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Research Article

Prevalence of mecA Gene in Methicillin Resistant *Staphylococcus aureus* Isolated from Different Clinical Specimens in Khartoum State, Sudan

Safa A Sherfi[1] and Ali M Badri[2]*

[1]*Head Department of Basic Sciences, Faculty of Medicinal Laboratory Sciences, International University of Africa, Khartoum, Sudan*

[2]*Department of Microbiology, Faculty of Medical Laboratory Sciences, International University of Africa, Khartoum, Sudan*

***Corresponding Author:** Ali M Badri, Department of Microbiology, Faculty of Medical Laboratory Sciences, International University of Africa, Khartoum, Sudan.

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Abstract

Background: Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the major agents for increasing number of serious hospital and community acquired infections. Rapid detection of MRSA is important for patient care and proper usage of infection control. The aim of this study was to determine the prevalence of mecA Gene of Methicillin Resistant *Staphylococcus aureus* isolated from different clinical specimens in Khartoum State Hospitals.

Methods: A total of 50 *S. aureus* isolated from different Clinical specimens were identified according to the standard microbiological procedures, oxacillin disk diffusion test were used to determination of MRSA by phenotypic methods. DNA was extracted from all isolates and the presence of mecA gene was detected by PCR method.

Results: Out of 50 *S. aureus* Isolated, 18 (36%) of isolates were considered to be MRSA by oxacillin disk diffusion test. PCR analysis showed that 21 (42%) of *S. aureus* isolates being mecA gene positive and were not found any VRSA strains.

Conclusions: Significant increase in the prevalence of methicillin-resistance in *S. aureus* strains represents an alarm emergence for the health authorities and community. Detection of mecA genes by PCR Method is useful, further studies are require about the distribution of isolates according to different variables.

Keywords: *Methicillin-Resistant Staphylococcus aureus; mecA Gene*

Abbreviations

MRSA: Methicillin Resistant *Staphylococcus aureus*; MSSA: Methicillin Sensitive *Staphylococcus aureus*; VRSA: Vancomycin-Resistant *Staphylococcus aureus*; CSF: Cerebrospinal Fluid; PCR: Polymerase Chain Reaction

Introduction

Staphylococcus aureus is the most common human bacterial pathogen and is an important cause of skin and soft tissue infections, toxic shock syndrome, meningitis, pneumonia, endovascular infections, tonsillitis, septic arthritis, pharyngitis, enterocolitis, endocarditis, osteomyelitis, sepsis. The resistance in these strains is increasing worldwide due to inappropriate use of antibiotics [1,2]. MRSA is strains of *S. aureus* resistant to semi-synthetic, penicillinase resistant, β-lactams such as methicillin, oxacillin or cloxacillin. MRSA strains are resistant to all cephalosporins, cephems and other β-lactams, such as ampicillin-sulbactam, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, piperacillin-tazobactam and the carbapenems. This group of organisms is also frequently resistant to most of the commonly used antimicrobial agents, including the aminoglycosides, macrolides, chloramphenicol, tetracycline and fluoroquinolones [3]. The resistance to methicillin in staphylococci is mediated by the mecA gene that encodes a modified penicillin-binding protein (PBP), the PBP2a or 2',

which shows reduced affinity to penicillins, such as methicillin and oxacillin and for all other beta-lactam antibiotics. mecA gene is located on a mobile genetic element (from 21-to 67-kb), called staphylococcal chromosomal cassette (SCCmec) [4]. MRSA cause the same type of infections as MSSA. Sometimes it causes serious and potential life-threatening infections like septicemia, deep abscesses. In the healthcare environment it causes nosocomial infections like surgical site infections. It is also a pathogen in biofilm-related infections. Both methicillin-sensitive *S. aureus* and methicillin-resistant *S. aureus* can cause mild to fatal diseases, spread locally and globally, colonize numerous human body parts, and persist in various environments outside of hosts. MRSA can be identified using phenotypic (antimicrobial susceptibility testing) or genotypic methods. In general, the genotypic methods are more discriminatory, but are expensive and technically demanding and the phenotypic methods are easier to perform and interpret, widely available and cost effective [5].

Materials and Methods

Sample collection

Out of 124 isolates a total of 50 *Staphylococcus aureus* was isolated from various clinical specimens (nasal swabs, surgical wounds, urine, semen, blood and CSF) of patients admitted to four hospitals (Soba University Hospital, Ribat University Hospital, Royal care hospital and Military Hospital) in Khartoum, during the period from January to April 2018 from Sudan.

Isolation and Identification of *Staphylococcus aureus* using Biochemical tests and selective medium

S. aureus were identified and differentiated from related organisms as per conventional methods on the basis of colony morphology, gram staining, catalase test, slide and tube coagulase, mannitol fermentation and DNase production following the standard procedures [6].

Detection of methicillin resistance by phenotypic method

The antimicrobial susceptibility testing of all identified isolates was done according to the criteria of the Clinical and Laboratory Standards Institute method (CLSI) [7].

All isolates were screened for Methicillin resistance by using Cefoxitin (30 mcg), Oxacillin (1 mcg) Erythromycin (15 mcg), Gentamycin (10 mcg), Fusidic acid (10 mcg) and Vancomycin (30 mcg). The inhibition zone diameters were measured and were interpreted according to the Clinical Laboratory Standards Institute guidelines [8].

DNA extraction

DNA for molecular detection was extracted after bacterial lysis according to the extraction protocol prepared by the Community Reference Laboratory for Antimicrobial Resistance (CRL, 2009). Briefly, a few colonies taken from fresh culture medium and transferred to phosphate buffered saline (pH 7.3). The suspension was then heated at 100°C for 15 minutes. Boiled suspension was transferred directly on ice, this was followed by vortexing and The suspension was then centrifuged at 12000 rpm for 5 minutes to sediment the debris, the clear supernatant was used as template DNA in PCR method [9].

Molecular Detection of mecA gene

PCR was performed and the test was carried out in a total volume of 25 µl, PCR reaction containing 5 µl of the extracted DNA, 2 µl from the primers forward (5'AAAATCGATGGTAAAGGTTGGC-3') and reverse (5'AGTTCTGCAGTACCGGATTTGC-3'), 13 µl of distilled water was added to the Dream Taq Green PCR Master Mix DNA marker "Gene Ruler" were provided by Thermo Scientific (Lithuania). The amplification was done by 35 cycles of PCR reaction (denaturation at 95°C for 1 minute, annealing at 54°C for 60 seconds and extension at 72°C for 1 minute. A final extension was performed at 72°C for 5 minutes). The amplification product was separated on 2% agarose gel electrophoresis and the product was visualized by staining with 0.15% ethidium bromide using UV gel documentation system.

Ethical consideration

This study was approved by the ethical committee of the International University of Africa, Faculty of Medical Laboratory Sciences, Department of Medical Microbiology. Permission from Soba University Hospital, Ribat University Hospital, Royal care hospital and Military Hospital Medical director was applied and verbal consent was obtained from all subjects enrolled in the study.

Data analysis

Statistical analysis was done by using Statistical Package for Social Science program (version 20).

Results

A total of 50 isolates were identified as *S. aureus* from (30 surgical wounds, 8 blood, 4 nasal swabs, 4 urines, 2 semen and 2 CSF) by routine phenotypic methods (Table 1) including Gram's staining, colony morphology and catalase and coagulase test. The results of culture on mannitol salt agar have showed grown on mannitol salt agar and fermentation of mannitol. Thus culture on mannitol salt agar to identify *S. aureus*, is not able to make a definite identification alone. among 50 isolates of *S. aureus*, 18 (36%) of isolates were determined MRSA by disk diffusion test (Table 2). By applying PCR method, among the 50 isolates were identified as *S. aureus* with phenotypic methods, 21 (42%) isolates were found to be mecA gene positive (Figure 1). The presence of some discrepancies between the results of phenotypic and genotypic methods for detection of Methicillin Resistant *S. aureus* strain, make it clear that, the method for identification of mecA gene is not sufficient alone. So, phenotypic and genotypic methods together were used for identification of Methicillin Resistant *S. aureus* strain.

Samples	Number of S. aureus results isolates	Frequency distribution of strains (%)
Wound swab	30	60
Nasal swab	4	8
Blood	8	16
Urine	4	8
Semen	2	4
CSF	2	4
Total	50	100

***Table 1**: Frequency of S. aureus isolates according to type of clinical specimens.*

Test	Frequency	Percentage
Disk diffusion test	18	36%
MecA gene detection	21	42%

***Table 2:** Number of MRSA strains detected by disk diffusion test and PCR method.*

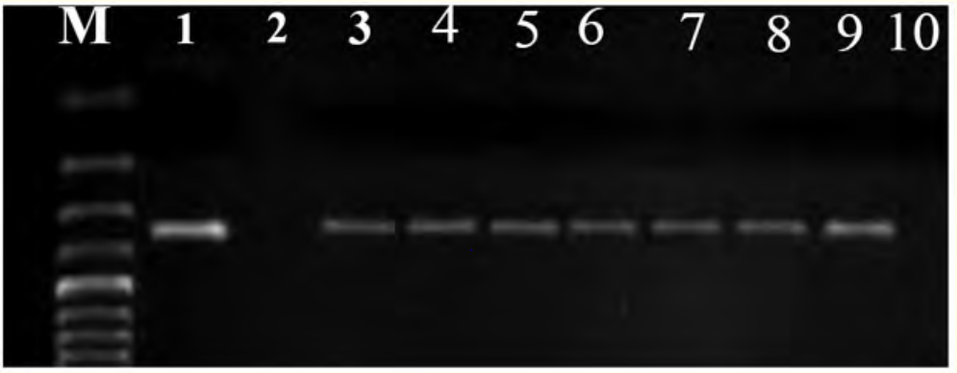

***Figure 1:** MecA gene DNA results (533 bp) on 2% agarose gel. Lane M shows 100 bp DNA marker, lane 1 shows positive control, lane 2 shows negative control, lanes 3, 4, 5, 6, 7, 8 and 9 show positive results and lane 10 show negative results.*

Discussion

Methicillin resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital acquired (HA-MRSA) infections and the most significant multi-resistant pathogens worldwide [10]. In this study, we used phenotypic (oxacillin disk diffusion test) and genotypic (PCR method for mecA gene) methods for detection of MRSA. Rapid and accurate detection of methicillin resistance in *S. aureus* is important for the control of nosocomial spread of MRSA strains and use of appropriate antimicrobial therapy. The oxacillin screen plate test is the gold standard for the phenotypic method [11,12]. The results of our study showed that 34% and 40% of *S. aureus* isolates were recognized as MRSA by disc diffusion test and PCR method. Whenever 12 (22%) of isolates had shown similar results in phenotypic and genotypic assays, 9 (18%) of isolates were mecA-positive in PCR but methicillin sensitive in disk diffusion test. This could be attributed to not consistently expression of mecA gene. Besides, 6 (12%) of the phenotypically methicillin-resistant strains were negative for mecA gene. This resistance can be due to lack of optimal PCR conditions or change in mecA gene due to the mutations or the presence of other resistance mechanisms. This prevalence is also similar to other studies [13]. This prevalence is high and comparable to results of other studies 51% in Saudi Arabia [14], 54% in Egypt [15], 57% in Jordan [16], in Tehran, Iran; 53% [17] and 88% [18], 61% in Taiwan [19], 61.8% in USA [20] and 69.4% and 78.0% in Sudan [21,22]. The rise of methicillin resistance may be due to antibiotic-resistant genes spread in the community, hospitals and healthy staff [23]. But the common thread among all of these studies, illustrate the variety of mecA gene in the risk of occurrence of resistant staph infections. Thus, health plans and control infection measures should be taken to prevent this problem. Vancomycin has been the drug of choice for MRSA infections, but vancomycin-resistant *S. aureus* (VRSA) also emerged as a new challenge in infection management [24]. However, we have not found any VRSA strains among the isolates included in the study [25,26].

Conclusion

Significant increase in the prevalence of methicillin-resistance in *S. aureus* strains caused by the indiscriminate and excessive use of antibiotics during the last decade. This study shows that PCR method is a useful method for detection of *mecA* genes which leads to rapid detection and identification of MRSA cultured from patient's specimens and may provide substantial benefits for infection control by allowing prompt and cost-effective implementation of contact precautions.

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