Original Article

Detection of methicillin-resistance gene in *Staphylococcus epidermidis* strains isolated from patients in Al-Zahra Hospital using polymerase chain reaction and minimum inhibitory concentration methods

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Abstract

Background: In recent years, antibiotic resistance of *Staphylococcus epidermidis* to methicillin has significantly increased, making it essential to study resistance to methicillin, which is a determining factor in the appropriate treatment pattern. The purpose of this study was to identify methicillin-resistant genes in *S. epidermidis strains* using polymerase chain reaction (PCR) and to determine their mean minimum inhibitory concentration (MIC) to methicillin using E-test method.

Materials and Methods: MIC was determined on 146 samples of *S. epidermidis* using E-test method. Moreover, all samples were tested for the presence of *mecA* gene using PCR.

Results: PCR test showed 75.34% of the samples to contain *mecA* gene. Methicillin resistance test was performed using E-test on all the samples, which showed resistance in different dilutions.

Conclusion: The frequency of *mecA* gene in *S. epidermidis* isolates was 75.34%. Among the various applied tests used for determining methicillin resistance, sensitivity and specificity of PCR were the highest and reached 100%. Sensitivity and specificity were found to be 95.3% and 94.7%, respectively, for phenotypic test (E-test) and 86.5% and 80.9%, respectively, for disk diffusion method. Based on the above results, it seems that resistance of *S. epidermidis* to methicillin is on the rise, and therefore more research is warranted.

Key Words: E-test, *mecA* gene, methicillin, *Staphylococcus epidermidis*

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INTRODUCTION

Coagulase-negative staphylococci (CNS) are considered important pathogens in nosocomial infections. About 80-90% of these bacteria are associated with nosocomial infections and show resistance to methicillin.[1] *Staphylococcus epidermidis* is a gram-positive and coagulase-negative bacterium which was initially considered a normal bacterial flora of healthy human skin and a commensal bacterium. In recent years, this bacterium has been known as the common cause of nosocomial infections.[2] These infections are mostly associated with using medical equipments such as intravenous and urinary catheters and joint shunts. *S. epidermidis* results in bacteremia,

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osteomyelitis, and peritonitis through these devices.[3,4]

S. epidermidis is more virulent in immunosuppressed patients and other patients who are hospitalized for a long time.[5] Among the CNS, they cause 74-92% blood infections in hospitals.[6] Based on the studies conducted in western countries, more than 70% of *S. epidermidis* isolates are resistant to methicillin or oxacillin.[7] This bacterium produces biofilm and easily adheres to catheters and shunts and, through this mechanism, protects itself from the effect of antimicrobial agents.[8-11]

The main compound of biofilm is cellular polysaccharide, which protects the bacterium against the body's immune system and also causes bacterial colonization on the surfaces of medical devices such as intravenous catheters and joint shunts, and creates resistance to external conditions.[12] It has been also proven that the bacteria inside the biofilm structure can easily exchange genetic information, such as antibiotic resistance genes, among themselves.[13] Resistance to methicillin in isolates which produce biofilm is considerably more than the other isolates which do not form biofilm.[14] Like *Staphylococcus aureus*, the mechanism of methicillin resistance occurs using the *mecA* gene which encodes penicillin binding proteins (PBPs) with little tendency to connect to the beta-lactam antibiotics.[15,16] This gene is located on chromosomal element named Staphylococcal Cassette Chromosome *mec* and is regulated by two other genes called *mec1* and *mecR1*.[17] Concerns in detecting methicillin resistance are on the point that the sensitivity tests may not be able to identify correct resistance to methicillin.[18]

Methicillin resistance is identified by phenotypic and genotypic methods.[19] Nowadays, phenotypic methods such as disk diffusion are mostly used in laboratories, in which different environmental factors can affect bacterial growth and results.[20] Although several studies have shown that standardized disk diffusion method has similar sensitivity level to that of *mecA* gene,[21-23] some errors have also been reported.[20,24-26] Therefore, it is essential to develop a rapid, sensitive, and accurate method to detect *mecA* gene, not affected by the conditions of the culture medium. In this study, we compared phenotypic (E-test) and polymerase chain reaction (PCR) genotypic methods to evaluate methicillin resistance in *S. epidermidis*. E-test method is derived from agar dilution and disk diffusion which has more advantages and requires less time compared to phenotypic methods.[27]

PCR is a rapid, sensitive, and accurate test for determination of *mecA* gene, and therefore methicillin resistance in these bacteria, and also used for confirming phenotypic methods which are less sensitive.[28]

Considering the high prevalence of *S. epidermidis* in various infections in infants, urinary infections, and its adherence to medical equipment and devices which leads to infection in different patients, and due to the considerable increase of methicillin resistance among patients, it was decided to compare the phenotypic and genotypic methods for evaluation of methicillin resistance in *S. epidermidis*.

MATERIALS AND METHODS

Bacterial isolates

A total of 146 *S. epidermidis* isolates were isolated from patients of different wards of Al-Zahra Hospital in Isfahan during 2009.

Laboratory methods

The collected isolates were identified by different conventional methods including gram staining, catalase test, tube test and slide coagulase test, DNAse, Novobiocin sensitivity, bacitracin and polymyxin B resistance, urea hydrolysis and Voges-Proskaer test, and finally culture in mannitol salt agar medium.

Antibiotic susceptibility test

Disk diffusion and minimum inhibitory concentration (MIC) determination with E-test method were performed for all isolates. Disk diffusion and MIC were accomplished according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) (ref. CLSI). We used 30 μg oxacillin disk (HiMedia Code: SD088, India, Mumbai) for disk diffusion test. An E-test stripe was utilized for MIC determination, and *S. aureus* ATCC 25923 and ATCC 33591 was used as *mecA* negative and positive control, respectively.

Genomic DNA extraction

Genomic DNA was extracted by conventional phenol–chloroform method.[29]

PCR

Thermal cycling for amplification of *mecA* gene was performed in an Eppendorf thermal cycler (Mastercycler® gradient). Amplification protocol consisted of 5 min initial denaturation at 94°C, followed by 35 cycles of denaturation (94°C/30 seconds), annealing (52°C/30 seconds), and extension (72°C/60 seconds), and an additional post-amplification extension step at 72°C for 5 min.

The following primers were used for PCR amplification of *mecA* gene:[29,30]

mecA-F: 5′-TGGCTATCGTGTCACAATCG-3′

mecA-R: 5′-CTGGAACTTGTTGAGCAGAG-3′

PCR was performed in a mixture of 25 μl volume containing: 2.5 μl 10 × buffer (Roche Germany, Berlin), 0.4 μl of each dNTP (200 μm), 2.5 μl (50 mm) $MgCl_2$, 2.5 U of Taq DNA polymerase, 10 pmol of each primer, and 5 μl of template DNA.

RESULTS

Of the total 146 *S. epidermidis* strains studied, 110 bacterial samples (75.34%) were methicillin resistant and contained *mecA* gene and 36 samples (24.66%) were methicillin sensitive not harboring *mecA* gene [Figures 1-3]. The specificity and sensitivity of E-test, disk diffusion, and PCR are compared in Table 1. Our results showed good correlation between phenotypic and genotypic methods for detection of antibiotic susceptibility.

DISCUSSION

Methicillin resistance in isolates of CNS has increased significantly in the recent years. Approximately 50-80% of it depends on the species containing *mecA* gene or show resistance to oxacillin. Among the CNS isolates, *Staphylococcus haemolyticus* is the most frequent species in nosocomial infections and shows more resistance to oxacillin.[31]

Resistance to methicillin was reported shortly after using this drug for the treatment of staphylococcal infections in 1961, and then it spread to hospitals around the world.[32] According to the studies conducted on resistance to methicillin in CNS, especially *S. epidermidis*, in different countries in recent years, resistance to methicillin is not usually below 50%.[33]The reports presented have indicated that methicillin resistance is increasing worldwide, causing great concern. Some of these studies are discussed below. In a study conducted in the United States in 1994, approximately 80% of *S. epidermidis* strains isolated from nosocomial infections showed resistance to methicillin and most of these strains also had resistance to other antibiotics.[19] According to a study conducted in Finland, methicillin resistance in *S. epidermidis* increased from 28% in 1938 to 77% in 1994.[34]

In a report published by Oliveria *et al*. in Brazil in 2007, methicillin resistance in *S. epidermidis* was reported as 78.3%.[35]

According to a survey conducted in South Korea in 2001, resistance rates of CNS and *S. epidermidis* to methicillin was found to be 60-90%.[36]

In India, CNS resistance to methicillin was 20.8% between 1997 and 1998.[19]

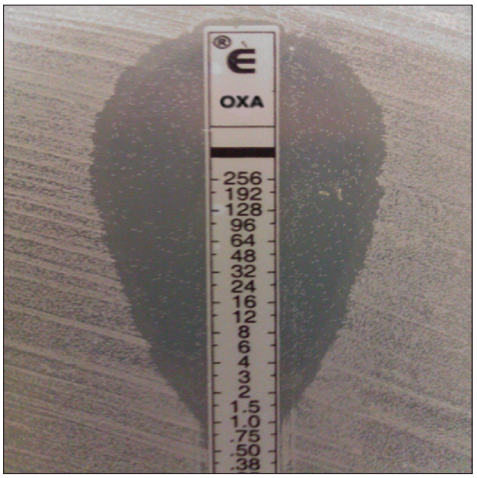

Figure 1: Negative results of the tested bacteria (methicillin-sensitive) in E-test method

Figure 2: Results of the tested bacteria (resistant to methicillin) in E-test method

Table 1: Comparing sensitivity and specificity of PCR, E-test, and disk diffusion method

Phenotypic method	Bacteria containing *mecA* gene		Bacteria without *mecA* gene		Sensitivity (%)	Specificity (%)
	Real positive	False negative	Real negative	False positive		
PCR	110	0	36	0	100	100
E-test	103	5	36	2	95.3	94.7
Disk diffusion	90	14	34	8	86.5	80.9

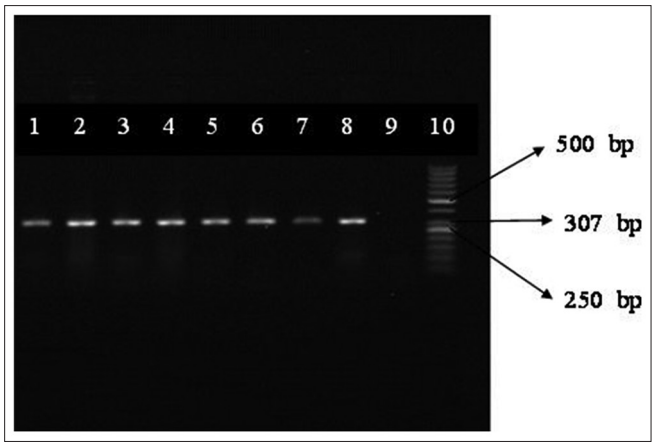

Figure 3: PCR results of mecA gene in 7 isolates staphylococcus epidermidis. Column 10: Size marker (50 bp). Column 8, 1-6: Isolates of staphylococcus epidermidis containing mecA gene. Column 7: Positive control of staphylococcus aureus ATC 33591. Column 9: Negative control of staphylococcus aureus ATCC 25923

During the present decade, resistance to methicillin has dramatically increased, causing various problems in the treatment.

In this study, among the 146 samples of *S. epidermidis* isolated from Al-Zahra Hospital in Isfahan, 110 cases carried the *mecA* gene or resistance to methicillin, which included 75.34% of all the samples.

From the results of the above-mentioned studies, it is seen that there is a correspondence between the present results and those of the above reports, which indicates that methicillin resistance in *S. epidermidis* is increasing worldwide.

Concerns in detecting methicillin resistance indicate that the available antimicrobial sensitivity tests are not able to detect this resistance correctly.[20] Identification of methicillin resistance includes phenotypic and genotypic methods.[21] Nowadays, the phenotypic methods such as disk diffusion are more used in the laboratories; different environmental factors affect the growth of bacteria and antibiogram results.[22]

Although several studies have indicated that standard method of disk diffusion is sensitive enough for detecting *mecA*-positive isolates,[23-25] some errors have also been reported by this method.[22,26-28] Hence, there is a need for rapid, accurate, and sensitive method which is not affected by the conditions of the medium.

In this study, E-test phenotypic method and PCR genotypic method were used to identify *mecA* gene and methicillin-resistant *S. epidermidis*. The E-test method was derived from agar dilution and disk diffusion, which has more advantages in comparison with other methods and requires less time.[24] In the present study, the frequency of *mecA* gene in disk diffusion and E-test methods was found to be 61.64% and 70.54%, respectively. E-test method is a simple and cheap phenotypic test which is used for detecting methicillin resistance. This method was first presented in 1988 and then introduced in 1991 by a business company called AB Biodisk.

Ferreira *et al*. studied methicillin resistance in 132 isolates of CNS using disk diffusion, E-test, and PCR methods, and reported that the sensitivity and specificity of disk diffusion test were 94.2% and 91.8%, respectively, and those of E-test were 100% and 71.4%, respectively.

While reviewing new techniques for determining methicillin resistance, Swenson reported that phenotypic methods had high sensitivity although they did not reach 100%. He also reported that disk diffusion tests had low sensitivity in the range of 61-85%.[37-39]

Considering the results obtained in other studies and the results of the present work, it could be stated that disk diffusion, as a phenotypic test, had slower sensitivity and specificity in comparison to E-test.

According to Table 1, in the false-negative and -positive phenotypic tests, it was observed that these results were more tangible in disk diffusion test.

Gerberding *et al*. and Chambers noted that the false-negative results were the heterogeneousness of *mecA* gene.[39,17]

Chambers noted (in the same year) that the false-positive results were due to two factors of producing excessive penicillinase and great variation of PBPs.[39]

CONCLUSIONS

In this study, three methods, E-test, PCR, and disk diffusion, were used to study methicillin resistance in *S. epidermidis* isolates. It was found that PCR was more precise and accurate than the other two methods; moreover, phenotypic method of E-test was a cheap and simple method for evaluating methicillin resistance. The result of this study indicated that resistance to methicillin in *S. epidermidis* in Iran is rapidly increasing, similar to other countries and even the developed ones.

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