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PREVALENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN CLINICAL SPECIMENS AND AMONG HOSPITAL STAFF NASAL CARRIERS IN KHARTOUM STATE

Ehssan H. Moglad [1, 2]

Department of Pharmaceutics [1], College of Pharmacy, Prince Sattam bin Abdulaziz University, P. O. Box 173 Alkharj 11942, Saudi Arabia.
Department of Microbiology [2], Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), P.O. Box 2404, National Center for Research, Khartoum, Sudan.

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Correspondence to Author:
Ehssan H. Moglad

Assistant Professor of Medical Microbiology, Department of Pharmaceutics, College of Pharmacy, Prince Sattam bin Abdulaziz University, P. O. Box 173 Alkharj 11942, Saudi Arabia.

E-mail: ehssanhassn@gmail.com

ABSTRACT: *Staphylococcus aureus* is a bacteria that causes a heavy epidemic disease burden worldwide. This study aimed to investigate the prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and the presence of *mecA* gene as a virulence factor of *S. aureus* isolates from clinical isolates and hospital staff nasal carriers using a conventional PCR. A cross-sectional study involving eighty-one *S. aureus* isolates, 60 clinical isolates, and 21 nasal swabs from hospital staff was conducted from January 2018 to April 2018. The samples were processed using standard microbiological procedures. The antibiotic resistance pattern was performed using the Kirby- Bauer disc diffusion method for cefoxitin (30 µg), Oxacillin (5 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), amoxicillin-clavulanate (20 + 10 µg), fusidic acid (10 µg) and Vancomycin (30 µg) according to the Clinical and Laboratory Standards Institute guidelines. The presence of the *mecA* gene in MRSA was detected by PCR. A total of 60 clinical samples were collected and analyzed; 42 (70%) resulted in Methicillin resistant and positive for mecA gene, and 18 (30%) isolates resulted in sensitive for Methicillin and negative for *mecA* presence. All samples from hospital staff were resistant for Methicillin and positive for *mecA* presence. The MRSA became resistant to methicillin due to an acquisition of the *mecA* gene. All isolated *S. aureus* strains among the hospital staff nasal carriers were MRSA may influence the spread of bacteria and increase the percentage of persistent nosocomial infections in hospitalized patients. More studies needed in order to understand the molecular characterization of MRSA in a setting better using different molecular typing tools.

INTRODUCTION: *Staphylococcus aureus* is a major human pathogen that causes a spectrum of infections; it has been increasingly problematic worldwide [1-5].

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S. aureus is reported as the second driving cause of nosocomial infections, especially in patients undergoing surgery, hemodialysis, cirrhosis, kidney transplant, and hospitalized patients [6-8].

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a severe public health concern, responsible for hospital and community-associated infections worldwide [7, 9-11]. MRSA infections are considered among the most life-threatening infections due to the limited therapeutic options [4, 5, 12, 13]. A systematic review of MRSA in Africa 13.8 billion

attributed to community-acquired MRSA [14]. Methicillin resistance is encoded by the *mecA* gene, borne on the staphylococcal cassette chromosome *mec* (SCC*mec*). The *mec*A gene codes for a78-kD a penicillin-binding protein (PBP2a), with decreased affinity to methicillin as well as all beta-lactam antibiotics [15-17]. Variable SCC*mec* types are circulating within different countries, reflecting the various MRSA strains [2, 18].

In Sudan, there is limited data on the prevalence of *S. aureus* in clinical samples and their resistance factors using molecular methods. This study aimed to screen the methicillin resistance gene (*mecA*) in *S. aureus* isolates from patients as well as health care workers. The findings from this study will contribute to the current knowledge on the prevalence and diversity of MRSA in Khartoum State.

MATERIALS AND METHODS:

Study Area and Clinical Isolates Collection: This study was a cross-sectional study conducted from January to May 2018. Sixty *S. aureus* clinical isolates were collected from Al Ribat Hospital, Bahri Hospital, Soba Hospital in Khartoum State. And Twenty-one nasal swabs were collected from health care workers and regarded as healthy carriers.

***S. aureus* Identification:** The bacterial isolates were collected and identified according to standard biochemical tests [19], which included morphological characteristics on sheep blood agar, gram stain, catalase, coagulase, deoxyribonuclease (DNase), and mannitol fermentation tests.

Cefoxitin Disc Screen Test for Detection of MRSA: Susceptibility profile of *S. aureus* isolates to cefoxitin (30µg) was implemented by the disc diffusion method on Mueller-Hinton agar plates. Bacterial suspension prepared on physiological saline and suspension turbidity equivalent to a 0.5 McFarland standard. Plates were incubated at 35°C for 24 h. According to CLSI guidelines, the interpretive criteria for cefoxitin were: *S. aureus*, sensitive≥22 mm, and resistant ≤21 mm. In contrast, MRSA was resistant to cefoxitin disc. *Staphylococcus aureus* ATCC BAA 2313 was used as a positive control for MRSA, and *Staphylococcus aureus* ATCC BAA 1026 was used as a quality control each time the tests were performed [20].

Antibiotics Susceptibility Testing: Susceptibility testing was performed on all *S. aureus* isolates using the Kirby- Bauer disc diffusion method to antibiotics from different categories, namely: Oxacillin (5 µg), Gentamicin (10µg), Ciprofloxacin (5µg), amoxicillin-clavulanate (20 + 10 µg), fusidic acid (10µg) and Vancomycin (30µg) (Himedia, India). This method was performed on Mueller-Hinton agar (Himedia, India) following the performance standards for antimicrobial susceptibility testing recommended by the Clinical and Laboratory Standards Institute guidelines [20]. ATCC 29213 *Staphylococcus aureus* strain was used for quality control for minimal antimicrobial susceptibility quality control testing.

DNA Extraction and PCR Detection of the Methicillin Resistance (*mec A)* Gene: DNA was extracted from all isolates using the Chelex method as previously described [21, 22]. The presence of the *mecA* gene was tested using conventional PCR. Briefly, 5 µl of template DNA was added to the PCR mixture containing a 1X master mix and 50 pmol of the forward and reversed *mec*A primers mecA F-GTAGAAATGACTGAACGTCCGATGA and *mec*A R-'CCAATTCCACATTGTTTCGGTCT AA'. Nuclease-free water was added to reach a final reaction volume of 50 µl. The optimal cycling conditions were as follows: initial denaturation 4 min at 94 ºC; 35 cycles of 45s denaturation at 94 ºC, annealing for 45s at 50 ºC, an extension for the 60s at 72 ºC, and final extension for 2 min at 72 ºC, performed on a Bio-Rad automatic thermal cycler. PCR performed in duplicates for each sample. The PCR products were resolved through gel electrophoresis (1.5% TBE agarose gel) to check for the specific product size of 310 pb. PCR products visualized under ultraviolet light (Transilluminator; Uvite, UK) **Fig. 1**.

Statistical Analysis: Data were analyzed using statistical package for social sciences (SPSS), version 25 (IBM, SPSS Inc., Chicago, IL). Descriptive data were presented as a percentage.

Ethical Approval: This study was carried out after ethical approval from the Institutional Ethics Committee, Deanship of Scientific Research,

Sudan University of Science and Technology. All participants were informed in detail before recruiting them, and the participation was voluntary, and informed consent was obtained from each participant prior to the start of this study.

RESULTS:

Phenotypic Detection of MRSA: A total of 60 clinical samples were collected and, and 43 (71.67%) resulted resistant for cefoxitin disc, while the 17 (28.33%) remains isolates resulted sensitive for cefoxitin. All nasal swabs from hospital staff were resistant to cefoxitin.

***S. aureus* Antimicrobial Susceptibility Testing:** Out of eighty-one isolate; 68 (83.9%) were resistant to Amoxicillin-clavulanate, followed by Oxacillin with resistant rate 64 (79.0%), while, *S. aureus* was most sensitive to Vancomycin 47(59.3%) **Table 1**.

TABLE 1: ANTIMICROBIAL RESISTANT RAT FOR ALL *S. AUREUS*

Classes of antibiotics	Name of Antibiotic	Count (%)
Fluoroquinolones	Ciprofloxacin	41 (50.6)
Aminoglycosides	Gentamicin	42 (51.9)
Glycopeptide	Vancomycin	33 (40.7)
Penicillins	Oxacillin	64 (79.0)
β-lactam/β-lactamase inhibitor complexes	Amoxicillin-clavulanate	68 (83.9)
Fusidane	Fusidic acid	48 (59.3)
Muti-drug reistant MDR		60 (74.1)

Key: The result interpreted according to CLSI guideline, N = 81.

PCR Detection of the Methicillin Resistance (*mecA)* Gene: Out of 81 *S. aureus* isolates, all clinical isolates and nasal swabs from hospital staff were identified as MRSA were positive for the mecA gene with the frequency rate of 64 (79.0%).

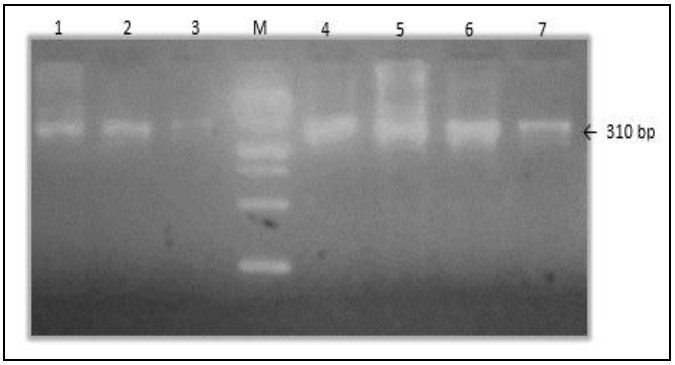

FIG. 1: A REPRESENTATIVE AGAROSE GEL ELECTROPHORESIS OF PCR PRODUCTS AFTER AMPLIFICATION OF THE *mecA* GENE. Lanes: 1- 7 positives mecA PCR products and (M) a molecular weight marker (O'Range Ruler 100 DNA Ladder, SM1143-Fermentas)

DISCUSSION: The prevalence of MRSA is high among clinical isolate and hospital staff nasal carriers who could consider as a potential source for the spread of resistant strains in nosocomial infection. This finding, like the previous studies, reported out that isolated *S. aureus* strains among the staff nasal carriers may lead to an increase the persistent nosocomial infections in hospitalized patients and also health care workers [1, 7].

In Africa, this study revealed a high prevalence of MRSA (79%), which was near to the prevalence rate in Egypt (82%), and unlike with the low prevalence (7%) reported in Madagascar [14]. The MRSA prevalence has been shown to vary across different countries; this marked variation might be due to different environmental determinants or only due to a difference in the genetic diversity of *S. aureus* [18]. For instance, in Kenya, there is a marked difference in reported MRSA prevalence in clinical isolates within Nairobi, with one recent study reporting a prevalence of 3.7% while another reported 87.2% [23, 24]. This finding was more than other studies in Saudi Arabia reported that the MRSA rate was 32% [25-27].

This study also reported the high resistant rate to other antibiotics from different categories used to treat the *S. aureus* infection. The highest resistance rate to Amoxicillin-clavulanate followed by Oxacillin; this is considered that β-lactam/β-lactamase inhibitor complexes and penicillins are not very active in the treatment. In contrast, vancomycin showed the highest sensitivity rate (59.3%) among all *S. aureus*, which means it still can be chosen as a therapeutic agent against *S. aureus* strains. This vancomycin sensitive rate was higher than other studies finding from Egypt that stated the sensitive rate of 45.5% [28]. Also, disagree with the study from Ethiopia stated that the percentage of VRSA was 65.1% [29]. While the other study from Tehran described that out of 1789 *S. aureus*, only four VRSA were noticed [30].

In this study *S. aurues* identified as Multi-drug resistant MDR when it developed resistant to at least one agent in three or more antimicrobial groups [31]. In overall, the increase in the resistance of isolated organisms to Penicillins, Fluoro-quinolones, Aminoglycosides, Glycopeptide, β-lactam/β-lactamase inhibitor complexes and

Fusidane in this study might be due to an increase in the usage of these antibiotic's classes in the hospital. The results of this study showed that 74.1% of the strains were resistant to more than two antibiotics, so due to the spread of MDR strains, this may become a significant challenge for the treatment process. This MDR situation might be due to acquiring of several resistant genes through R plasmid [32]. Furthermore, throughout the latest several decades, the incidence of MDR organisms in hospitals and health centers has increased steadily. So, this study reported the developments of multi-drug resistance among *S. aureus* and illustrative an alarming threat of the appearance of multi-drug resistant pathogens.

This study illustrated that all resistant bacteria have *mecA* gene; this agreed with previous studies demonstrated it as a possible cause of bacteria to be resistant for Methicillin, which was by the acquisition of *mecA* gene [4, 5, 12, 13]. The methicillin-resistant gene (*mecA*) plays a significant role in the resistance to lactam antibiotics. Because of its resistance to multiple antibiotics, MRSA is challenging to eliminate [10]. This study entirely agrees with previous studies which they reveal a markedly heterogeneous population of SA isolates [15, 16, 33].

CONCLUSION: This study has demonstrated that the prevalence of MRSA and MDR strains was high, also has revealed that in order to increase the accuracy of the identification results of MRSA, next to the detection of the methicillin resistance gene (*mecA*) by PCR. It is also essential to characterize the *S. aureus* strains using different molecular typing tools as well as we need for continuous surveillance in order to keep track of emerging clones.

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