Original Article

Detection of Methicillin Resistance in *Staphylococcus aureus* by Disk Diffusion and PCR Methods

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ABSTRACT

Background and Objective: **Methicillin resistance in *Staphylococcus aureus* is an increasingly important clinical problem. A chromosomal gene, mecA, mediates resistance to penicillinase-resistant penicillins such as methicillin and oxacillin in *Staphylococcus aureus*. We evaluated the validity of disk diffusion test by using oxacillin, methicillin and cefoxitin disks with consideration of the presence of mecA gene as the reference method for detection of methicillin resistant *Staphylococcus aureus* (MRSA).**

Materials and Methods: **The susceptibility testing of 222 *S. aureus* clinical isolates to oxacillin (1 µg), cefoxitin (30 µg) and methicillin (5 µg) was carried out by the disk diffusion method according to the Clinical Laboratory Standards Institute guidelines. Detection of mecA gene was performed using PCR method.**

Results: **An amplified mecA gene of 310 bp was detected in 55% of examined strains by PCR, thus 55% strains were considered MRSA. Sensitivity of oxacillin, methicillin and cefoxitin disks were determined 100%, 99.1% and 98.3% respectively.**

Conclusion: **All MRSA strains in PCR had shown resistance to penicillinase-resistant penicillins by oxacillin disk, but two and one strains were sensitive by cefoxitin and methicillin disk respectively. Thus, oxacillin was the most appropriate disk for detecting MRSA. The prevalence of MRSA in this study is comparable to that found in United States, Canada, Europe and Iran, but the percentage of MRSA isolates is almost twice of percentage reported from Japan.**

Key words: *Staphylococcus aureus*, PCR, mecA, Disk diffusion

Introduction

Methicillin resistance in *Staphylococcus aureus* is now common in many areas of the world and following its initial isolation in the UK in 1961, the frequencies of infections and outbreaks due to methicillin resistant *S. aureus* (MRSA) have continued to increase (1-4). It is noteworthy that the prevalence of MRSA varies from one geographic region to another and between different institutions

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in a given area. MRSA is an increasingly important clinical problem since MRSA is often multidrug resistant and therapeutic options are limited (5). For this, accuracy and promptness in the detection of methicillin resistance is important in treating, controlling and preventing MRSA infections (6,7). All MRSA produce an additional penicillin-binding protein, PBP2a or PBP2' which confers resistance to all currently available β-lactam agents, including penicillinase-resistant penicillins (such as oxacillin, methicillin) and cephalosporins (such as cefoxitin). PBP2a is encoded by the mecA gene that causes resistance to this agent due to low affinity for β-lactam agents (7,8). There are several methods such as disk diffusion and polymerase chain reaction (PCR) of the mecA gene for detecting MRSA, but detection of the mecA gene by PCR is considered as the reference method (7). However, many laboratories do not have the capacity to use molecular methods and therefore phenotypic methods such as disk diffusion are incorporated into routine clinical practice (6,7). Traditionally, oxacillin or methicillin disks have used for detection of MRSA, although cefoxitin has recently been investigated as an alternative agent for detection of MRSA (6-8).

In this study, we evaluated the validity of disk diffusion test by using oxacillin, methicillin, and cefoxitin disks for establishing the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each disk with consideration to the presence of mecA gene by PCR as the reference method.

Materials and Methods

A total of 222 *staphylococcus aureus* isolates were collected from four hospitals in Tehran, Iran. Identification and confirmation of the isolates was done by routine laboratory tests, including cultural characteristics, Gram's stain, mannitol fermentation, catalase, DNase and coagulase tests.

The susceptibility testing of *S. aureus* isolates to penicillinase-resistant penicillins was carried out by the disk diffusion method according to the Clinical Laboratory Standards Institute guidelines (9) using commercial disks (MAST Diagnostics, Merseyside, UK). The antibiotics used and their disk potencies were as follows: oxacillin (1 µg), cefoxitin (30 µg) and methicillin (5 µg). The test medium was Mueller-Hinton agar (Merck, Darmstadt, Germany) and were overlaid with the inoculum (turbidity equivalent to that of a 0.5 McFarland Standard) of the *S. aureus* strains. Zone diameters were measured at 24 h following CLSI criteria (9). *S. aureus* ATCC 25923 was used as reference strain.

Detection of mecA gene was performed by using PCR method. For this, DNA was extracted from all isolates by rapid DNA extraction method (10), after overnight culture on brain heart infusion agar plates, one colony of each sample was suspended in 25 µl of sterile distilled water and the suspension was then placed in a 100 °C heat block for 15 min. After centrifuge at 14000 rpm for 5 min, the supernatant was used as the template. The primers used in this study have been previously described (10) and were purchased from IsoGene, Netherlands: MecA1 (5'-GTA GAA ATG ACT GAA CGT CCG ATA A-3') and MecA2 (5'-CCA ATT CCA CAT TGT TTC GGT CTA A-3'). PCR reaction was performed in a 20 µl volume, 2 µl of DNA template was added to 18 µl of PCR mixture consisting of 2 µl of PCR buffer (10x), 1 µl of MgCl2 (50mM), 4 µl of dNTPs (1mM), 4 µl of each primers (10 Pmol), 0.25 µl of Taq DNA polymerase (5 u/µl) (Cinnagen) and 2.75 µl of double distilled water. DNA amplification was carried out in a thermocycler (Touchgene Gradient, Techne, UK) with the following thermal cycling profile: an initial denaturation step at 94 °C for 5 min was followed by 30 cycles of amplification (denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 30 s) ending with a final extension step at 72 °C for 5 min. After PCR amplification, 5 µl was removed and subjected to agarose gel electrophoresis (2% agarose, 1x Tris-acetate-EDTA, 100 V, 100 min). The gel was stained with ethidium bromide, and the 310 bp amplicons were visualized using a gel documentation system by comparison with a molecular size marker (100 bp ladder, Eurobio, UK).

Results

An amplified mecA gene of 310 base pairs (bp) was detected in 122 strains by PCR (Figure 1), thus 55% of studied strains were considered MRSA. In disk diffusion method, these 122 strains were shown resistance by oxacillin disk but 121 and 120 strains were considered MRSA by methicillin and cefoxitin disks respectively. Therefore, sensitivity of oxacillin, methicillin and cefoxitin were determined 100, 99.1 and 98.3% respectively. However, specificity of all disks was 100%. The overall results obtained with each disk are shown in Table 1.

Table 1. Parameters of different disks for detecting MRSA compred to PCR

Disk	False negative	False positive	Sensitivity (%)	Specificity (%)	NPV	PPV
Oxacillin	0	0	100	100	100	100
Methicillin	1	0	99.1	100	99	100
Cefoxitin	2	0	98.4	100	98	100

NPV, Negative predictive value; PPV, Positive predictive value

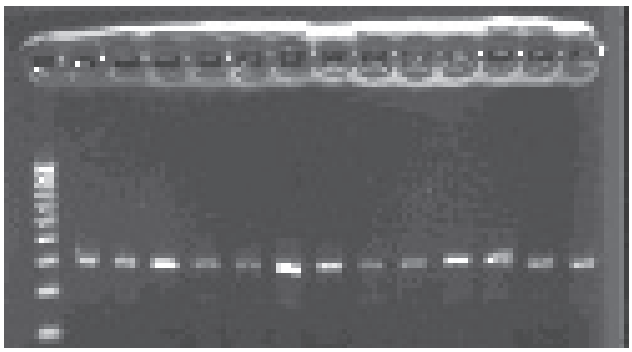

Figure 1. Results of gel electrophoresis of PCR product of mecA gene in clinical isolates of *Staphylococcus aureus*

The prevalence of MRSA was 55% among all studied strains but was highest among *S. aureus* isolated from respiratory tract (66.2%) and was lowest among isolates associated with urine infections (2.9%). The prevalence of methicillin resistance was 20.6% and 5.9% among wound and blood infection isolates respectively. Considerable differences were observed when the distributions of MRSA isolates in different wards were compared. Almost 21% of the *S. aureus* isolates from intensive care units (ICUs) and 11.3% of the isolates from operation wards were MRSA, whereas only 1.4% of the isolates from emergency rooms were MRSA.

Discussion

Laboratory diagnosis is a crucial step in treating, controlling and preventing MRSA infections (6,7). Identification of the mecA gene is the most reliable method of detecting MRSA isolates, however not all laboratories can include molecular methods. For this, it is essential that phenotypic methods such as disk diffusion able to detect MRSA isolates in a rapid and accurate manner (6,7). Out of the 122 strains that were positive for the mecA gene, two and one yielded false negative results with cefoxitin and methicillin disk respectively. The lower sensitivity may be explained by the absence of or reduced expression of the mecA encoded protein, PBP2a (6). Thus, in this study oxacillin was the most appropriate disk for detecting MRSA, as all mecA-positive isolates were detected with oxacillin disk, but in study of David Valesco (6) cefoxitin was the appropriate disk for detecting MRSA isolates. In general, our study was similar to other studies that showed disk diffusion and PCR of mecA gene can be used for detecting MRSA.

The prevalence of MRSA in this study is comparable to that found in United States, Canada, Europe and Iran, but the percentage of MRSA isolates is almost twice of percentage reported from Japan (1,3,4, 5,11). Differences in prevalence of methicillin resistance among *S. aureus* isolated from various specimens might be due to prolonged antibiotic treatment of severely sick patients, which generally have longer hospital stays, resulting in enhanced selection pressure. Distributions of MRSA isolates were varied in different wards which partly reflects the fact that some patients, e.g., critically ill patients in ICUs, have a greater chance of becoming colonized or infected (5). Our results concerning the high prevalence of MRSA in ICUs are largely in accordance with other studies (2,12); as CDC reported that more than 50% of *S. aureus* isolates from ICUs are now resistant to methicillin and, in other hospital units, this rate is close behind at 40% (2).

Conclusion

The low prevalence of MRSA in emergency rooms suggests that the level of MRSA in the community is still lower than that in hospitals.

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