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Virulence factors and antibiotic resistance properties of the *Staphylococcus epidermidis* strains isolated from hospital infections in Ahvaz, Iran

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Abstract

Background: Resistant *Staphylococcus epidermidis* strains are considered to be one of the major causes of human clinical infections in hospitals. The present investigation was done to study the pattern of antibiotic resistance and the prevalence of virulence and antibiotic resistance genes amongst the *S. epidermidis* strains isolated from human hospital infections.

Methods: One hundred hospital infectious samples were collected and *S. epidermidis* strains were identified using culture and biochemical tests. Isolated strains were subjected to disk diffusion and PCR.

Results: Forty-six out of 100 hospital infectious samples (46%) were positive for *S. epidermidis*. *S. epidermidis* strains harbored the highest prevalence of resistance against penicillin (95.65%), tetracycline (91.30%), erythromycin (82.60%), cefazolin (78.26%), and trimethoprim-sulfamethoxazole (73.91%). All *S. epidermidis* strains had resistance against at least three different types of antibiotics, while the prevalence of resistance against more than seven types of antibiotics was 17.39%. *AacA-D* (69.56%), *tetK* (56.52%), *mecA* (45.65%), *msrA* (39.13%), and *tetM* (39.13%) were most commonly detected antibiotic resistance genes. The prevalence of *vatC* (4.34%), *ermA* (8.69%), *vatA* (8.69%), *vatB* (13.04%), *ermC* (13.04%), and *linA* (10.86%) were lower than other detected antibiotic resistance genes. *ClfA* (32.60%), *agrIII* (17.39%), and *etB* (13.04%) were the most commonly detected virulence factors.

Conclusions: The presence of virulent and multi-drug resistance *S. epidermidis* strains showed an important public health issue in hospitals.

Keywords: *Staphylococcus epidermidis*, Antibiotic resistance, Antibiotic resistance genes, Hospital infections, Iran

Background

Hospital infections are considered as a major issue all around the world. Bacteria have the highest impact on the occurrence of hospital infections [1–7]. *Staphylococcus* spp. are commensal bacteria of human skin and have been isolated from diverse clinical sources such as urinary tract infections (UTIs), respiratory tract infections (RTIs), wound infections (WIs), soft tissue infections, blood infections, and endocarditis [3, 8–10]. It has been suggested that *Staphylococcus epidermidis* (*S. epidermidis*) is one of the most important species of this group.

It is a Gram-positive, non-spore forming, nonmotile, facultative anaerobic, and catalase-positive and coagulase-negative bacterium responsible for different types of hospital and nosocomial infections. Indwelling medical devices are considered a major vector of *S. epidermidis* in hospitalized patients [11, 12]. *S. epidermidis* results in approximately 13% of prosthetic valve endocarditis infections, with a high rate of intracardiac abscess formation (38%) and mortality (24%) [13].

S. epidermidis strains usually resist against several types of antibiotic classes such as tetracyclines, aminoglycosides, cephalosporins, fluoroquinolones, penicillins, and macrolides [14–17]. Nowadays, resistant *S. epidermidis* has become a serious problem in hospitals [14–16]. Resistant

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staphylococcal strains are responsible for about 100,000 cases of infections with around 10% mortality rate each year in the USA [16]. The presence of certain antibiotic resistance genes is responsible for the occurrence of antibiotic resistance [14–17]. *MecA*, *aacA-D*, *tetK* and *tetM*, *ermA* and *ermC*, *msrA* and *msrB*, *linA* and *vatA*, and *vatB* and *vatC* antibiotic resistance genes are responsible for the occurrence of resistance against methicillin, aminoglycosides, tetracyclines, macrolide-lincosamide-streptogramin B, macrolides, lincosamides, and streptogramin A groups of antibiotics, respectively [3, 14–17] (Table 1).

Some potential virulence factors including toxic shock syndrome toxin-1 (TSST-1 encoded by *tst*), exfoliative toxins A and B (*eta* and *etb*), clumping factor (*clfA*), and types I, II, and III of the accessory gene regulator (*agr*) are responsible for virulence characters of the *S. epidermidis* strains isolated from human clinical infections [10]. The *X-region* gene of *Staphylococcus* strains has a high degree of importance in the occurrence of diseases, and it may have a variation rate (or clock speed) that provides suitable discrimination for outbreak investigation [10]. The *IgG-binding region* is responsible for causing host specificity and various immunological responses against *Staphylococcus* strains. The *X-region* and *IgG-binding region* have been detected in various types of staphylococcal infections [10].

Scarce researches have been conducted on epidemiological and molecular aspects of the *S. epidermidis* strains in hospital infections in Iran. Thus, the current research was done to study the prevalence rate, distribution of virulence factors, and antimicrobial resistance properties of *S. epidermidis* strains isolated from various types of human clinical infections collected from Ahvaz city, Iran.

Results

The present investigation was done to assess the antibiotic resistance properties and distribution of virulence genes amongst the *S. epidermidis* strains isolated from different types of hospital infectious samples. Forty-six out of 100 hospital infectious samples (46%) were positive for *S. epidermidis*.

Table 2 represents the antibiotic resistance pattern of the *S. epidermidis* strains isolated from hospital infectious samples. *S. epidermidis* strains harbored the highest prevalence of resistance against penicillin (95.65%), tetracycline (91.30%), erythromycin (82.60%), cefazolin (78.26%), and trimethoprim-sulfamethoxazole (73.91%) antibiotic agents. Reversely, *S. epidermidis* strains harbored the lowest prevalence of resistance against nitrofurantoin (34.78%) and mupirocin (50%) antibiotic agents. The prevalence of resistance against ciprofloxacin, clindamycin, azithromycin, and rifampin antibiotic agents were 69.56%, 65.21%, 60.86%, and 60.86%, respectively.

Figure 1 represents the prevalence of multi-drug resistant *S. epidermidis* strains isolated from hospital infectious samples. Multidrug-resistant *S. epidermidis* strains were determined as those who had at least simultaneous resistance against three or more than three types of antibiotics. All *S. epidermidis* strains had resistance against at least three different types of antibiotics, while the prevalence of resistance against more than seven types of antibiotics was 17.39%.

Table 3 represents the distribution of antibiotic resistance genes amongst the *S. epidermidis* strains isolated from hospital infectious samples. We found that *aacA-D* (69.56%), *tetK* (56.52%), *mecA* (45.65%), *msrA* (39.13%), and *tetM* (39.13%) were most commonly detected antibiotic resistance genes amongst the *S. epidermidis* strains. The prevalence of *vatC* (4.34%), *ermA* (8.69%), *vatA* (8.69%), *vatB* (13.04%), *ermC* (13.04%), and *linA* (10.86%) were lower than other detected antibiotic resistance genes. The prevalence of *msrA* and *msrB* antibiotic resistance genes were 36.13% and 26.08%, respectively.

tetK (56.52%), *mecA* (45.65%), *msrA* (39.13%), and *tetM* (39.13%) were most commonly detected antibiotic resistance genes. The prevalence of *vatC* (4.34%), *ermA* (8.69%), *vatA* (8.69%), *vatB* (13.04%), *ermC* (13.04%), and *linA* (10.86%) were lower.

Table 4 represents the distribution of virulence factors amongst the *S. epidermidis* strains isolated from hospital infectious samples. We found that *clfA* (32.60%), *agrIII*

Table 1 Antibiotic resistance pattern of the *S. epidermidis* strains isolated from different types of hospital infectious samples

Samples (no. positive samples for <i>S. epidermidis</i>)	Antibiotic resistance pattern (%)										
	P10*	Cef	Cip5	Clin	Az	Eryt	Mup	Rif	Tet30	Tri-Sul	N/F300
UTIs (10)	9 (90)	7 (70)	5 (50)	5 (50)	5 (50)	8 (80)	2 (20)	5 (50)	8 (80)	6 (60)	2 (20)
WIs (20)	19 (95)	16 (80)	15 (75)	13 (65)	12 (60)	16 (80)	11 (55)	12 (60)	19 (95)	15 (75)	8 (40)
RTIs (16)	16 (100)	13 (81.25)	12 (75)	12 (75)	11 (68.75)	14 (87.50)	10 (62.50)	11 (68.75)	15 (93.75)	13 (81.25)	6 (37.50)
Total (46)	44 (95.65)	36 (78.26)	32 (69.56)	30 (65.21)	28 (60.86)	38 (82.60)	23 (50)	28 (60.86)	42 (91.30)	34 (73.91)	16 (34.78)

*P10 penicillin (10 µg/disk), Cef cefazolin (30 µg/disk), Cip5 ciprofloxacin (5 µg/disk), Clin clindamycin (2 µg/disk), Az azithromycin (15 µg/disk), Eryt erythromycin (15 µg/disk), Mup mupirocin (30 µg/disk), Rif rifampin (5 µg/disk), Tet30 tetracycline (30 µg/disk), Tri-Sul trimethoprim-sulfamethoxazole (25 µg/disk), and N/F300 nitrofurantoin (300 µg/disk) antibiotic agents

Table 2 Distribution of antibiotic resistance genes amongst the *S. epidermidis* strains isolated from different types of hospital infectious samples

Samples (no. positive samples for <i>S. epidermidis</i>)	Antibiotic resistance genes (%)											
	<i>mecA</i>	<i>msrA</i>	<i>msrB</i>	<i>AacA-D</i>	<i>tetK</i>	<i>tetM</i>	<i>vatA</i>	<i>vatB</i>	<i>vatC</i>	<i>ermA</i>	<i>ermC</i>	<i>linA</i>
UTIs (10)	2 (20)	2 (20)	1 (10)	5 (50)	4 (40)	2 (20)	1 (10)	1 (10)	–	1 (10)	1 (10)	1 (10)
WIs (20)	10 (50)	9 (45)	7 (35)	16 (80)	12 (60)	8 (40)	2 (10)	3 (15)	1 (5)	2 (10)	3 (15)	3 (15)
RTIs (16)	9 (56.25)	7 (43.75)	4 (25)	11 (68.75)	10 (62.50)	8 (50)	1 (6.25)	2 (12.50)	1 (6.25)	1 (6.25)	2 (12.50)	1 (6.25)
Total (46)	21 (45.65)	18 (39.13)	12 (26.08)	32 (69.56)	26 (56.52)	18 (39.13)	4 (8.69)	6 (13.04)	2 (4.34)	4 (8.69)	6 (13.04)	5 (10.86)

(17.39%), and *etB* (13.04%) were the most commonly detected virulence factors amongst the *S. epidermidis* strains isolated from hospital infectious samples. Virulence factors for *coa*, *X-region*, and *IgG-binding region* were negative.

Discussion

S. epidermidis is a common commensal bacterium of the human skin and mucosa. While *S. epidermidis* has long been considered nonpathogenic, it is now recognized as a relevant opportunistic pathogen [11, 12, 14]. Most *S. epidermidis*-related not only are associated with intra-vascular devices (prosthetic heart valves, shunts, etc.) but also commonly occur in prosthetic joints, catheters, and large wounds. However, recently published data revealed the high prevalence of *S. epidermidis* in the cases of human clinical infections [11, 12, 14]. Additionally, nosocomial *S. epidermidis* isolates were characterized by their pronounced resistance against many commonly used antibiotics [11, 12, 14].

The present study was done to assess the antibiotic resistance pattern and distribution of antibiotic resistance and virulence genes amongst the *S. epidermidis* strains isolated

from different types of human clinical infections. Findings showed that 46% of human clinical infection samples were positive for *S. epidermidis* strains. Relatively high prevalence of *S. epidermidis* in the human clinical infections of the present study is may be due to the ubiquitous presence of the bacterium in the hospital environment, its ability to bio-film formation, and finally failure to observe sanitary and disinfection principles in hospitals in Ahwaz city, Iran. Because of the ubiquitous prevalence of *S. epidermidis* as a commensal bacterium, it is often difficult for a clinician to decide whether an isolate represents the causative agent of an infection or an unspecific culture contamination.

S. epidermidis strains harbored the highest prevalence of resistance against penicillin, tetracycline, erythromycin, ceftazolin, and trimethoprim-sulfamethoxazole antibiotic agents. All *S. epidermidis* strains had resistance against at least three different types of antibiotics. Unauthorized and illegal prescription of antibiotics is the main reason for the high prevalence of antibiotic resistance. Mohaghegh et al. [18] reported that the prevalence of antibiotic resistance of the *S. aureus* strains against ampicillin, amoxicillin-clavulanic acid, ceftazidime, nalidixic acid, and penicillin were

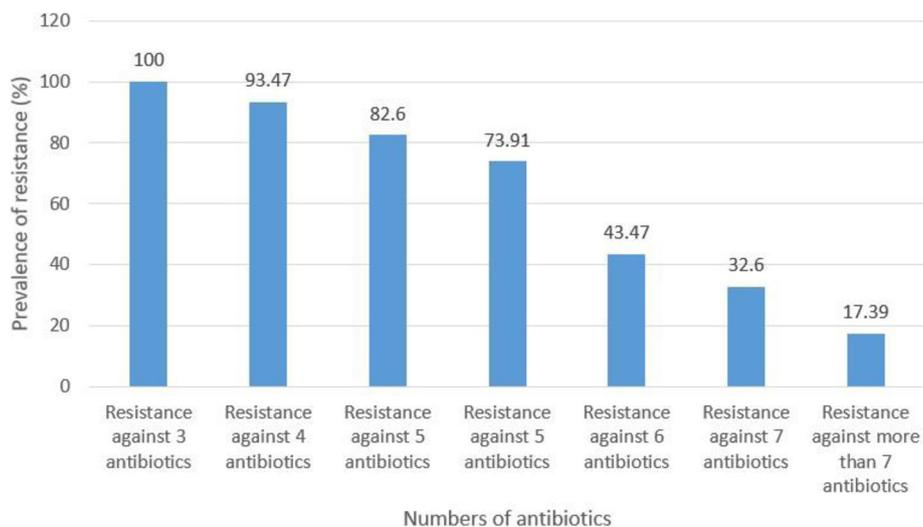


Fig. 1 Prevalence of multidrug-resistant *S. epidermidis* strains in hospital infectious samples

Table 3 Distribution of virulence factors amongst the *S. epidermidis* strains isolated from different types of hospital infectious samples

Samples (no. positive samples for <i>S. epidermidis</i>)	Virulence factors (%)									
	<i>coa</i>	<i>clfA</i>	<i>X-region</i>	<i>IgG-binding region</i>	<i>tsst-1</i>	<i>etA</i>	<i>etB</i>	<i>agrI</i>	<i>agrII</i>	<i>agrIII</i>
UTIs (10)	–	2 (20)	–	–	–	–	1 (10)	1 (10)	1 (10)	2 (20)
WIs (20)	–	8 (40)	–	–	1 (5)	2 (10)	3 (15)	2 (10)	2 (10)	4 (20)
RTIs (16)	–	5 (31.25)	–	–	1 (6.25)	1 (6.25)	2 (12.50)	1 (6.25)	2 (12.50)	2 (12.50)
Total (46)	–	15 (32.60)	–	–	2 (4.34)	3 (6.52)	6 (13.04)	4 (8.69)	5 (10.86)	8 (17.39)

85%, 80%, 100%, 98.30%, 90.90%, and 90%, respectively. Moreover, the prevalence of resistance against tetracycline (51%), erythromycin (41.20%), oxacillin (97.70%), cephalosporin (58.30), and norfloxacin (51.90%) was entirely high [18]. Eladli et al. [19] reported that the prevalence of antibiotic resistance of the *S. epidermidis* strains against amoxicillin-clavulanic acid, ciprofloxacin, clindamycin, erythromycin, gentamicin, levofloxacin, mupirocin, oxacillin, rifampin, tetracycline, and trimethoprim-sulfamethoxazole antibiotics was 100%, 100%, 37%, 0%, 33%, 16%, 0%, 80%, 0%, 80%, and 0%, respectively. Ma et al. [20] reported that the prevalence of antibiotic resistance of the coagulase-negative staphylococci strains against penicillin, oxacillin, erythromycin, tetracycline, clindamycin, ciprofloxacin, trimethoprim-sulfamethoxazole, chloramphenicol, ceftizoxime, gentamicin, rifampin, teicoplanin, and vancomycin antibiotics were 94.20%, 79.10%, 89.50%, 59.50%, 53.70%, 52.80%, 58.50%, 39.10%, 26.70%, 29.50%, 18.40%, 2.30, and 0%, respectively. High prevalence of multidrug-resistant *S. epidermidis* was also reported in their investigation. Similar patterns of antibiotic resistance of the *S. epidermidis* strains were reported from Mexico [21], Spain [22], Iran [23], USA [24], Belgium [25], and Ireland [26].

S. epidermidis strains isolated from the clinical infection samples harbored the high distribution of antibiotic resistance genes, especially *aacA-D*, *tetK*, *mecA*, and *tetM*. *S. epidermidis* strains had a considerable prevalence of resistance against clindamycin (65.21%). One of the most imperative mechanisms involving resistance against clindamycin is modulated by methylase enzyme which is often encoded by *ermA* and *ermC* genes [27]. The prevalence of *ermA* and *ermC* antibiotic resistance genes amongst the *S. epidermidis* strains of our research were 8.69% and 10.86%, respectively. The majority of isolates carried two tetracyclines, two erythromycins, one macrolide, and several streptogramin resistance determinants revealed a great diffusion of these types of resistance. The literature-based studies did not indicate any report on the prevalence of *vatA*, *vatB*, *vatC*, *msrA*, *ermA*, *ermC*, *linA*, *aacA-D*, *tetK*, and *tetM* antibiotic resistance genes amongst the *S. epidermidis* strains isolated from hospital infectious samples. Eksi [28] revealed

the higher prevalence of *ermA* than *ermC* antibiotic resistance genes amongst the clindamycin, erythromycin, and telithromycin-resistant and also higher prevalence of *tetM* than *tetK* antibiotic resistance genes amongst the tetracycline-resistant MRSA strains. Duran et al. [17] reported that the distribution of *mecA*, *femA*, *ermA*, *ermB*, *ermC*, *tetK*, *tetM*, *msrA*, and *blaZ* antibiotic resistance genes amongst the coagulase negative staphylococci strains isolated from human clinical infections were 29.60%, 7.50%, 33.10%, 5.80%, 21.60%, 13.70%, 28.80%, 9.40%, and 93.50%, respectively. Adwan et al. [29] reported that the prevalence of *ermA*, *ermC*, *tetK*, *tetM*, *aacA-aphD*, *vatA*, *vatB*, and *vatC* genes amongst the staphylococci strains isolated from human infections were 30.90%, 74.50%, 76.40%, 16.40%, 74.50%, 1.80%, 0%, and 5.50%, respectively. High prevalence of *tetK* and *tetM* antibiotic resistance genes in the *S. epidermidis* isolates can be clarified by their usual genetic locations. The presence of *tetK* gene on small multicopy plasmids and *tetM* on conjugative transposons contribute to the spread of these determinants [30]. Some of the *S. epidermidis* strains harbored *ermC* gene. This gene is often located on small multicopy plasmids which are present in many different staphylococcal species [30]. The *ermA* gene is usually carried by transposons which could explain its high prevalence amongst the *S. epidermidis* strains. Resistance to aminoglycosides which are encoded by the *aacA-D* gene (69.59%) is more prevalent. It is because this gene is usually more diffused in staphylococci of human origin [30].

Amongst all virulence markers found in the *S. epidermidis* strains, genes encoding clumping factor (*clfA*), *IgG-binding region*, toxic shock syndrome toxin (*tst*), exfoliative toxins (*eta* and *etb*), accessory gene regulator (*agr*), and *X-region* were recognized as the most important markers in occurrence of infectious diseases caused by *S. epidermidis* [10]. Eftekhari et al. [31] reported that the frequency of the *spa*, *fnbB*, *fnbA*, *clfB*, *clfA*, *can*, *bbp*, *ebp*, *etb*, *eta*, *pvl*, and *tst* virulence genes amongst the *S. aureus* strains isolated from hospitalized patients was 100%, 75.70%, 74.30%, 78.60%, 71.40%, 24.30%, 0%, 58.60%, 2.90%, 7.10%, 21.40%, and 51.40%, respectively. Additionally, amongst all the examined

Table 4 Target genes, oligonucleotide primers, and PCR conditions used for the detection of virulence factors and antibiotic resistance genes in the *S. epidermidis* strains isolated from various types of hospital infectious samples

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50 µL)
<i>vatA</i>	F: TGGTCCCGGAACAACATTTAT R: TCCACCGACAATAGAATAGGG	268	1 cycle: 94 °C, 5 min	5 µL PCR buffer 10X 1.5 mM MgCl ₂
<i>vatB</i>	F: GCTGCGAATTCAGTTGTTACA R: CTGACCAATCCCACCATTTTA	136	30 cycles: 94 °C, 60 s 60 °C, 60 s	200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.25 U Taq DNA polymerase (Fermentas)
<i>vatC</i>	F: AAGGCCCAATCCAGAAGAA R: TCAACGTTCTTTGTCACAACC	467	72 °C, 90 s 1 cycle: 72 °C, 7 min	2.5 µL DNA template
<i>mecA</i>	F: AAAATCGATGGTAAAGGTTGGC R: AGTTCTGCAGTACCGGATTTGC	532		
<i>tetK</i>	F: GTAGCGACAATAGGTAATAGT R: GTAGTGACAATAAACCTCCTA	360		
<i>tetM</i>	F: AGTGGAGCGATTACAGAA R: CATATGTCCTGGCGTGCTA	158	1 cycle: 94 °C, 5 min	5 µL PCR buffer 10X 2 mM MgCl ₂
<i>msrA</i>	F: GGCACAATAAGAGTGTAAAGG R: C AAGTTATATCATGAATAGATTGCCTGTT	940	30 cycles: 94 °C, 60 s 60 °C, 60 s	200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas)
<i>msrB</i>	F: TATGATATCCATAATAATTATCCAATC R: AAGTTATATCATGAATAGATTGCCTGTT	595	72 °C, 90 s 1 cycle: 72 °C, 7	5 µL DNA template
<i>aacA-D</i>	F: TAATCCAAGAGCAATAAGGGC R: GCCACACTATCATAACCACTA	227		
<i>ermA</i>	F: AAGCGGTAACCCCTCTGA R: TTCGCAAATCCCCTTCTCAAC	190		
<i>ermC</i>	F: AATCGTCAATTCCTGCATGT R: AATCGTCAATTCCTGCATGT	229		
<i>linA</i>	F: GGTGGCTGGGGGTAGATGTATTAACCTGG R: GCTTCTTTGAAATACATGGTATTTTCGA	323		
<i>tsst-1</i>	F: ATGGCAGCATCAGCTTGATA R: TTTCCAATAACCACCCGTTT	350	1 cycle: 94 °C, 6 min	5 µL PCR buffer 10X 2 mM MgCl ₂
<i>etA</i>	F: CTAGTGCATTTGTTATTCAA R: TGCATTGACACCATAGTACT	119	30 cycles: 94 °C, 2 min 55 °C, 2 min	200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas)
<i>etB</i>	F: ACGGCTATATACATTCAATT R: TCCATCGATAATATACCTAA	200	72 °C, 1 min 1 cycle: 72 °C, 8 min	5 µL DNA template
<i>agrI</i>	F: ATGCACATGGTGACATGC R: GTCACAAGTACTATAAGCTGCGAT	441	1 cycle: 94 °C, 6 min	5 µL PCR buffer 10X 2 mM MgCl ₂
<i>agrII</i>	F: ATGCACATGGTGACATGC R: TATTAATAATTGAAAAGTGCCATAGC	575	26 cycle: 94 °C, 30 s 55 °C, 30 s	200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas)
<i>agrIII</i>	F: ATGCACATGGTGACATGC R: GTAATGTAATAGCTTGTATAATAATACCCAG	323	72 °C, 1 min 1 cycle: 72 °C, 8 min	5 µL DNA template
<i>coa</i>	F: CGAGACCAAGATTCAACAAG R: AAAGAAAACCACTCACATCA	970	1 cycle: 95 °C, 2 min 30 cycles: 95 °C, 30 s 58 °C, 2 min 72 °C, 4 min 1 cycle: 72 °C, 7 min	5 µL PCR buffer 10X 2 mM MgCl ₂ 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 5 µL DNA template
<i>clfA</i>	F: GGCTTCAGTGCTTGTAGG R: TTTTCAGGGTCAATATAAGC	980	1 cycle: 94 °C, 4 min 35 cycles: 94 °C, 1 min 57 °C, 1 min 72 °C, 1 min 1 cycle: 72 °C, 5 min	5 µL PCR buffer 10X 2 mM MgCl ₂ 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 5 µL DNA template
<i>X-region</i>	F: CAAGCACCAAAGAGGAA R: CACCAGGTTAAACGCAT	320	1 cycle: 95 °C, 4 min	5 µL PCR buffer 10X 2 mM MgCl ₂

Table 4 Target genes, oligonucleotide primers, and PCR conditions used for the detection of virulence factors and antibiotic resistance genes in the *S. epidermidis* strains isolated from various types of hospital infectious samples (*Continued*)

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50 µL)
			25 cycles: 95 °C, 1 min 60 °C, 1 min 72 °C, 1 min 1 cycle: 72 °C, 3 min	200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 5 µL DNA template
IgG-binding region	F: CACCTGCTGCAAATGCTGCG R: GGCTTGTTGTGTCTTCCTC	920	1 cycle: 94 °C, 2 min 30 cycles: 94 °C, 1 min 58 °C, 1 min 72 °C, 1 min 1 cycle: 72 °C, 5 min	5 µL PCR buffer 10X 2 mM MgCl ₂ 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 5 µL DNA template

genes, *clfB* (78.60%) and *etb* (2.90%) had the highest and lowest prevalence, respectively. The prevalence of *tsst-1* gene amongst the *S. epidermidis* strains of our research was low (4.34%). Similar findings have also been reported from Iran (11.60%) [32], Sweden (22.00%) [33], Malaysia (0.50%) [34], and Colombia (10.00%) [35]. *Tsst-1* gene is a pyrogenic toxin that encodes a 21.9 KDa extracellular toxin causing toxic shock syndrome (TSS). It is known as a severe acute disease distinguished by symptoms such as fever, rash, hypotension, and dysfunction of multiorgan systems. In addition, TSS secretion into the human blood may raise the rate of neonatal TSS-like exanthematous disease, Kawasaki syndrome, and sudden infant death syndrome [32]. Regarding the other detected genes, the *eta* gene was presented in 6.52% of strains. The prevalence of *etb* gene was 13.04%. The incidence rate of the *eta* and *etb* in the present study was higher than that reported in other investigations conducted on Iran (0.68%) [32], Colombia (3.00%) [35], and Malaysia (0%) [34]. A higher prevalence of *eta* gene was reported in studies conducted in Czech (10.00%) [36] and Turkey (19.20%) [37]. It was detected that the prevalence of the *etb* gene differs amongst numerous investigations, ranging from 0% in Colombia [35] and Malaysia [34] to 9.20% in Turkey [37]. Ghasemian et al. [38] reported the high prevalence of the *clfA* and *clfB* genes (100%). The incidence of the *clfA* gene in the bacterial strains of our research was relatively high (32.60%). A higher prevalence of this gene was reported from Brazil [39] and China [40]. Another important detected gene amongst the *S. epidermidis* strains was *agr*. The prevalence of *agrI*, *agrII*, and *agrIII* virulence genes amongst the *S. epidermidis* strains were 8.69%, 10.86%, and 17.39%, respectively. *Agr* virulence gene was also predominant amongst the *S. epidermidis* strains isolated from clinical samples recovered from China [40], USA [41], and Iran [42]. The accessory gene regulator (*agr*) locus

influences the expression of many virulence genes in the *S. epidermidis*. Four allelic groups of *agr*, which generally inhibit the regulatory activity of each other, have been identified within the species. Interference in virulence gene expression caused by different *agr* groups has been suggested to be a mechanism for isolating bacterial populations and a fundamental basis for subdividing the species [43]. It encodes a two-component signal transduction system that leads to downregulation of surface proteins and upregulation of secreted proteins during in vitro growth. A role for *agr* in virulence has been demonstrated by the attenuated virulence of *agr* mutants in different animal infection models [43].

Conclusions

The present investigation is the first report of the phenotypic and genotypic analysis of antibiotic resistance in the *S. epidermidis* strains isolated from human hospital infectious samples in Iran. The total prevalence of *S. epidermidis* strains in hospital infectious samples was 46%. Considerable prevalence of resistance against penicillin, tetracycline, erythromycin, cefazolin, and trimethoprim-sulfamethoxazole and high distribution of *aacA-D*, *tetK*, *mecA*, and *tetM* antibiotic resistance genes may pose a potential public health threat. Additionally, *clfA*, *agrIII*, and *etB* were the most commonly detected virulence factors. A high prevalence of multi-drug resistant *S. epidermidis* in the human clinical infectious samples is another important finding of the present study. Moreover, the presence of antibiotic resistance genes and also virulence factors in some *S. epidermidis* strains should be considered as a serious health hazard. Further researches are required to understand additional epidemiological aspects such as the exact relations between antibiotic resistance genes and virulence factors of the *S. epidermidis* strains in hospital infectious samples.

Methods

Samples

From February to July 2018, a total of 100 various types of hospital infectious samples were randomly collected from several private hospitals of the Ahvaz city, Iran. Hospital infectious samples were defined as those which were collected from hospitalized patients with severe infections such as UTIs, WIs, and RIs. Furthermore, samples were taken from the site of infection. Samples were immediately transferred to the Clinical Microbiology Research Center of the Islamic Azad University of Shahrekord in a cooler with ice packs.

Bacterial isolation

S. epidermidis was identified by conventional bacteriological tests. The sample was enriched in a tryptic soy broth, and grown on mannitol salt agar, and then catalase, tube coagulase and urease tests, and carbohydrate fermentation were performed. *S. epidermidis* is catalase-positive, coagulase-negative, urease-positive, unable to ferment D-mannitol and D-trehalose, and able to ferment D-mannose and D-maltose [44, 45].

Antibiotic resistance pattern

Patterns of antimicrobial resistance of the *S. epidermidis* strains were studied using the Kirby-Bauer method. A simple disk diffusion technique on the Mueller-Hinton agar (Merck, Germany) medium was used for this purpose. susceptibility of *S. epidermidis* isolates was tested against several types of antibiotic agents including penicillin (10 µg/disk), cefazolin (30 µg/disk), clindamycin (2 µg/disk), mupirocin (30 µg/disk), azithromycin (15 µg/disk), erythromycin (15 µg/disk), tetracycline (30 µg/disk), ciprofloxacin (5 µg/disk), trimethoprim-sulfamethoxazole (25 µg/disk), nitrofurantoin (300 µg/disk), and rifampin (5 µg/disk) (Oxoid, UK). The instructions of the Clinical and Laboratory Standards Institute were used for this purpose [46]. The plates containing the disks were allowed to stand for at least 30 min before incubated at 37 °C for 24 h. The diameter of the zone of inhibition produced by each antibiotic disc was measured and interpreted using the CLSI zone diameter interpretative standards [46]. *S. epidermidis* ATCC 12228 was used as a quality control organism in antimicrobial susceptibility determination.

DNA extraction and amplification of virulence factors and antibiotic resistance genes

S. epidermidis isolates were sub-cultured on TSB media (Merck, Germany) and further incubated for 48 h at 37 °C. Genomic DNA was extracted from bacterial colonies using the DNA extraction kit (Fermentas, Germany) according to the manufacturer's instruction. Table 1 represents the list of primers and PCR conditions used for the amplification of virulence factors and antibiotic resistance genes [47]. A

programmable DNA thermo-cycler (Eppendorf Master-cycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) was used in all PCR reactions.

Statistical analysis

Statistical analysis was done using the SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA). Chi-square test and Fisher's exact two-tailed test were used to assess any significant relationship between the prevalence of *S. epidermidis* strains, virulence factors, and their antibiotic resistance properties. *P* value < 0.05 was considered as statistical significant level.

Abbreviation

CLSI: Clinical and Laboratory Standards Institute

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Authors' contributions

HM and RC carried out the molecular genetic studies, participated in the primers sequence alignment, and drafted the manuscript. HM and RC carried out the sampling and culture method, participated in the design of the study, and performed the statistical analysis and writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data analyzed during this study are included in this published article.

Ethics approval and consent to participate

The present study was approved by the Ethical Council of Research of the Faculty of Basic Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran. Verification of this research project and the licenses related to the sampling process were approved by Prof. Hassan Momtaz (approval ref. number MM 2017/10). Samples were collected from volunteer patients hospitalized in the hospitals. Written informed consents were taken from the participants and their personal information was kept confidential.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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