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Recognition of (*Sesc*) for Easy Identification of *Staphylococcus Epidermidis* and Molecular and Phenotypic Study of B-Lactam Resistance in *Staphylococcus Epidermidis* Isolates in Isfahan

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Abstract

Background: Not only is it crucial to rapidly detect *Staphylococcus epidermidis* (*S. epidermidis*) isolates from a broad range of bacteria, but recognizing resistance agents can greatly improve current diagnostic and therapeutic strategies.

Methods: The current cross-sectional study investigated 120 clinical isolates from a nosocomial *S. epidermidis* infection. The isolates were identified using common biochemical tests, and specific *S. epidermidis* surface protein C (*SesC*) primers were used to confirm the presence of *S. epidermidis*. PCR and special primers were used to detect the β-lactamase gene (*blaZ*). Methicillin resistance was measured using the agar screening method and antibiotic susceptibility was measured by disk diffusion.

Results: 100 samples were characterized as *S. epidermidis* using a phenotypic and genotypic methods. From the 100 specimens examined, 80% contained *blaZ*. According to agar screening, 60% of isolates were methicillin-resistant. *S. epidermidis* isolates demonstrated the highest resistance to penicillin (93%) and the highest sensitivity to cefazolin (39%).

Conclusions: The increased resistance to β-lactam antibiotics in *S. epidermidis* isolates is alarming, and certain precautions should be taken by healthcare systems to continuously monitor the antimicrobial pattern of *S. epidermidis,* so that an appropriate drug treatment can be established.

Keywords: Antibiotic resistance, β-lactam, *Staphylococcus epidermidis*.
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Introduction

It is important to identify bacteria in clinics and medical diagnostic laboratories to provide appropriate therapeutic solutions (1). In most laboratories across the country, the identification process of isolates is based on phenotype or experimental biochemical test (1, 2).

Unfortunately, common biochemical tests are often Laborious and technical. The exact and definite diagnosis of isolates requires multiple tests that demand extensive preparation and isolated cultures (1). When the number of laboratory visits patients increases, it is practically impossible for everyone to provide and prepare media and other biochemistry tests, therefore, incomplete testing may occur. It actually reduces the required number of tests and the accuracy of identifying isolates by providing incorrect answers (1, 2). The biofilm interconnectedness and densification of gram-positive bacteria under a microscope are more complex than gram-negative bacteria. So that recognition of these bacteria based on morphology and biochemistry test is hard and time-consuming, and also some tests for them such as PYR and fermentation of different sugars, are not possible in laboratories due to their high cost (1, 2, 5, 18). *Staphylococcus epidermidis* (*S. epidermidis*) has

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become an important member of the coagulase-negative staphylococci (CNS) group within the last decade, is the third cause of nosocomial infections, and one of the most common causes of blood infections. *S. epidermidis* has been isolated from wound infections, skin infections, endocarditis, bacteremia, pneumonia, infected urinary tracts and soft tissue (1-4). The most important factor in the success of *S. epidermidis* stems from its ability to produce biofilm on the catheter. Today, *S. epidermidis* surface proteins have been found in biofilms, including *S. epidermidis* surface protein C (*SesC)* and *S. epidermidis* surface protein I (*SesI*). The *SesC* gene is necessary for biofilm creation and encodes for *SesC*, which contains 676 amino acids (2, 5). Anti- *SesC* antibodies are used to prevent the formation of biofilms caused by different strains of *S. epidermidis*, as these sequences are highly protected within this particular species (1, 5). Today, *SesC* is used as a rapid identification marker for *S. epidermidis* from other isolates (2). β-lactam drugs are used to treat most *S. epidermidis* infections (6, 7). Resistance to β-lactam antibiotics is commonly associated with staphylococci resistance. This can be observed in hospital pathogens such as CNS (6). In staphylococci, the main mechanism of resistance against β-lactam antibiotics is generally divided into two categories (6, 8). The first category is characterized by the deactivation of penicillin by β-lactam ring hydrolysis. The second category, mostly observed in humans, includes the production of β-lactamase enzymes and penicillin-binding proteins (PBPs), both of which reduce the response to medication and increases resistance to semi-synthetic penicillin, cephalosporins, and carbapenems (9). A study conducted on the β-lactamase gene (*blaZ*) showed that it has structural, suppressor and signal transducer sequences coded by different *blaZ* groups (10). Not only does the secretion of the β-lactamase enzyme causes increased resistance to β-lactam drugs, but at high concentrations, some non-methicillin-resistant gene strains may confer false resistance to methicillin (6, 10, 11). Methicillin-resistant isolates are created as a result of *S. epidermidis* conferring resistant genes, such as mecA. Often, this type of resistance leads to connects mecA gene or penicillin-binding protein (PBP2' or PBP2a). This protein is a transpeptidase that has low attraction ability for β-lactams (6, 12). The resistance of the CNS group has been observed in both humans and animals (6, 13).

The identification of these pathogens is important to eliminate the development of hospital infections (6, 14). The aim of this study was to detect *SesC* and *blaZ* in clinical specimens of *S. epidermidis* and determine resistance to methicillin and antibiotic susceptibility pattern of β-lactam drugs.

Materials and methods

Sample collection and bacterial characterization

In a span of six months, from January to June 2019, 120 clinical samples were collected and examined. The identification of isolates was conducted using common biochemical tests. The isolates were stored in brain heart infusion (BHI) media and 15% glycerol at -20 °C**.**

Confirm of Staphylococcus epidermidis isolates

To confirm the identity of strains, isolates were exposed to polymerase chain reaction (PCR) for the *SesC* gene. The genomic DNA of isolates was purified using a DNA extraction kit (CinnaGen, Iran) which was performed according to the manufacturer's protocol. Specific PCR primers used in this study can be found in Table 1. PCR was used to detect *SesC* which was previously described (2).

Polymerase chain reaction for detection of blaZ

PCR reaction was performed for detection of *blaZ* gene. DNA of the isolates were derived using DNA derivation kits according to the manufacturer's instructions (CinnaGen, Iran). The quality and quantity of DNA were determined using a spectrometer (Thermo Scientific, Waltham, MA, USA). Specific primer sequences were used to amplify *blaZ* (Table 1). The PCR protocol of this study followed the protocol described by Zehra et al (15). The PCR mixture was performed in a total volume of 25 μl. Primer sets containing *blaZ* (1 μl of each primer of 20 pmol concentration) were added to the reaction volume. PCR cycling conditions included an initial denaturation step at 94 °C for 45 s, followed by 30 cycles each of denaturation at 94 °C for 20 s, then, annealing at 55 °C for 15 s and extension at 70 °C

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for 15 s, followed by a final extension step at 72 °C for 2 mins (15). Amplicons were stored at 4 °C.

Table 1. Primer sequences used for PCR reaction.

Size	Target gene	Sequence	Reference
388bp	***SesC***	F: 5'- GTTGATAACCGTCAACAAGG -3' R:5'CATGTTGATCTTTGAATCCC-3'	(2)
173bp	***blaZ***	F: 5'- ACT TCA ACA CCTGCTGCTTTC-3' R:5'TGA CCA CTT TTA TCA GCA ACC -3'	(15)

Resistance to methicillin (Agar screening method)

For separation of isolates resistant to methicillin, agar screening method was used. For this purpose, after preparing of Muller Hinton agar Medium (*Himedia, India*) containing four percent salt, before distributing it on the plate, after the temperature of the autoclaved culture media reached about 45 °C, the solution containing 6µl/mg oxacillin (Sigma - Aldrich USA), was added to the autoclaved culture media by 0.2 µm syringe filter. The bacterial suspension was prepared by previous incubation in BHI broth for 24 h and turbidity was set to equivalent to 0.5 McFarland standard. After preparation of the bacterial suspension, 10 µl suspension was seeded in spots on the agar surface with a swab and it was incubated for 24 h at 37 °C. Finally, the separation of resistance isolates was according to the Clinical and Laboratory Standard Institute (CLSI) guidelines. Any growth on the medium was considered as a positive test outcome (16, 22).

Antimicrobial susceptibility test

Antibiotic susceptibility pattern of β-lactam drugs was performed using the Kirby-Bauer disk diffusion method. The following antibiotics were tested: Penicillin (10 µg), Ceftriaxone (30 µg), Amoxycillin (10 µg), Cefoxitin (30 µg), Cefotaxime (30 µg), Cephalexin (30 µg), and Cephazolin (30 µg) (*Mast, UK*).

Results

Among the 120 specimens obtained from several different nosocomial infections, 100 samples were characterized as *S. epidermidis* following phenotypic analysis. All of the isolates were confirmed by *SesC* PCR (Fig. 1). Data introduced in Fig. 3 illustrates the distribution of *S. epidermidis* in various clinical specimens, and among the 100 *S. epidermidis* isolates, the highest percentage of specimens were found in urine (35%) and blood (25%) samples and lowest percentage of specimens were found in lung (3%) and eye (2%) samples (Fig. 3).

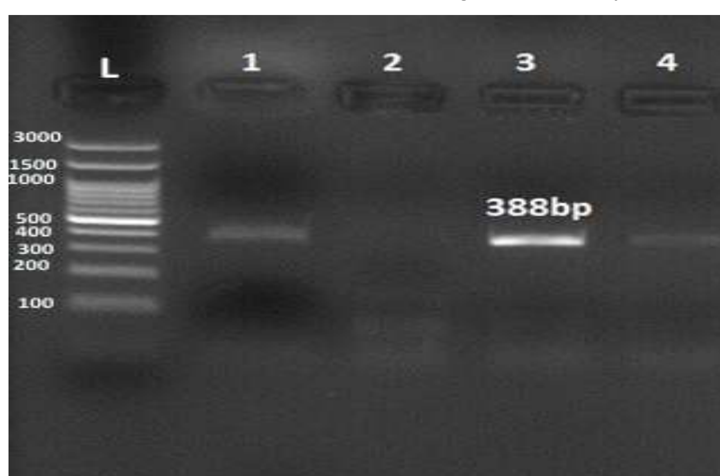

Fig. 1. *SesC* amplicon in PCR product of *Staphylococcus epidermidis* isolates. Lane L: Size marker (100 bp), Lane 2: Negative Control, Lanes 1, 3, and 4 (388 bp): Positive samples.

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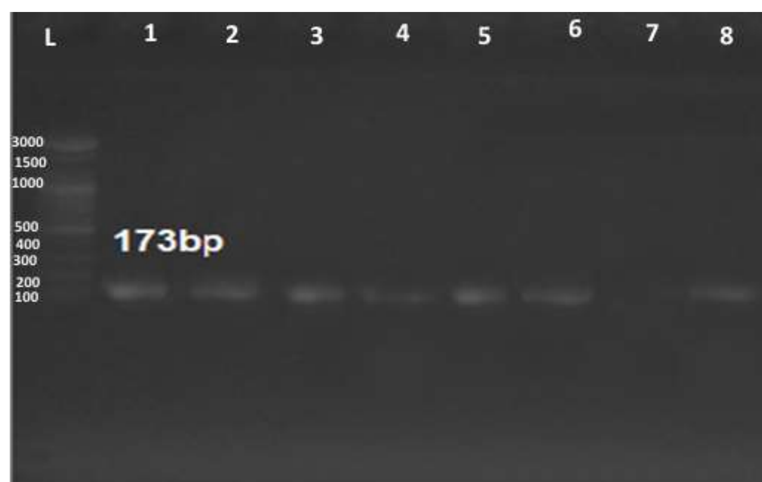

Fig. 2. *blaZ* amplicon in PCR product of *Staphylococcus epidermidis* isolates. Lane L: Size marker (100 bp), Lane 7: Negative Control, Lanes 1, 2, 3, 4, 5, 6, and 8 (173 bp): Positive samples.

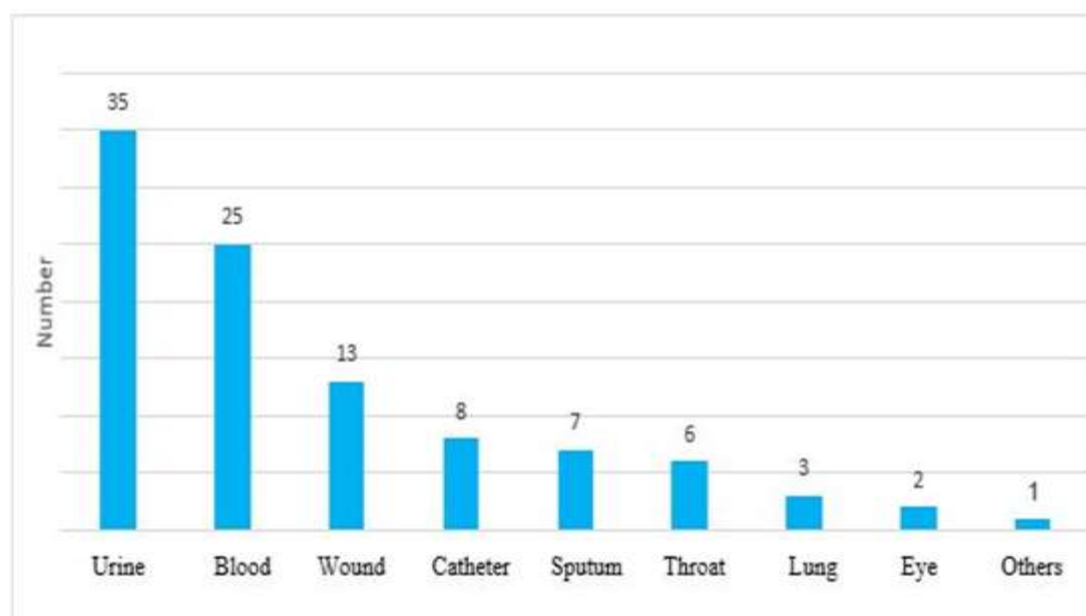

Fig. 3. Distribution of *Staphylococcus epidermidis* isolated from clinical samples.

Among the 100 specimens examined, 80 (80%) samples contained *blaZ* (Fig. 2), and according to agar screening, 60% of the isolates were found to be methicillin-resistant. Furthermore, isolates showed higher resistance to penicillin (93%) compared to ceftriaxone (85%), and higher sensitivity to cephalexin (40%) compared to Cephazolin (39%) (Table 2).

Table 2. Antibiotic resistance pattern *Staphylococcus epidermidis* isolates to group of β-lactam antibiotics.

Antibiotic	No. of strains	Resistance (%)	No. of strains	Semi resistance (%)	No. of strains	Sensitivity (%)
Penicillin	93	93	0	0	7	7
Ceftriaxone	85	85	0	0	15	15
Amoxycillin	80	80	10	10	10	10
Cefoxitin	65	65	0	0	35	35
Cefotaxime	50	50	2	2	48	48
Cephalexin	40	40	1	1	59	59
Cefazolin	39	39	4	4	57	57

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Discussion

Accurately identifying different strains of bacteria in laboratories has crucial implications in healthcare settings. The ability to rapidly recognize bacterial strains, while maintaining accuracy and simplicity, can improve current diagnostic approaches (2, 17). In this study, the *SesC* gene was present in all *S. epidermidis* isolates. Therefore, the *SesC* gene can be used as a genetic marker to distinguish *S. epidermidis* from other isolates (2). Khodaparast et al. (2016) reported *SesC* as a genetic marker that can easily identify *S. epidermidis*, since all of the *S. epidermidis* isolates contained *SesC*. Furthermore, the results of this study were consistent with our study (5, 18). Previous studies show that 60–85% of *S. epidermidis* isolates were methicillin-resistant in various geographical locations (12). Following an agar screen test, Shamansouri et al. (2016) reported that 68 out of 120 *S. epidermidis* isolates were methicillin-resistant, similar to our findings (16). Among the 100 *S. epidermidis* isolates of the present study, the highest percentage of specimens were found in urine (35%) and blood samples (25%). Shamansouri et al. (2016) reported the highest isolation percentage of specimens in urine (77.94%) but only 10.29% in the blood (16).

Additionally, Tahmasebi et al. (2016) found the highest percentage of isolates in the urine (49.49%). Together, these two studies results align with our observations (6). The increased prevalence of resistance to β-lactam in *S. epidermidis* should be noted. In the present study, according to phenotype, 93% and 85% of *S. epidermidis* showed resistance to Penicillin and Ceftriaxone, and among the 100 specimens, 80 (80%) contained *blaZ*, which is comparable to the outcome described by both Du et al. (England) and Nahaei et al. (Iran). They reported a varying range of resistance to β-lactams in *S. epidermidis*, which is in agreement with our observations (19, 20). Tahmasebi et al. (2016) reported the highest resistance to Penicillin (85%) and Ceftriaxone (42.42%) and the least resistance to Ciprofloxacin (30.81%) and Cephalexin (21.71%). They also found that 149 specimens out of 198 specimens (75.25%) contained *blaZ*. These results were similar to our observations (6). Raei et al. (2007) reported the highest resistance to Penicillin (98.1%), Methicillin (90.9%), Ceftriaxone (52.7%) and Ceftizoxime (49.09%), and the lowest to Cephazolin (30.9%), Cephalexin (23.6%). Furthermore, an iodometric test showed that all of the isolates were β-lactamase producers and, moreover, PCR analysis confirmed the presence of *blaZ* in all isolates. Although the antibiotic resistance pattern was similar to our findings, the results of *blaZ*, however, differed (21). The propagation of resistant isolates to various β-lactam antibiotics is on the rise. Therefore, the therapy of infections related to *S. epidermidis* infections requires a definite and accurate diagnosis. Ultimately, we can prevent the unnecessary use of prescribed antibiotics by developing rapid, and more accurate treatments.

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No conflict of interest was announced by the authors.

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