Original Article

Molecular Identification of Methicillin-Resistant *Staphylococcus aureus* in Khartoum Teaching Hospital

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Abstract:
Antimicrobial resistance has become a great public health problem worldwide and multi-drug resistant *Staphylococcus aureus* has been widely reported.
Methods: The presence or absence of methicillin resistance gene *(mecA)* in 48 clinical wound isolates of *S. aureus* was examined by the polymerase chain reaction (PCR). The results were analyzed in parallel to the disk diffusion method by oxacillin (1 µg). Polymerase chain reaction was amplified at a sequence of *mecA* gene at 1319 bp.
Results: Nine (18.75%) out of the 48 isolates and were found to be identical to those of disk diffusion test. All strains were studied for their susceptibility to traditionally used antibiotics. The results revealed that multi-drug resistance was common among MRSA strains. The drug of choice for the treatment of MRSA and MSSA was vancomycin.
Conclusion: The study concluded that multiple antibiotic resistance was common, and the PCR assay can be used to confirm MRSA infection.

Key words: MRSA, polymerase chain reaction (PCR).

Resistance to methicillin was first described for *Staphylococcus aureus* in 1960, shortly after the introduction of the drug into clinical practice[1]. Since then, methicillin-resistant *S. aureus* (MRSA) has become a widely recognized cause of morbidity and mortality throughout the world[2]. Staphylococcal strains usually have penicillin-binding proteins (PBPs). In the absence of β-lactam antibiotics, the *Staphyolococci* utilize PBPs to synthesize the cell wall which is composed of peptidoglycan[3]. In contrast, an additional low-affinity penicillin binding protein designated PBP 2a is encoded by a unique MRSA-PBP gene[4] and is the main factor responsible for the expression of methicillin resistance[5]. The β-lactam resistance of MRSA to all β-lactam antibiotics is mediated by the methicillin resistance determinant[6].

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Traditionally, methicillin or oxacillin has been tested and the results are representative of resistance to all β-lactam agents [7]. Disk diffusion method against bacterial isolates has been used routinely for the choice of appropriate chemotherapy. In the case of MRSA strains, the disk diffusion is influenced by growth conditions, pH and NaCl [8]. Therefore, it is important to find out whether the isolated *S. aureus* possesses MRSA-PBP gene, in order to confirm the results of the disk diffusion method. In this study the presence of MRSA-PBP gene in clinical isolates of *S. aureus* was investigated by comparing PCR results and disk diffusion method of oxacillin.

Materials and Methods

In this study, 48 *S. aureus* isolates were obtained from infected wound specimens, such as ulcers, burns, abscesses and post-operative wounds from Khartoum Teaching Hospital. *S. aureus* isolates were identified by the standard microbiological methods including gram strains, catalase, coagulase and DNase tests. MRSA was determined by the disk diffusion method to oxacillin according to the National

Committee for Clinical Laboratory Standard (NCCLS) guidelines [9].

Antimicrobial susceptibility testing was performed by the disk diffusion method for cephalexin (30 µg), cotrimoxazole (25 µg), clindamycin (20 µg), erythromycin (15 µg), vancomycin (30 µg), tetracycline (30 µg), rifampicin (5 µg), amoxycillin (10 µg), and ciprofloxacin (5 µg). A suspension of the tested organism was adjusted against 0.5 MacFarland's standard turbidity. It was then inoculated onto Mueller-Hinton agar, then incubated at 37 C for 16-18 hours and examined for sensitivity.

Genomic DNA was isolated by using chloroform DNA purification protocol. With 1 µl loop, a small quantity of growth (equivalent to 2 small colonies) was mixed into 100 µl of sterile distilled water in a micro-centrifuge tube. Chloroform 100 µl *(Sigma)* was added, and the mixture was vortexed for 10 seconds. The mixture was heated at 80 C for 20 minutes, after which it was held at -20 C for another 20 minutes. The sample was allowed to thaw while still cold and was centrifuged at 12,000 g for 3 minutes in a mini-centrifuge. The extracted DNA was stored at -20 C ready for use [10].

Two 20-mer PCR primers of methicillin resistance *(mecA)* gene were chosen. These oligonucleotides were complementary to the target segment of the MRSA-PBP gene sequence as follows: primer MR1, 5'-GTG GAA TTG GCC AAT ACA GG- 3' (478 TO 497) and primer MR2, 5' TGA GTT CTG CAG TAC CGG AT- 3' (1816 to 1797) [11].

The PCR reaction mixture contained reaction buffer (10 µl), 500 U Taq DNA polymerase (Promega) (0.3 µl), 10 m/M dNTP mix (1 µl) 25 mM $MgCl_2$ (3 µl), 10 mM each primer (10 µl), template DNA (1 µl), and distilled water (14.7 µl) to a final volume of 40 µl.

The amplification procedure was as follows: an initial step at 94 C for 30 seconds; 55 C for 30 seconds and 72 C for 1 min (each step was repeated 40 times) and a final step at 72 C for 4 minutes.

The amplification products were subjected to electrophoresis on 0.4% agarose gel with ethidium bromide incorporated for DNA strainsing. The PCR products were visualized and photographed on an UV trans-illuminator and the bands of the PCR products were determined by using the 1 kb ladder DNA marker (Promega).

Results

Nine (18.75%) isolates out of the 48 *S. aureus* strains were resistant to oxacillin. The oxacillin resistant *S. aureus* was found to be identical by genotyping to the amplified DNA fragment of MRSA-PBP gene. None of the other 39 MSSA strains showed similar gene. The size of the DNA fragment was 1319 bp. (Fig 1).

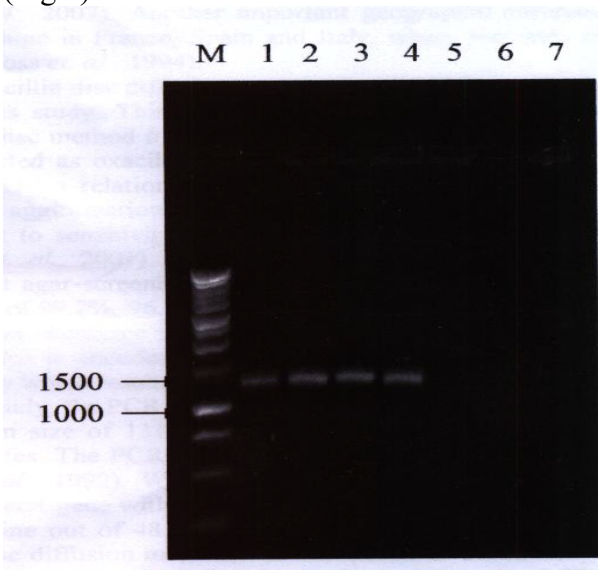

Fig 1: Agarose gel electrophoresis of PCR products of mecA gene.
Lanes 1-4: Methicillin resistant S. aureus (1319 bp) DNA fragment.
Lanes 5-7: Methicillin sensitive S. aureus (MSSA).
Lane M: (1kb ladder) DNA marker.

All isolates were found to be sensitive to vancomycin (100%). The sensitivity for the other antibiotics was as follows: co-trimoxazole 27 (56.25%), rifampicin 35 (72.9%), clindamycin 33 (68.75%), tetracycline 16 (33.3%), erythromycin 31 (64.6%), cephalexin 29 (60.4%), oxacillin 39 (81.25%), amoxycillin 19 (39.6%) and ciprofloxacin 32 (66.7%).

Twenty isolates (41.7%) including the nine MRSA showed multiple antibiotic resistances to four or more of the antimicrobial agents (Table 1).

Table 1: Antibiotic resistances to four or more of the antimicrobial agents.

Resistance	MRSA	MSSA	Total(%)
Fully sensitive	0	0	0 (0)
Resistant to 1 agent	0	6	6 (12.5)
Resistant to 2 agents	0	9	9 (18.75)
Resistant to 3 agents	0	13	13 (27.1)
Resistant to 4 agents	0	6	6 (12.5)
Resistant to 5 agents	1	5	6 (12.5)
Resistant to 6 agents	3	0	3 (6.25)
Resistant to 7 agents	5	0	5 (10.4)
Total	9	39	48(100.0)

Discussion

Antimicrobial resistant and multi-drug resistant *S. aureus* have become a great public health problem worldwide. This study determined the pattern of resistance to the commonly used antibiotics for MRSA and MSSA.

Multi-drug resistance to different antimicrobial agents among MRSA strains is significantly higher than MSSA. MRSA in nosocomial infections is among the most important multi-resistant pathogens worldwide [12].

The frequency of antibiotic resistance among *S. aureus* in the current study was in accordance with other studies. The results from intensive care units showed that 78% of 32 *S. aureus* isolates were with multiple antimicrobial resistances as follows: 12% co-trimoxazole, 25% teicoplanin, 46% erythromycin, 50% clindamycin, 68% gentamicin, 71% ciprofloxacin, 81% oxacillin, and 100% penicillin resistant [13]. Higher resistance of *S. aureus* isolates was reported in a study from Nigeria showing resistance to ampicillin 91.7%, clindamycin 78.3%, cephalexin 75%, methicillin 71.7% and vancomycin 68.3%, but had very low resistance to Gentamicin 3.3%, ciprofloxacin 3.3%, ofloxacin 3.3%, sparfloxacin 3.3%, and pefloxacin 10.0%. As many as 71.7% of the isolates in the current study showed multi-drug resistances [14], whereas in Peru only 25% of the MRSA strains were resistant to multiple drugs [15].

Table 2: Comparison between MRSA and MSSA in relation to antibiotic susceptibility.

No. of Isolates	Antibiotic Resistance									
	R	Am	T	Cl	Co	E	Cp	Cd	Ox	Va
MRSA (9)	5	9	9	9	4	3	5	5	9	-
	55.6%	100%	100%	100%	44.4%	33.3%	55.6%	55.6%	100%	-
MSSA(39)	8	20	23	10	17	14	11	10	-	-
	20.6%	51.3%	59.0%	25.6%	43.6%	35.9%	28.2	25.6%	-	-

(R) Rifampicin, (Am) Amoxycillin, (T) Tetracycline, (Cl) Cephalexin, (Co) Co-trimoxazole, (E) Erythromycin, (Cp) Ciprofloxacin, (Cd) Clidamycin, (Va) Vancomycin, (Ox) Oxacillin.

MRSA strains were also resistant to multiple antibiotics in Algiers Hospitals. Isolates (97.6%) were resistant to kanamycin, 86% to fusidic acid, 73% to tetracycline, 25% to erythromycin, 16% to ofloxacin, 11.3% to clindamycin, 7% to Gentamicin, 4.5% to pristinamycin, 2.3% to chloramphenicol, and 2.3% to refampicin [16]. The rate of antibiotic resistant strains of MRSA was higher for penicillin and cephalosporins in Japan, whereas over 95% of MRSA were resistant to amoxycillin and piperacillin. [17]. In India, the resistance to all antibiotics tested among MRSA and MSSA were found to be 23.2% and 6.6% respectively. Higher resistance to multiple antibiotics in MRSA isolates was

also reported [18]. In Egypt, MRSA strains showed multi-drug resistance to many antibiotics [19].

Since the emergence of MRSA in 1960s, tiecoplanin and vancomycin were the drugs of choice and commonly used for the treatment of the Nosocomial MRSA and other gram positive infections [20, 21]. In this study, the only antibiotic to which all MRSA and MSSA strains were still sensitive was vancomycin. These results are similar to the studies carried out in Scotland, Bulgaria and Egypt [19, 21].

MRSA now commonly accounts for 20% to 40% of all *S. aureus* isolates [22]. Generally, high rates of about 71% have been reported in the United States [23], Malaysia 84% [24], Peru 68% [15], Japan 56.9% [25], Iran 53.6% [26], and in the Latin America 31% [27]. In India, methicillin resistance among the *S. aureus* isolates was 39.5% [28]. Another important geographic difference for MRSA prevalence was also found in France, Spain and Italy, where rates of 30-35% of MRSA were reported [29].

S. aureus resistance to methicillin occurs when an isolate carries an altered PBP 2a which is encoded by the *mecA* gene. The new PBP 2a binds β-lactam with lower affinity which results in resistance to this class of antimicrobial agents. In this study the conventional method for the determination of susceptibility to oxacillin was confirmed by the amplification of *mecA* gene by PCR. Nine out of 48 *S. aureus* isolated initially classified as methicillin resistance based on disk diffusion method were confirmed by the PCR to carry *mecA* gene. None of MSSA showed the *mecA* gene. Therefore, detection of *mecA* gene by the PCR is considered to be the gold standard assay for resistance.

Conclusion

In conclusion, the prevalence of the multiple antibiotic resistant MRSA was common in wound infections, and the injudicious use of antibiotics will lead to the development of more drug resistance. The PCR assay appears to be a very reliable and accurate test for the detection of MRSA-PBP.

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