs were applied, the plate was incubated aerobically at a temperature of 37°C for 24 h and the results interpreted according to the performance standards for antimicrobial susceptibility testing by the Clinical Laboratory Standards Institute (CLSI) [13]. Quality control strains used for the disc diffusion susceptibility testing of the isolates were *S. aureus* strains ATCC 43300 and ATCC 25923.

(iv) Determination of mecA genes by PCR method

Methicillin resistance in the isolates were confirmed by determination of the *mec*A gene using PCR at the Department of Bacteriology, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Accra. PCR method used was as described by Larsen *et al*. [15]. Preparation of DNA templates was done in accordance to Kumari *et al*. [16]. Each PCR contained 0.45 µM *mec*A primers (*mec*A P4, 5'-TCCAGATTACAACTTCACCAGG; *mec*A P7, 5'-CCACTTCATATCTTGTAACG), 1 × Multiplex PCR Master Mix (Qiagen, Valencia, CA, USA), and 1 µL of DNA template preparation, using the following cycling conditions: 15 min at 94°C, followed by 30 cycles of 30 sat 94°C, 1 min at 59°C and 1 min at 72°C and a final 10 min at 72°C in a DNA Engine DYAD (Bio-Rad, Hercules, CA, USA) thermal cycler. The PCR products were visualized on 2% agarose gel.

STATISTICAL ANALYSIS

Results obtained from the experiment were analysed using descriptive statistics, Chi-square test (X^2), p-value ($p < 0.05$ was considered significant), and Statistical Package for Social Sciences (SPSS) statistical software (16th version).

RESULTS

(i) Isolation and characterization

In all, a total of 11 (16.9%) isolates of *S. aureus* were isolated from 65 samples investigated (Table 1). When the *S. aureus* isolates were screened for oxacillin-resistance, only 1 (9.1%) was positive on ORSAB medium. The *mec*A gene was also detected by PCR in that isolate (Fig. 1). The number of isolates of MSSA and MRSA from the staffs, patients, and the different sites in the University of Ghana Hospital, Legon, Accra are also presented in Table 1. From the Table it can be observed that the highest number of MSSA (66.7%) were isolated from nasal swabs with only 1 (8.3%) MRSA isolate also from a nasal swab. The prevalence rate of MSSA and MRSA at the University of Ghana Hospital, Legon are also presented in Table 2. The prevalence of MSSA and MRSA were found to be 90.9% and 9.1% respectively.

(ii) Antibacterial susceptibility testing

The only 1 *mec*A positive strain isolated in this study was susceptible to cotrimoxazole, tetracycline, and gentamicin but resistant to ampicillin, cephalexin, cefotaxime, ciprofloxacin, prulifloxacin, ofloxacin, cloxacillin, roxithromycin, lincomycin, and cefoxitin antibiotics used in this investigation (Table 3). The overall resistance rate of the 10 isolates of MSSA to the various antibiotics, that is ampicillin (100%), cotrimoxazole (36.4%), cephalexin (36.4), tetracycline (18.2), cefotaxime (100%), ciprofloxacin (9.1%), prulifloxacin (45.5%),

ofloxacin (9.1%), cloxacillin (100%), roxithromycin (54.5%), lincomycin (27.3%), gentamicin (0%), and cefoxitin (9.1%) are as presented in Fig. 2. All isolates were, however susceptible to gentamicin.

(iii) Description of PCR Bands

Top band: *spa* type: confirms that isolate is *S. aureus* (variable size 200-600bp depending on the type of *S. aureus*) as presented in Fig. 1. Middle band: confirms that isolate has *mecA* gene (MRSA): 162bp. Lower band: confirms that isolate has a toxin (Panton Valentine Leukocidin): 80bp. From the study, only sample 8 was an MRSA isolate.

Table 1

Isolates of MRSA from the University of Ghana Hospital, Legon, Accra.

Type of swab	No. of samples	No. of *S. aureus*	No. of MRSA
Nasal	35	8	1
Wound	6	1	0
Taps	5	0	0
Sinks	6	1	0
Bed sheets	8	0	0
Treatment room materials	5	1	0
Total	**65**	**11**	**1**

Table 2

Prevalence rate of MSSA and MRSA at the University of Ghana Hospital, Legon, Accra.

	Frequency	Percentage (%)
MSSA	10	90.9
MRSA	1	9.1
Total	**11**	**100.00**

MSSA, methicillin-sensitive Staphylococcus aureus
MRSA, methicillin-resistant Staphylococcus aureus

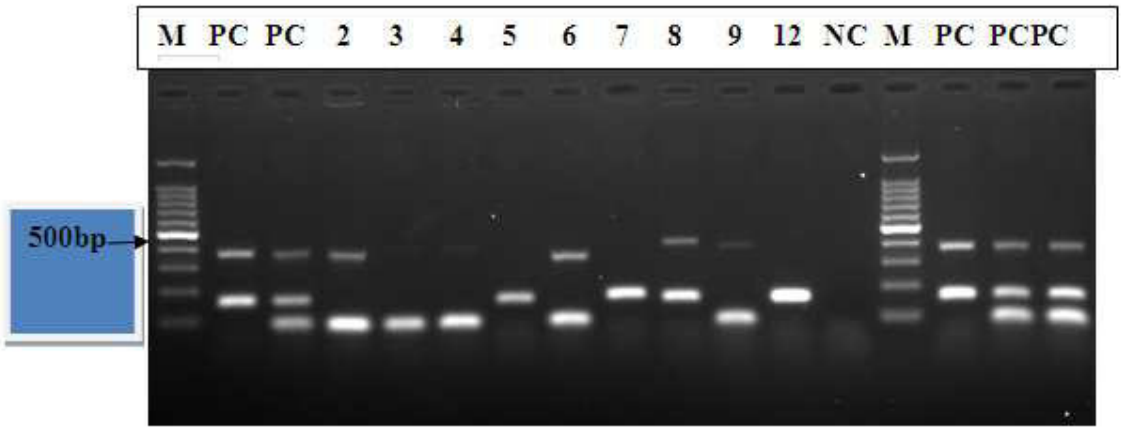
Table 3

Antibiotic susceptibility of MSSA and MRSA isolates.

		MSSA (n=10)			**MRSA (n=1)**		**Total (n=11)**
Antibiotic	S	I	R	S	I	R	Resistance rate (%)
Ampicillin	0	0	10	0	0	1	100
Cotrimoxazole	6	0	4	1	0	0	36.4
Cephalexin	3	4	3	0	0	1	36.4
Tetracycline	8	0	2	1	0	0	18.2
Cefotaxime	0	0	10	0	0	1	100
Ciprofloxacin	10	0	0	0	0	1	9.1
Prulifloxacin	1	5	4	0	0	1	45.5
Ofloxacin	10	0	0	0	0	1	9.1
Cloxacillin	0	0	10	0	0	1	100
Roxithromycin	4	1	5	0	0	1	54.5
Lincomycin	6	2	2	0	0	1	27.3
Gentamicin	10	0	0	1	0	0	0
Cefoxitin	10	0	0	0	0	1	9.1

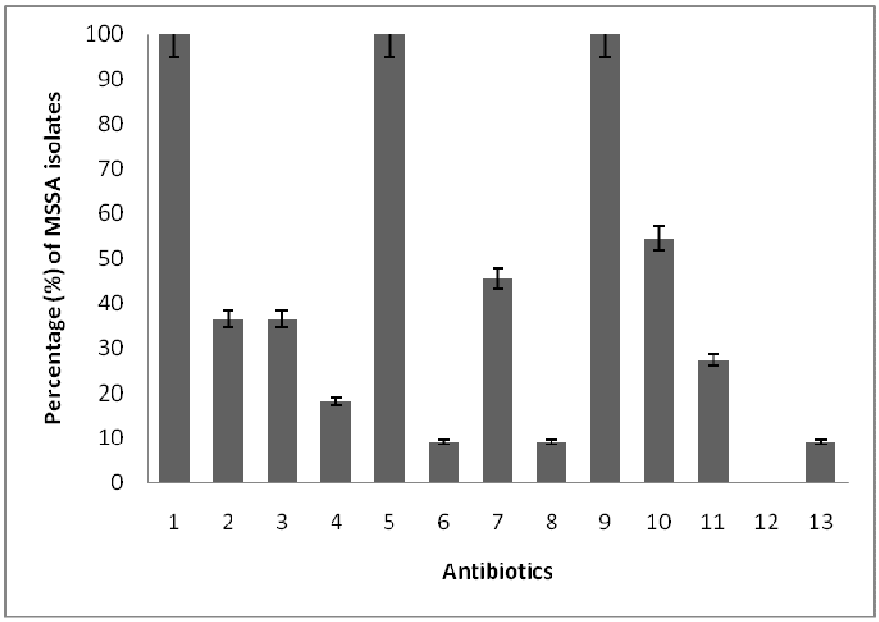
S, susceptible; I, intermediate resistance; R, resistance

Figure 1
Molecular analysis of MRSA and MSSA



[From left of photograph, lane M: 100 base-pair marker, lanes 2 and 3: positive controls (PC) of MRSA, lanes 4-12: Samples 2, 3, 4, 5, 6, 7, 8, 9, 12, lane13: Negative control (NC), lane 14: 100 base-pair marker, lanes 15-17: Positive controls of MRSA].

Figure 2
Percentages of MSSA isolates and their resistance to the various antibiotics



[1-ampicillin, 2-cotrimoxazole, 3-cephalexin, 4-tetracycline, 5-cefotaxime, 6-ciprofloxacin, 7-prulifloxacin, 8-ofloxacin, 9-cloxacillin, 10-roxithromycin, 11-lincomycin, 12-gentamicin, and 13-cefoxitin]. Mean Error bars in the graph represents the mean ± standard error from duplicate samples that were tested. $P < 0.05$ was taken as significant differences.

DISCUSSION

In the past, strains of *S. aureus* used to respond to antimicrobial agents but the acquisition of methicillin-resistance and other genes have provided the bacteria with mechanisms that have made all members of the largest and most useful family of

antimicrobial agents including the β-lactams antibiotics no longer effectiveas therapeutic agents against these bacteria [17,18]. It is in view of this that, this study was done to determine the prevalence of MRSA in the University of Ghana Hospital, Legon, Accra. One strain of MRSA was isolated from 11 *S. aureus* isolates which represents a prevalence of 9.1% of the total number of samples (n=65) in the present investigations. However, in a previous study by Odonkor *et al*. [19], they collected 250 isolates from 5 hospitals in Accra, Ghana and found the prevalence rate of 84 (33.6%) of MRSA in the Accra Metropolis. In another study by Tsering *et al*. [20] to determine the prevalence of MRSA in a referral tertiary care teaching hospital of Sikkim, India, they found out that 152 (52.2%) out of 291 isolates of *S. aureus* from 827 clinical specimens were methicillin-resistant. Joshi *et al*. [21] also using 26310 isolates found the prevalence of MRSA to be 41% in India. The low prevalence of MRSA in the University of Ghana Hospital, Legon, Accra might be due to small sample size used in the present study. The bacteria were classified as being susceptible, intermediate or resistant to the antibiotics used depending on the size of inhibition zone diameters compared to the standard provided by the CLSI [13]. It was found out in this study that, all the 10 MSSA isolates showed 100% resistant to ampicillin, cefotaxime, and cloxacillin (Table 3). These MSSA isolates were also found to show levels of intermediate resistance to cephalexin, prulifloxacin, roxithromycin, and lincomycin. However, none of the MSSA isolates were resistant to gentamicin. The MRSA isolate, on the other hand was resistant to the following antibiotics, ampicillin, cephalexin, cefotaxime, ciprofloxacin, prulifloxacin, ofloxacin, cloxacillin, roxithromycin, lincomycin, and cefoxitin (Table 3). It was however, susceptible to cotrimoxazole, tetracycline, and gentamicin. In a similar work by Odonkor *et al*. [19] in Accra, they found the susceptibility of MRSA isolates against gentamicin as being 54.7%, cotrimoxazole as 49%, ampicillin as 15.5%, and tetracycline as being 7.1%. Also Egyir *et al*. [22] working on the prevalence of nasal carriage of *S. aureu*s at the KBTH, Accra, Ghana reported the susceptibility of MRSA isolates to tetracycline as 72% in their study.

Odonkor *et al*. [19] have recommended that though some work have been done on the isolation and identification of MRSA isolates in Ghana, molecular analysis of *S. aureus* before confirming the isolates as MRSA is of great scientific importance. It is of this fact that the isolates in this study were analysed using PCR methods for the identification and characterization. From the results of the molecular analysis (Fig. 1) in the present study it was observed that sample 8 was positive for the *mecA* gene, hence, methicillin-resistant. This has confirmed the phenotypic microbiological analysis, which showed sample 8 as being the only isolate resistant to cefoxitin which is among the antibiotics used to show methicillin-resistance during the screening. However, from the molecular analysis (Fig. 1), 3 out of the 11 samples were non-*S. aureus* but carried the *mecA* gene. Also when the 11 strains of *S. aureus* were investigated for oxacillin-resistance by growing on ORSAB medium, it was observed that the detection of *mec*A gene indicated an excellent relationship between ORSAB medium and PCR analysis. This relationship is due to the fact that it was only one isolate that was able to grow on the ORSAB medium and the PCR method was also able to detect the *mec*A gene in that isolate as has been previously proposed by Suleiman and co-workers [23].

CONCLUSION

Although the data from the present study indicates low prevalence rate (9.1%) of MRSA in the University of Ghana Hospital, Legon, Accra. The MRSA isolate was susceptible to only 3 (23.1%) of the 13 antibiotics used in the investigation which calls for continues surveillance and monitoring in order to prevent the outbreak of a highly resistant strains of *S. aureus* or MRSA in the hospital and Ghana as a whole.

ACKNOWLEDGEMENT

We thank Ms. Marjorie Ntiwaa Quarchie of the School of Allied Health Sciences (SAHS), College of Health Sciences, University of Ghana during the collection and analysis of the samples.

CONFLICT OF INTEREST

Conflict of interest declared none.

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