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Antibiotic Susceptibility and *mecA* Frequency in *Staphylococcus epidermidis*, Isolated From Intensive Care Unit Patients

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Background: Coagulase-negative staphylococci (CoNS), especially *Staphylococcus epidermidis*, are considered as normal flora of human epithelia and also important opportunistic pathogens for nosocomial infections. *S. epidermidis* can also act as a reservoir for *mecA*, responsible for high-level resistance to methicillin and transferring it to *S. aureus*.
Objectives: The aim of this study was to determine the prevalence of *S. epidermidis* as well as antibiotic susceptibility pattern and *mecA* prevalence in *S. epidermidis* isolated from intensive care unit (ICU) patients.
Materials and Methods: A cross-sectional study was conducted from September 2010 to September 2011 and 184 coagulase-negative staphylococci were collected from different clinical samples in three hospitals. *S. epidermidis* was identified by conventional bacteriological tests. Antibiotic susceptibility testing was performed using disk diffusion method. Frequency of *mecA* was detected by specific PCR.
Results: Frequency of *S. epidermidis* was 34.8%, the most susceptibility was seen to linezolid and vancomycin, and the least susceptibility was seen to tetracycline. Majority of the *S. epidermidis* isolates carried *mecA* (92.2%). The most common resistant pattern was trimethoprim-sulfamethoxazole, tetracycline, erythromycin, and methicillin resistance, found in 23.4% of the isolates, followed by resistance to methicillin as the second-most common resistant pattern, observed in 20.3% of the isolates.
Conclusions: Frequency of *S. epidermidis* was significantly lower, compared to other studies. Presence rate of *mecA* and susceptibility to linezolid and vancomycin did not show significant differences with other investigations, while resistant to trimethoprim-sulfamethoxazole was significantly lower compared to other investigations, and resistance to tetracycline was significantly higher in comparison to other investigations. Presence of methicillin-resistant *S. epidermidis* in ICU patients, especially in individuals with compromised immune systems, may cause infection and would be more complicated in the case of antibiotic resistance.

Keywords:Staphylococcus epidermidis; *mecA*; Coagulase; Methicillin-Resistant

1. Background

Coagulase-negative staphylococci (CoNS), especially *Staphylococcus epidermidis*, are considered as normal epithelial flora of every human in different parts of the body such as nares, head and axilla, with an essential role in maintaining the normal flora of healthy skin (1). *S. epidermidis* is also recognized as an important opportunistic pathogen in nosocomial infections, particularly in indwelling medical device users and contaminant agent of blood cultures. Nosocomial infection with *S. epidermidis* should be more complicated when bacteria are resistant to beta-lactam antibiotics such as methicillin. Methicillin-resistant CoNS show a higher resistance rate to antibiotics than *S. aureus*. These bacteria are common causes of nosocomial infections worldwide in immunocompromised patients or patients who carry indwelling devices (2).

Reduced affinity for beta-lactam antibiotics in methicillin-resistant staphylococci mediates by a penicillin-binding protein (PBP2a) encoded by *mecA* gene located in staphylococcal chromosome cassette *mec* (SCC*mec*). Furthermore, it is believed that *S. epidermidis* acts as a reservoir for *mecA* and transfers it to *S. aureus* by horizontal gene transfer. Recent data indicate that not only *mecA*, but also other mobile genetic elements can be transferred from *S. epidermidis* to *S. aureus* (3).

2. Objectives

The aim of this study was to determine the prevalence of *S. epidermidis* as well as antibiotic susceptibility pattern and *mecA* prevalence in *S. epidermidis* isolated from intensive care unit (ICU) patients.

3. Materials and Methods

3.1. Bacterial Isolates

A cross-sectional study was conducted from September 2010 to September 2011. A collection of 184 coagulase-negative staphylococci isolated from blood (n = 135), urine (n =

17), tracheal (n = 19), wound (n = 6), cerebrospinal fluid (CSF) (n = 2), pleural fluid (n = 2), synovial fluid (n = 2), and peritoneal fluid (ascitic fluid) (n = 1), from three different teaching hospitals of Tehran, Iran, was included in this study. *S. epidermidis* was identified by conventional bacteriological tests. The sample was enriched in tryptic soy broth, and grown on mannitol salt agar, then catalase, tube coagulase and urease tests, and carbohydrate fermentation were performed. *S. epidermidis* is catalase positive, urease positive, unable to ferment D-mannitol and D-trehalose, and able to ferment D-mannose and D-maltose (4, 5).

3.2. Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed for *S. epidermidis* isolates using disk diffusion method on Mueller-Hinton agar plates (Merck, Germany) and commercial antibiotic disks including: vancomycin (30 μg), linezolid (30 μg), rifampin (5 μg), ciprofloxacin (5 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), tetracycline (30 μg), and erythromycin (15 μg), (Mast, UK), according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (6).

3.3. DNA Extraction

DNA of each *S. epidermidis* isolate was extracted from 1mL of overnight bacterial culture. Extraction was performed by adding TE [10 mM Tris, 1 mM EDTA (pH = 8)] buffer and lysostaphin, 95°C for 5 minutes, then 0°C for 5 minutes, and centrifuged in 10000 rpm for 5 minutes. The supernatant was used as the template DNA in PCR reactions. DNA was measured using a BioPhotometer (Eppendorf, Germany) to determine the concentration and purity.

3.4. Detection of mecA

PCR was performed as described by Zhang et al. (7) with the following specific primers: forward: 5'-GTGAAGATATACCAAGTGATT-3' reverse: 5'-ATGCGCTATAGATTGAAAGGAT-3'. Amplification was performed in a MJ mini. Gradiant thermal cycler PTC- 1148. U.S.A ; initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of 45 seconds at 94°C,45 seconds at 55°C,and 90 seconds at 72°C, ending with a final extension step at 72°C for 10 minutes, followed by a hold at 4°C. The amplified products were visualized by UV light after electrophoresis on 1% agarose gel. The amplicon size was 147 bp (Figure 1). In the PCR reaction, *S. epidermidis* ATCC 12228 and *S. aureus* ATCC 33591 were used as negative and positive controls, respectively (7).

3.5. Statistical Analysis

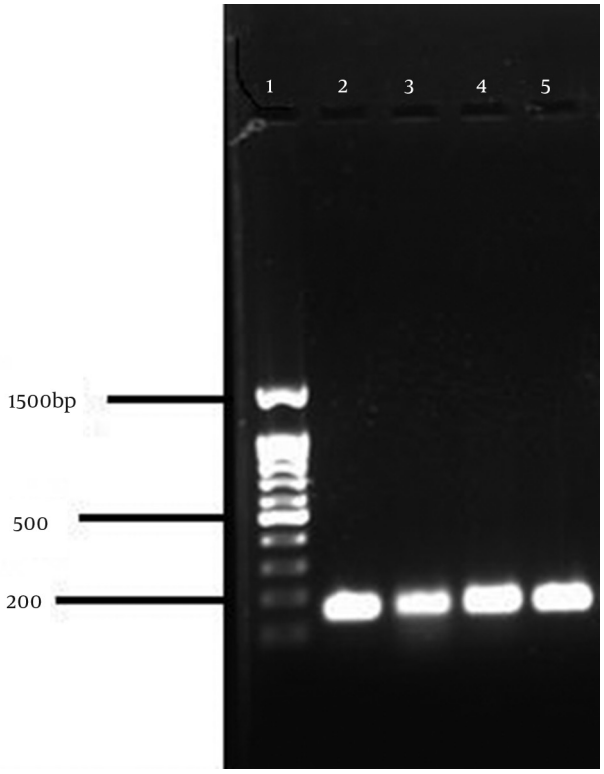
Confidence interval test was used to assess the statistical significance with confidence level of 95% (α = 0.05).

4. Results

In this study, frequency of *S. epidermidis* was 34.8% (95% CI: 46.5-23.1). Of the 64 *S. epidermidis* isolates, 52 (81.3%) were from blood, 6 (9.4%) tracheal, 4 (6.2%) urine, and 2 (3.1%) wound samples. *mecA* was detected in 92.2% (95%CI: 98.8-85.6) of *S. epidermidis* isolates. The results of antibiotic susceptibility testing are shown in Table 1. All of 64 *S. epidermidis* isolates were susceptible to vancomycin and linezolid. Resistance to rifampin and ciprofloxacin were 9.4% (95%CI: 16.5-2.3) and 23.4% (95%CI: 33.8-13), respectively. Resistance to trimethoprim-sulfamethoxazole was observed in 51.6% (95% CI: 63.8-39.4) of *S. epidermidis* isolates, while 46.9% (95% CI: 59.1-34.7) were susceptible to trimethoprim-sulfamethoxazole. Susceptibility to tetracycline was observed in 42.1% (95% CI: 54.2-30) of *S. epidermidis* isolates, while resistance was observed in 57.8% (95%CI: 69.9-45.7) and 59.4% (95%CI: 71.4-47.4) to tetracycline and erythromycin, respectively. Resistance pattern of 64 *S. epidermidis* isolates is shown in Table 2.

Three Of the 64 isolates (4.7%) were resistance to six antibiotics, 4 (6.3%) to five antibiotics, 20 (31.3%) to four antibiotics, 9 (14%) to three antibiotics, 13 (20.3%) to two antibiotics, 14 (21.9%) to one antibiotic, and 1 (1.7%) was susceptible to all the antibiotics. The most common resistance pattern was trimethoprim-sulfamethoxazole, tetracycline, erythromycin, and methicillin resistance, found in 15 isolates (23.4%), followed by resistance to methicillin only, the second-most common resistance pattern, observed in13 (20.3%) of the isolates.

Figure 1. PCR for *mecA* Gene



1, Marker, 100 bp; 2, *S. aureus* ATCC 33591 (147 bp); 3-5, Positive clinical strains.

Table 1. Antimicrobial Susceptibility Rate of *S. epidermidis* Isolates by Disk Diffusion Method

	Blood	Urine	Trachea	Wound	Total, No. (%)
Vancomycin	52	4	6	2	64 (100)
Linezolid	52	4	6	2	64 (100)
Rifampin	48	4	4	2	58 (90.6)
Ciprofloxacin	41	2	3	2	48 (75)
Trimethoprim-sulfamethoxazole	26	3	0	1	30 (46.9)
Tetracycline	21	3	1	2	27 (42.2)
Erythromycin	21	2	1	1	25 (39.1)

Table 2. Resistance Pattern of 64 *S. epidermidis* Isolates [a]

Resistance Pattern	Number
Resistance to 6 antibiotics	
RP, CIP, SXT, Te, E, *mecA*	3
Resistance to 5 antibiotics	
CIP, SXT, Te, E, *mecA*	2
RP, CIP, Te, E, *mecA*	2
Resistance to 4 antibiotics	
SXT, Te, E, *mecA*	15
CIP, Te, E, *mecA*	2
CIP, SXT, E, *mecA*	1
CIP, SXT, Te, *mecA*	1
RP, CIP, E, *mecA*	1
Resistance to 3 antibiotics	
Te, E, *mecA*	3
SXT, E, *mecA*	3
CIP, E, *mecA*	1
SXT, Te, *mecA*	2
Resistance to 2 antibiotics	
Te, E	3
SXT, E	2
Te, *mecA*	3
SXT, *mecA*	4
CIP, *mecA*	1
Resistance to 1 antibiotic	
mecA	13
Te	1
Non	1
Total	64

[a] Abbreviations: CIP, ciprofloxacin; E, Erythromycin; *mecA*, presence of *mecA*; RP, rifampin; SXT, trimethoprim-sulfamethoxazole; Te, tetracycline,

5. Discussion

In the recent years, *S. epidermidis* has been isolated as an infective agent in nosocomial infections. Some reasons for such infections increasing are increased number of immune-deficient patients, use of indwelling medical devices, and use of antibiotics and disinfectants (8). Methicillin resistance was observed in 75-90% of isolated *S. epidermidis* from nosocomial infections, which was higher than the resistance rate for *S. aureus* isolates (40-60%). Noticing the presence of *S. epidermidis* as a human commensal flora, it is assumed to be carrier and reservoir for different genes such as antimicrobial resistance gene (2).

Frequency of *S. epidermidis* isolates in this study was significantly lower than reports of Okee et al. (9), Eftekhar and Raei (10), Bouchami et al. (11), Barbier et al. (12), Ruppe et al. (13), and Mombach Pinheiro Machado AB et al. (14). In the present study, all the *S. epidermidis* isolates were susceptible to vancomycin, which was in accordance with the result of Mendes et al. (15), Eftekhar and Raei (10), Abd EL Hafez et al. (16), Hellmark et al. (17), and Zhanelet al. (18), and significantly higher than report of Delgado et al. (19). In our study, all the *S. epidermidis* isolates were susceptible to linezolid, which was in accordance with the results of Hellmark et al. (17) and Zhanel et al. (18), but significantly higher than Delgado et al. (19) report.

In the present study, resistance to rifampin was significantly higher than Abd EL Hafez et al. (16) report, but significantly lower than reports of Bouchami et al. (11) and Hellmark et al. (17). In addition, resistance to ciprofloxacin was in accordance with Haque et al. (20) report, but significantly lowers than Mendes et al. (15), Hellmark et al. (17), and Bouchami et al. (11) reports, and it also was significantly higher than Abd EL Hafez et al. (16) reports. Susceptibility to trimethoprim-sulfamethoxazole was in accordance with Mendes et al. (15) report, but significantly higher than reports of Hellmark et al. (17), Bouchami et al. (11), Abd EL Hafez et al. (16), and Zhanel et al. (18). Furthermore, resistant to trimethoprim-sulfamethoxazole was significantly lower than report of Delgado et al. (19). In this study, resistance to erythromycin did not show significant difference with results of Mendes et al. (15), Bouchami et al. (11), and Hellmark et al. (17), but was significantly higher than the report of Haque et al. (20) and significantly lower than Abd EL Hafez et al. (16) report. In this study, susceptibility to tetracycline was significantly lower than the report of Mendes et al. (15) and Delgado et al. (19); also, resistance to tetracycline was significantly higher than the report of Bouchami et al. (11).

In this study, 92.2% of *S. epidermidis* isolates carried the

mecA gene. Rohde et al. (21) reported that 87.5% of *S. epidermidis* isolates harbored *mecA*. Pourmand et al. (8) also reported that 95.8% of *S. epidermidis* isolates harbored *mecA*. Eftekhar and Raei (10) reported 90.9% methicillin resistance. Our data did not show significant difference with these studies, but was significantly higher than Līduma et al. (22) and Okee et al. (9) results, which reported only 10% *mecA* distribution, and Mendes et al. (15) study, which reported 73.2% methicillin resistant. In our study, 56.3% of *S. epidermidis* isolates were resistant to three antibiotics or more. Considering the presence of *S. epidermidis* in ICU patients, multidrug-resistant bacteria can cause infection and would be more complicated in treatment.

In conclusion, frequency of *S. epidermidis* was significantly lower compared to other studies. Presence rate of *mecA* and susceptibility to linezolid and vancomycin did not show significant differences with other investigations, while susceptibility to trimethoprim-sulfamethoxazole was significantly higher, and susceptibility to tetracycline was significantly lower than other investigations. Presence of *mecA*-positive *S. epidermidis* isolates in ICU patients, especially in ones with compromised immune systems, may cause infection and would be more complicated in the case of antibiotic resistance.

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Authors' Contributions

Here we confirm that all authors participated in the research design and contributed to sections of the research.

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The authors declare that there is no conflict of interests to publish this article.

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