Frequency of *MecA* Gene in the Clinical Isolates of *Staphylococcus epidermidis* in Isfahan, Iran

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ABSTRACT

***Background*:** *Staphylococcus epidermidis* is an important agent for nosocomial infections in infants and people with permanent prostheses. Its increased resistance to antibiotics has created a serious challenge for healthcare system. This study was conducted to determine the pattern of antibiotic-resistance of *S. epidermidis* isolates from clinical samples.

***Methods*:** During nine months, 251 clinical samples isolated from strains of *S. epidermidis* were examined. Following identification of isolates, their antibiotic sensitivity was determined using disc diffusion method. Resistance to vancomycin was assessed using agar screening method, and its MIC values were measured using episilometry (Etest). Methicillin-resistant gene (*mecA*) was traced using PCR.

***Results*:** A total of 120 *S. epidermidis* strains were isolated from the 251 clinical samples, mostly associated with urine samples. In this study, 95 isolates (79%) were found resistant to cefoxitin, 66 (55%) to vancomycin, and 94 (78.33%) to multiple drugs. In molecular assessment, 37 isolates (54.41%) contained *mecA* gene, of which, 32 isolates showed resistance to vancomycin.

***Conclusion*:** Increased resistance to methicillin and vancomycin in *S. epidermidis* isolates represents a serious warning to the healthcare system. Thus, careful and appropriate choice of treatment is imperative for reducing medication resistance.

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Introduction

In the past two decades, *Staphylococcus epidermidis* and other coagulase-negative Staphylococci have been recognized as the main cause of nosocomial infections (1).

The horizontal transmission of antibiotic resistant markers between these bacteria has hindered the treatment of such infections. These elements are carried on the plasmid or the chromosome.

Methicillin resistance in staphylococci is due to expression of PBP 2a, a novel, low-affinity PBP for which there is no homologue in methicillin-susceptible strains. PBP 2a is encoded by *mecA* gene which is carried by a mobile genetic element called the SCCmec (2). The clinical resistance of *S. epidermidis* strains has increased in recent years to glycopeptide antibiotics and methicillin (3). The emergence of multiple drug-resistant isolates has made the treatment of bacterial infections difficult, as a reservoir of antibiotic-resistant genes, has also spread resistance across the health systems. The present study was conducted to investigate vancomycin-resistance and carry out a molecular analysis of methicillin in isolates of *S. epidermidis* isolated from clinical samples in Isfahan.

A total of 251 clinical samples were collected from outpatients (n=50) and hospitalized patients (n=201) in Askarieh Hospital and Nobel Laboratory in Isfahan, including urinary, blood, spinal fluid, wound, tracheal and sputum samples. After the initial isolation, the isolates were identified through conventional biochemical methods (4). The antibiotic-resistance pattern of isolates was determined through the Kirby-Bauer method and according to the CLSI protocol (5). The MIC values of vancomycin was measured using an Epsilometer test (Himedia, India). A 0.5 McFarland suspension was again prepared from the 24-hour bacterial culture and was then lawn-cultured on Mueller-Hinton Agar medium. The tapes were then placed on the medium, and the plates were incubated at 37 °C for 24 hours. The MIC was then read from the tape according to the CLSI protocol. A vancomycin MIC ≥ 32 µg/ml was taken to indicate a resistant strain and a vancomycin MIC ≤ 12 µg/ml then to indicate a susceptible strain (5).

A 24-hour bacterial culture in Mueller-Hinton Agar medium was used for detecting the *mecA* gene. First, the DNA was extracted by boiling. A PCR was then performed using primer sequences for the *mecA* gene (F: TGCTATCCACCCTCAA ACAGG; R: AACGTTGTAACCACC CCAAGA) as per the following instructions: 1 µl of the extracted DNA and 1 µl of each primer was added to 12 µl the PCR Master kit (Kappa, USA), making for a final volume of 25 µl. This gene was amplified by PCR through the following steps: Initial denaturation at 96 °C for 5 minutes, 35 cycles of denaturation at 94 °C for 45 seconds, annealing at 45.5 °C for 60 seconds and extension at 72 °C for 10 minutes. The PCR products were then separated using electrophoresis on agarose gel 1% with Midori viewer and were detected by a 100 bp DNA ladder as a molecular weight marker using the gel documentation system. Staphylococci aureus ATCC 33591 and ATCC 25923 were used as positive and negative controls (6). The data obtained were analyzed in SPSS-16.

Table 1 shows these isolates divided by their clinical samples. The majority of the strains were isolated from urinary samples.

The disc diffusion method revealed multiple drug-resistance in 94 strains (78.33%), with the highest resistance observed to Oxacillin (79%) and the lowest to Levofloxacin (18%); (Table 2).

According to the Etest results, 68 (56.6 %) isolates were resistant to cefoxitin and 65 (54.16%) (MIC ≥32) to vancomycin, 27 (12 ≤ MIC ≤ 32) vancomycin- relatively susceptible and 28 (MIC ≤32) vancomycin- susceptible. The PCR results showed thirty seven out of 68 methicillin resistant isolates (54.4%) were *mecA* positive, 37 isolates harbored *mecA* gene. Among them 32 isolates were also resistant to vancomycin (figure 1). A total 68 isolates, 13 (34.21%) isolates were false positive and 10 (26.31 %) were false negative.

Table 1. The frequency of the *S. epidermidis* strains isolated from the clinical samples.

Clinical sample	No.	*S. epidermidis* (%)
Urinary Tract	205	93 (77.5)
Blood	24	13(10.83)
Vaginal Smear	9	6 (5)
Wound	8	5 (416)
Spinal Fluid	1	1 (0.83)
Tracheal	1	1 (0.83)
Throat	1	1 (0.83)
Sputum	2	–
Total	251	120

Table 2. Antimicrobial susceptibility of the *S. epidermidis* strains.

Antibiotic	Susceptible	Relatively Susceptible	Resistant
Vancomycin	19 (15.83)	–	70 (58.33)
Cefoxitin	29 (24.16)	15 (12.50)	72 (60)
Oxacillin	15 (12.50)	–	95 (79.16)
Gentamicin	64 (53.33)	28 (23.33)	38 (31.66)
Rifampicin	31 (25.83)	84 (70)	44 (36.66)
Cefazolin	62 (51.66)	22 (18.33)	46 (38.33)
Tetracyclin	40 (33.33)	57 (47.50)	30 (25)
Levofloxacin	57 (47.50)	38 (31.66)	22(18.33)

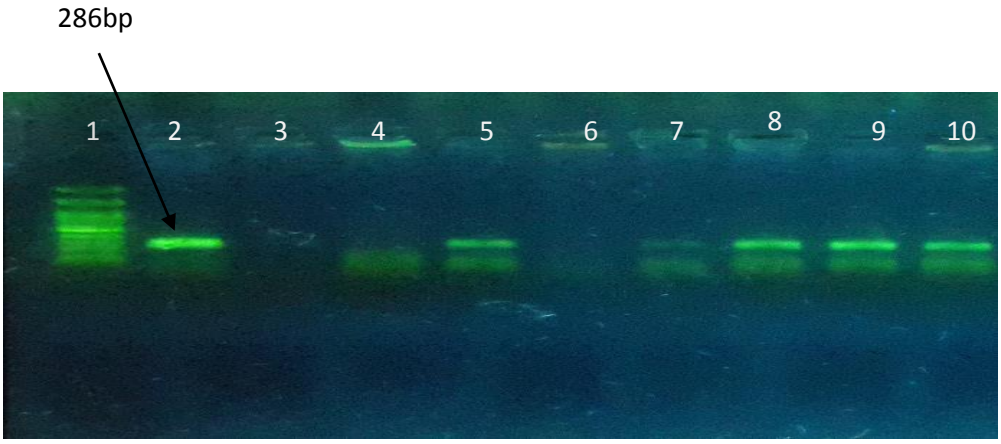

Figure 1. The *mecA* gene PCR in the MRSE isolates. Lake 1: 100 bp molecular weight marker; Lake 2: *S. aureus* ATCC 33591 as the positive control; Lake 3: *S. aureus* ATCC 25923 as the negative control; Lakes 3-10: clinical samples.

In the present study, the highest resistance in isolates was observed to oxacillin (79%) and the lowest to Levofloxacin (18%). Multiple drug-resistance was observed in 77% of the isolates. In a study conducted by Sedighian et al. (2008) in Babol in northern Iran, oxacillin-resistance was observed in 89.2% of the isolates and vancomycin-resistance in 6.7% (7). In one study, Bahamer et al. (2014) reported multiple drug-resistance in 66% of the samples, vancomycin-resistance using Agar screening in 55% and cefoxitin-resistance in 5% (8). Shojae et al. conducted a study (2007) in Tabriz using Agar screening and reported oxacillin-resistance as 89% (9), which is higher than the amount detected in the present study. According to the results obtained, vancomycin-resistance increased significantly in *S. epidermidis* strains. This study used an Etest to measure MIC values due to its easy interpretation and great accuracy; the results obtained revealed vancomycin-resistance in 55% of the isolates and cefoxitin-resistance in 56%. In a study conducted in Tehran, Rahimi et al. (2011) reported Agar dilution resistance to oxacillin in 42% of the samples (10). In a similar study conducted by Pishva et al. (2013) in Isfahan, oxacillin-resistance was reported in 80.9% of the cases using Agar dilution and in 86.5% using the Etest (11). In a study conducted in India, Hajera et al. (2014) reported vancomycin-resistance in 53.34% of the samples using the Etest (12).

The disparity of results may be attributed to the methods used for detecting resistance among the isolates and their different degrees of accuracy, making molecular PCR techniques imperative to the identification of this gene.

In the present study, *mecA* gene was present in 54.4% of the cefoxitin-resistant isolates. In a study conducted by Japoni et al. (2003) in Tehran, methicillin-resistance was reported in 56.5% of the samples using PCR (13). Methicillin-resistance was reported as 73.34% by Pishva et al. (2013) in Isfahan by PCR (11). In a study conducted by Nowak et al. (2006) in the Netherlands, methicillin-resistance was reported as 45.94% (14).

Comparing the results obtained in this and other studies suggests varying degrees of resistance in *S. epidermidis* isolates in different geographical regions and even in different hospitals within the same city.

Conclusion

Given the increasing prevalence of nosocomial infections and drug-resistance, a quick and accurate detection method should be used for detecting and treating infections in the shortest possible time and at the lowest cost. Replacing phenotype tests with genotype ones (especially PCR) is the best measure that should be taken. Moreover, hand hygiene and compliance with health and safety guidelines can help reduce the spread of infection.

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Conflict of interest

No conflict of interests is declared.

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