ISSN: 2588-4115; Infection Epidemiology and Microbiology. 2021;7(1):1-15 10.29252/iem.7.1.1

Characterization of Biofilm Producing *Staphylococcus epidermidis* Strains Isolated from Patients and Healthy People

ARTICLEINFO

Article Type
Original Research

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How to cite this article
Danesh M., Rahimi F. Characterization of Biofilm Producing *Staphylococcus epidermidis* Strains Isolated from Patients and Healthy People. Infection Epidemiology and Microbiology. 2021;7(1): 1-15

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Article History
Received: November 05 2020
Accepted: December 15 ,2021
Published: January 23 ,2021

ABSTRACT

Background: *Staphylococcus epidermidis* isolates are among the most important causes of nosocomial infections and could be classified as health threatening agents. This study aimed to determine the biofilm formation ability and clonal dissemination of *S. epidermidis* strains isolated from patients and healthy people in Isfahan during 2016 and 2017.

Materials & Methods: A total of 139 and 123 suspected colonies of *S. epidermidis* were collected from different clinical specimens and the arm of healthy people, respectively. The ability to form biofilm was determined using a combination of Congo-red agar (CRA) and microtiter plate (MTP) assays. The presence of genes involved in biofilm formation was also tested by the polymerase chain reaction (PCR) test. The susceptibility of all strains to 12 antibiotics was evaluated using the disk diffusion method according to the Clinical & Laboratory Standards Institute (CLSI) guidelines. Moreover, all biofilm-producing strains were typed using PhenePlate system as well as cassette chromosome *mec* (SCC*mec*) and accessory gene regulator (*agr*) locus typing method.

Findings: A total of 43 biofilm-producing *S. epidermidis* strains were identified among 107 and 123 confirmed strains isolated from hospitalized patients and healthy people, respectively; all of which were positive for *aap* gene, and the presence of *ica* operon was limited to 86 and 27% of the strains isolated from patients and healthy people, respectively. All the strains showed susceptibility to vancomycin, quinupristin-dalfopristin, and linezolid. Moreover, SCC*mec* Types III, IV, and V were detected among all methicillin-resistant *S. epidermidis* (MRSE) strains, and *agr* Type I was the most frequent one. Among all biofilm-positive strains, 3 common types (CTs) and 7 single types (STs) were determined; CT1 and CT2 were the most common types among the strains isolated from hospitalized patients and healthy people.

Conclusion: These findings indicated the presence and persistence of diverse clone types of biofilm-producing *S. epidermidis* strains with common types of PhP, *agr,* and SCC*mec* in the hospital and the community of Isfahan.

Keywords: *Staphylococcus epidermidis*, Biofilm, Congo red, Hospitals, Healthy people, Bacterial typing.

CITATION LINKS

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Introduction

Coagulase-negative staphylococci are widely dispersed on the human body and are known as the major commensal bacterial microbiota. Among coagulase-negative staphylococci (CoNs), *Staphylococcus epidermidis* is the most common opportunistic pathogenic species often isolated from human skin, it mainly colonizes the nose, head, and armpit and is also associated with bacteremia and nosocomial infections, especially in patients using catheter or other medical devices [1-2]. For many years, *S. epidermidis* isolates were considered as common contaminant and noninfectious agents in laboratories [3], but during the past two decades, *S. epidermidis* and other coagulase-negative staphylococci have been considered among the most important causes of nosocomial infections and classified as health threatening agents [4]. Most infections caused by *S. epidermidis* are persistent and recurrent [5]. Underlying diseases, immunodeficiency disorders (e.g. leukemia, or other malignant cancers), and the use of indwelling devices such as urinary catheters, mechanical heart valves, intravascular catheters, and orthopedic implants increase the risk of infection with *S. epidermidis* [6]. Unlike *S. aureus*, *S. epidermidis* strains lack virulence factors, and the main cause of their pathogenicity is the ability to form biofilm on the surface of indwelling medical devices [7].

Bacterial biofilm formation mechanism in *S. epidermidis* strains consists of primary attachment via microbial surface components recognizing adhesive matrix molecules (MSCRAMM); the production of extracellular polymers, proliferation, and formation of microcolonies, which result in biofilm maturation; and finally biofilm shedding which is necessary for maintenance of the biofilm life cycle [8].

Polysaccharide intercellular adhesin (PIA) plays an important role in cell-to-cell adhesion and is essential for the production of biofilm by *S. epidermidis* isolates. *lux*S quorum sensing system controls exopolysaccharide formation through the regulation of *ica* gene which controls the production of PIA [9]. PIA synthesis is accomplished by the product of the chromosomal complex genes of *ica* operon. The *ica* operon consists of *ica*A-D and *ica*R genes, the expression of which is essential for PIA synthesis [8]. Accessory gene regulator (*agr*) locus is a general staphylococcal regulator involved in the secretion of surface proteins in response to auto-inducing peptides (AIPs) [10]. During biofilm formation, this system controls the primary attachment and final detachment of *S. epidermidis* cells. Isolates could be classified into three *agr* groups based on the sequence differences. Isolates belonging to one *agr* group could activate the *agr* response in isolates belonging to the same group, but inhibit the *agr* response in members belonging to the other groups [11].

Objectives: The aim of this study was to determine the biofilm formation ability of *S. epidermidis* strains isolated from patients and healthy people in Isfahan, Iran. Moreover, the antibiotic resistance patterns and clonal dissemination of these strains were also investigated.

Materials and Methods

Isolation and identification of *S. epidermidis*: During November 2016 to July 2017, a total of 139 suspected *S. epidermidis* isolates were collected from clinical specimens (blood, urine, abscess, wound, CSF, sputum, ascites) of patients in a referral hospital in Isfahan, Iran. Moreover, 100 healthy people (50 men and 50 women) were selected, and sampling was carried out from the skin of arm using a sterile swab. Healthy people had no symptoms of any infection and were not hospitalized during the past six months. All collected

samples were cultured on mannitol salt agar (Scharlau, Barcelona, Spain) medium and incubated at 37ºC for 48 hrs. After incubation, the suspected red colonies were cultured on brain heart infusion (BHI) agar (Scharlau, Barcelona, Spain) and identified at the species level by the polymerase chain reaction (PCR) test using specific primers for *gse*A gene (encode a serine protease) as described previously [8].

Biofilm assay among *S. epidermidis* strains

Qualitative biofilm assay by Congo-red agar method: The qualitative Cong-red (CR) method was used to evaluate the ability of *S. epidermidis* isolates to form slime as described previously [8]. Briefly, bacteria were cultured on CR agar plates and incubated at 37 ˚C for 72 hrs. Bacterial colonies were classified as slime positive, suspected colonies, and slime negative strains based on their black, dark red, and red appearance, respectively [8]. *S. epidermidis* ATCC 35984 and *S. epidermidis* ATCC 12228 were used as positive and negative controls [12].

Quantitative biofilm assay by microtiter plate method: To quantitate the biofilm formation capacity of *S. epidermidis* strains, crystal violet microtiter plate (MTP) method was employed according to Stepanović et al. [13]. For this purpose, a culture of bacteria in trypticase soy broth (TSB) (Biolife, Bolzano, Italy) containing 25% sucrose was prepared, and finally, the OD_{570} nm was measured using ELISA reader (Stat Fax 2100). Bacteria were classified into four groups based on the OD values as strong ($4\times ODc \leq OD_s$), moderate ($2\times ODc \leq OD_s \leq 4\times ODc$), weak ($ODc \leq OD_s \leq 2\times ODc$), and non ($OD_s \leq ODc$) biofilm-producing strains.

Antimicrobial susceptibility testing: Antibiotic susceptibility of all *S. epidermidis* strains to 11 antibiotics was determined by the disk diffusion method according to the Clinical and Laboratory Standard Institute guidelines [14]. The antibiotics tested in this study included cefoxitin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), linezolid (30 µg), quinupristin-dalfopristin (15 µg), teicoplanin (30 µg), trimethoprim-sulfamethoxazole (23.5/1.25 µg), and tetracycline (30 µg) (Neo-Sensitabs, Rosco, Denmark). Moreover, the susceptibility of *S. epidermidis* strains to vancomycin was measured by broth micro dilution assay according to CLSI recommendations [14].

Detection of biofilm associated genes: In this study, DNA of all bacteria was extracted using the boiling method according to the protocol described previously for *S. aureus* strains [15]. The biofilm producing strains were selected, and the presence of *ica* operon as well as *ica*A, *ica*B, *ica*C, *ica*D, IS*256,* and *aap* genes among which was detected by the PCR test using the specific primers introduced previously [16-18].

Typing of biofilm producing strains: Biofilm producing strains isolated from both sources in this study were typed by the PhP-CS plates (PhPlate AB, Stockholm, Sweden) system according to the manufacturer's instructions as described previously [15, 19].

SCC*mec* typing: All cefoxitin-resistant strains were checked for the presence of *mec*A gene as published previously [19]. Moreover, all *mec*A positive strains were typed using multiplex-PCR assay by specific primers for SCC*mec* Types I-V [19].

***agr* typing:** To achieve the *agr* diversity among biofilm producing *S. epidermidis* strains, a multiplex-PCR assay using specific primers (*agr* Types I-III) was employed according to the previously published protocol [20].

Statistical analysis: GraphPad Prism software Version 5.0 (GraphPad Software) was employed for statistical analysis to evaluate data for univariate comparisons of

categorical results by Fisher's exact test. A *p* value ≤ .05 was considered as statistically significant.

Findings

Identification of bacteria: In this study, a total of 123 suspected red colonies isolated from healthy people were collected from mannitol salt agar medium. All the isolates were identified and confirmed as *S. epidermidis* strains using specific *gse*A primers. Moreover, out of the 139 suspected strains isolated from patients in the referral hospital, only 107 (77%) strains were confirmed as *S. epidermidis*, of which 75 (70%) strains were isolated from inpatients hospitalized for at least 72 hrs. No specific band was observed for 32 (23%) strains in PCR test.

Out of the 107 clinical strains, 56 (52%) and 51 (48%) strains were isolated from women and men, respectively. The distribution of *S. epidermidis* strains among females and males in different age groups is shown in Table 1. The findings revealed that the highest number of strains was isolated from patients in the age ranges of 61-70 and 31-40 years. On the other hand, 20% of *S. epidermidis* strains were isolated from men >70 years; also, among females, the highest frequencies of strains belonged to the patients less than1 year old and in the age range of 31-40 years. Moreover, no significant difference was observed among men and women in different age groups (Table 1). As shown in Table 2, 42% (n=45) and 35% (n=38) of strains were isolated from blood and urine cultures, respectively. The other strains (n=24, 22%) were isolated from wound, abscess, cerebrospinal fluid (CSF), peritoneal, sputum, and bronchus specimens. Moreover, 34% (n=36), 25% (n=27), and 14% (n=15) of the strains were also isolated from urology, emergency, and intensive care unit (ICU) wards, respectively.

Biofilm formation

Qualitative Congo-red agar assay: Among 123 *S. epidermidis* strains isolated from healthy people, eight (7%) strains had black colonies and were slime positive. Moreover, three (2%) strains showed bright red colonies and were slime negative. Also, 112 strains (91%) had dark red colonies and classified as suspected colonies. On the

Table 1) Distribution of *S. epidermidis* strains among males and females according to the age.

Age (year)	Females (%)	Males (%)	N (%)	P Value
<1	9 (16)	5 (9)	14 (13)	.1989
1-10	6 (11)	2 (4)	8 (7)	.1046
11-20	2 (4)	2 (4)	4 (4)	1.0000
21-30	6 (11)	3 (6)	9 (8)	.3106
31-40	9 (16)	7 (14)	16 (15)	.8433
41-50	5 (9)	7 (14)	12 (11)	.3757
51-60	7 (13)	7 (14)	14 (13)	1.0000
61-70	8 (14)	8 (16)	16 (15)	.8433
70>	4 (7)	10 (20)	14 (13)	.0119
Total	56 (52)	51 (48)	107	.6715

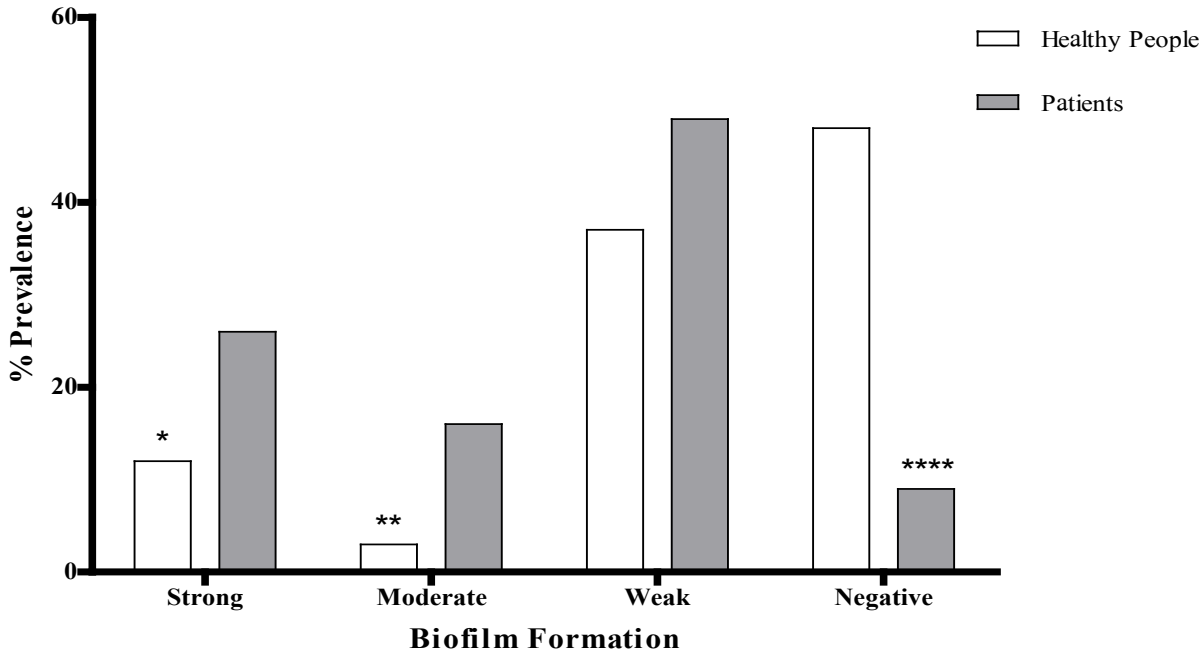


Fig 1) Frequency of biofilm producing *S. epidermidis* strains isolated from healthy people and patients by microtiter plate assay.
*: Significant at $p \leq .0183$, **: Significant at $p \leq .0029$, ****: Significant at $p < .0001$.

Table 2) Distribution of *S. epidermidis* strains isolated from different samples and different wards.

Sample	Urology	Emergency	ICU	NICU	Surgery	Gynecology	Internal	Infectious diseases	Respiratory	Dermatology	Gastroenterology	n (%)
Blood	15	17	6	2	3	1	1	-	-	-	-	45 (42)
Urine	21	6	4	4	-	2	1	-	-	-	-	38 (35)
Wounds	-	-	1	1	1	1	-	1	-	1	-	6 (6)
Abscess	-	1	1	1	1	1	1	-	-	-	-	6 (6)
CSF	-	1	1		1		-	-	-	-	-	3 (3)
Peritoneal specimen	-	1	1	1	-	-	-	-	-	-	1	4 (4)
Sputum and bronchus specimens	-	1	1	-	2	-	-	-	1	-	-	5 (4)
Total	36 (34%)	27 (25%)	15 (14%)	9 (8%)	8 (8%)	5 (5%)	3 (3%)	1 (1%)	1 (1%)	1 (1%)	1 (1%)	107

Abbreviations are: ICU, Intensive care unit; NICU, neonate intensive care unit; CSF, cerebrospinal fluid.

Table 3) The prevalence of genes involved in biofilm formation among clinical isolates and healthy people.

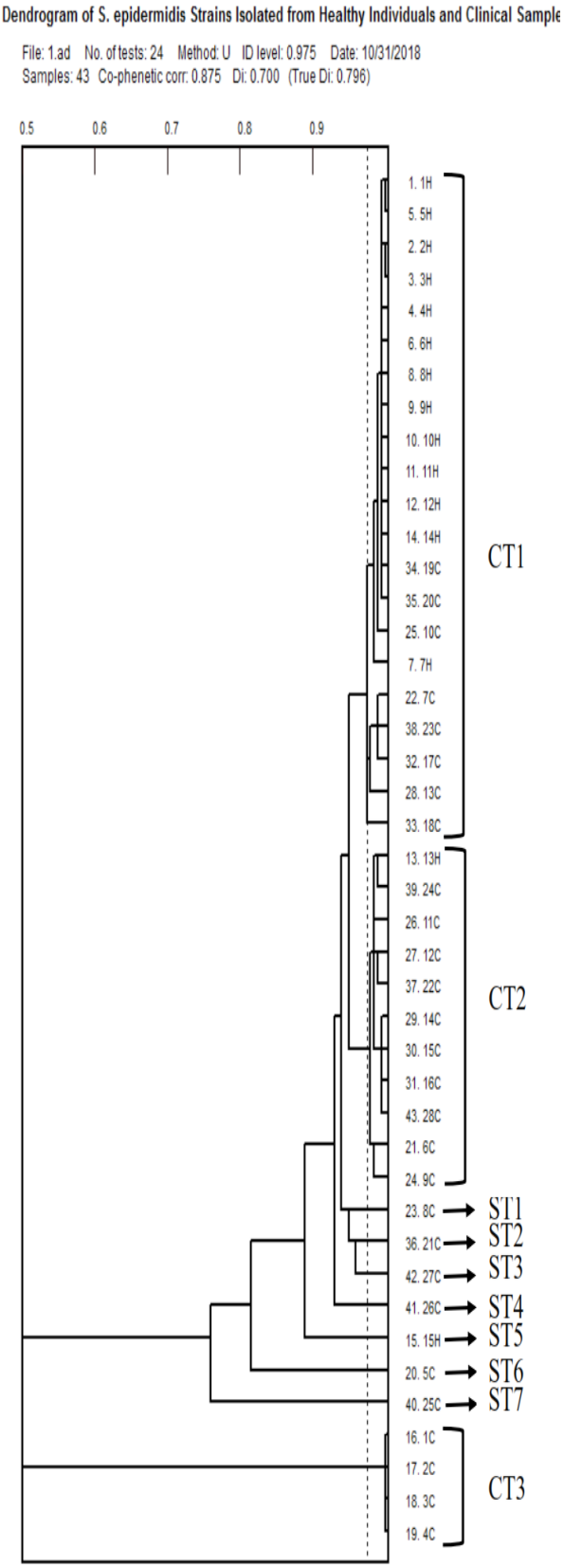
Biofilm-Associated Genes	Clinical Isolates (%)	Healthy Isolates (%)	P Value
icaA	19 (68)	4 (27)	< .0001
icaB	10 (36)	4 (27)	-
icaC	0	0	-
icaD	19 (68)	4 (27)	< .0001
IS256	2 (7)	0	.0140
Aap	28 (100)	15 (100)	-
ica operon	24 (86)	4 (27)	< .0001
agr type I	18 (64)	11 (74)	.1686
agr type II	7 (25)	2 (13)	.0464
agr type III	3 (11)	2 (13)	.8282

Table 4) Frequency of antibiotic resistance percentage of *S. epidermidis* strains isolated from healthy people and patients.

Antibiotics	All Strains		Biofilm Positive	
	Patients (%)	Healthy People (%)	Patients (%)	Healthy People (%)
E	92 (86)****	47 (38)	22 (79)	12 (80)
CIP	31 (29)**	14 (11)	17 (61)	9 (60)
CD	72 (67)****	20 (16)	14 (50)	10 (67)*
FOX	56 (52)****	28 (23)	25 (89)****	9 (60)
T	49 (46)****	22 (18)	15 (54)	7 (47)
TS	44 (41)	36 (29)	13 (46)	11 (73)***
GM	32 (30)****	9 (7)	19 (68)****	4 (27)
C	-	-	-	-
VAN	-	-	-	-
TE	-	-	-	-
SYN	-	-	-	-
LZD	-	-	-	-

Abbreviations are: E, erythromycin; CIP, ciprofloxacin; CD, clindamycin; FOX, cefoxitin; T, tetracycline; TS, trimethoprim-sulfamethoxazole; GM, gentamicin; C, chloramphenicol; VAN, vancomycin; TE, teicoplanin; SYN, quinupristin-dalfopristin; LZD, linezolid.
*: Significant at $p \leq .0214$, **: Significant at $p \leq .0024$, ***: Significant at $p \leq .0002$, ****: Significant at $p < .0001$.

Accessory File 1) Different PhP types among biofilm producing *S. epidermidis* strains isolated from clinical and healthy people samples. Abbreviations are H: healthy people, C: clinical samples, St: single type and CT: common type.



other hand, 23 (21%) strains isolated from the clinical samples were able to produce slime and black colonies, and also four (4%) strains were slime negative. Furthermore, 75% (n=80) of the strains produced dark red colonies.

Quantitative microtiter plate assay: The results of MTP assay revealed that 12% (n=15) and 26% (n=28) of the strains isolated from healthy people and patients were able to attach to the polystyrene plates and were biofilm positive, respectively (Fig. 1). On the other hand, the rate of non-biofilm producing strains isolated from healthy people was significantly higher than those isolated from patients ($p < .0001$). Among all 28 (26%) biofilm-positive strains isolated from patients, 14 (50%), 8 (29%), and 2 (7%) strains were isolated from blood, urine, and wound samples, respectively. Moreover, the biofilm-producing strains were isolated from patients in surgery (25%, n=7), neonate intensive care unit (NICU) (21%, n=6), emergency (21%, n=6), urology (14%, n=4), ICU (7%, n=2), internal (7%, n=2) and gynecology (4%, n=1) wards. Furthermore, in this study, 6 (21%) strains were isolated from outpatients, and 22 (79%) were from inpatients.

Detection of biofilm associated genes and *agr* typing: Among the 28 biofilm-producing *S. epidermidis* clinical strains, *aap* gene was the most prevalent gene detected in all the strains (Table 3). Moreover, *ica* operon was present in 86% of clinical strains, and the prevalence of *ica*A and *ica*D was 68% (19 strains). On the other hand, all of the 15 strains isolated from healthy people were positive for *aap* gene, but *ica*A, *ica*B, and *ica*D genes were present in 27% (n=4) of the strains. Furthermore, none of the biofilm-producing strains harbored the *ica*C gene, and the presence of IS*256* transposon gene was limited to clinical isolates.

The results of *agr* typing revealed the

Table 5) Prevalence of different PhP types among biofilm producing *S. epidermidis* strains isolated from healthy people and clinical samples.

PhP Types	Source	ica operon	icaA	icaD	aap	agr type	IS256	Cefoxitin resistant	SCCmec type
CT1	Healthy	+	+	+	+	I	-	+	III
CT1	Healthy	+	+	+	+	I	-	+	III
CT1	Healthy	-	-	-	+	I	-	-	-
CT1	Healthy	-	-	-	+	I	-	+	III
CT1	Healthy	-	-	-	+	I	-	+	III
CT1	Healthy	-	-	-	+	I	-	-	-
CT1	Healthy	-	-	-	+	II	-	-	-
CT1	Healthy	-	-	-	+	I	-	-	-
CT1	Healthy	-	-	-	+	II	-	-	-
CT1	Healthy	+	+	+	+	I	-	+	III
CT1	Healthy	-	-	-	+	I	-	+	III
CT1	Healthy	-	-	-	+	I	-	-	-
CT1	Healthy	-	-	-	+	I	-	+	III
CT1	Clinic	+	+	+	+	I	-	-	-
CT1	Clinic	+	+	+	+	I	-	+	III
CT1	Clinic	+	-	-	+	II	+	+	III
CT1	Clinic	+	+	+	+	I	-	+	III
CT1	Clinic	+	+	+	+	I	-	+	III
CT1	Clinic	+	+	+	+	I	-	+	III
CT1	Clinic	+	+	+	+	II	-	+	III
CT1	Clinic	+	-	-	+	I	-	+	III
CT2	Healthy	+	+	+	+	III	-	+	III
CT2	Clinic	-	-	-	+	II	-	-	-
CT2	Clinic	+	+	+	+	I	-	+	III
CT2	Clinic	+	+	+	+	I	-	+	III
CT2	Clinic	+	+	+	+	II	-	+	III
CT2	Clinic	+	+	+	+	I	-	+	III
CT2	Clinic	+	+	+	+	I	-	+	III
CT2	Clinic	+	+	+	+	I	-	+	III
CT2	Clinic	+	+	+	+	II	-	+	III
CT2	Clinic	+	-	-	+	I	-	+	III
CT2	Clinic	+	-	-	+	I	-	+	III
CT3	Clinic	-	-	-	+	I	-	-	-
CT3	Clinic	+	+	+	+	I	-	+	III
CT3	Clinic	+	+	+	+	I	-	+	III
CT3	Clinic	+	+	+	+	I	-	+	III
ST1	Healthy	-	-	-	+	III	-	+	IV
ST2	Clinic	-	-	-	+	III	-	+	IV
ST3	Clinic	-	-	-	+	I	+	+	IV
ST4	Clinic	+	+	+	+	III	-	+	IV
ST5	Clinic	+	+	+	+	III	-	+	IV
ST6	Clinic	+	+	+	+	II	-	+	IV
ST7	Clinic	+	-	-	+	II	-	+	V

Accessory File 2) Primers used in this study.

Primer	Sequence (5´ to 3´)	Size (bp)	Reference
*gse*A-F	ATGAAAAAGAGATTTTTATCT	504	[8]
*gse*A-R	GTTTGGTGACACTCTTAAG		
*ica*A-F	TCTCTTGCAGGAGCAATCAA	188	[14]
*ica*A-R	TCAGGCACTAACATCCAGCA		
*ica*B-F	ATGGCTTAAAGCACACGACGC	527	[15]
*ica*B-R	TATCGGCATCTGGTGTGACAG		
*ica*C-F	ATAAACTTGAATTAGTGTATT	990	[15]
*ica*C-R	ATATATAAAACTCTCTTAACA		
*ica*D-F	ATGGTCAAGCCCAGACAGAG	198	[14]
*ica*D-R	CGTGTTTTCAACATTTAATGCAA		
ica-F	CTCGAATTTGTTACATACTAG		[16]
ica-R	CCATAGCTTGAATAAGGGAC		
*IS*256-F	GCGCAGCTTTACGACACGTGTAGGC	409	[15]
*IS*256-R	CATGAAGCCGATAATTTCACGGTCGCC		
aap-F	ATACAACTGGTGCAGATGGTTG	399	[15]
aap-R	GTAGCCGTCCAAGTTTTACCAG		
SCC*mec* type I-F	GCTTTAAAGAGTGTCGTTACAGG	613	[17]
SCC*mec* type I-R	GTCTCTCATAGTATGACGTCC		
SCC*mec* type II-F	CGTTGAAGATGATGAAGCG	398	[17]
SCC*mec* type II-R	CGAAATCAATGGTTAATGGACC		
SCC*mec* type III-F	CCATATTGTGTACGATGCG	280	[17]
SCC*mec* type III-R	CCTTAGTTGTCGTAACAGATCG		
SCC*mec* type IVa-F	GCCTTATTCGAAGAAACCG	776	[17]
SCC*mec* type IVa-R	CTACTCTTCTGAAAAGCGTCG		
SCC*mec* type IVb-F	TCTGGAATTACTTCAGCTGC	493	[17]
SCC*mec* type IVb-R	AAACAATATTGCTCTCCCTC		
SCC*mec* type IVc-F	ACATATTTGTATTATCGGAGAGC	200	[17]
SCC*mec* type IVc-R	TTGGTATGAGGTATTGCTGG		
SCC*mec* type IVd-F	CTCAAAATACGGACCCCAATACA	881	[17]
SCC*mec* type IVd-R	TGCTCCAGTAATTGCTAAAG		
SCC*mec* type V-F	GAACATTGTTACTTAAATGAGCG	325	[17]
SCC*mec* type V-R	TGAAAGTTGTACCCTTGACACC		
agr type I-F	GGCATTAGTCGGATTAATTATTACG	438	[19]
agr type I-R	TGTAGGCCTGCAAACGG		
agr type II-F	TTTACCATTTGCAGCTATACAAGTG	575	[19]
agr type II-R	ATAACAATAATATAACCAAACTCAAAAGTACAG		
agr type III-F	GAAAGAGTGTATTCAATGGATGAGC	338	[19]
agr type III-R	TAAATATTATGTATTATATCTTCAGTATATAAAGAGATGA		

presence of 3 different *agr* types among biofilm-producing *S. epidermidis* strains, and *agr* Type I was the dominant type among the strains isolated from healthy and clinical samples (Table 3). On the other hand, *agr* Type II was also detected in 25% (n=7) and 13% (n=2) of the strains isolated from clinical samples and healthy people, respectively.

Antibiotic susceptibility testing: The results of antibiotic susceptibility test revealed that none of the strains in this study were resistant to vancomycin, linezolid, teicoplanin, quinupristin-dalfopristin, and chloramphenicol (Table 4). On the other hand, among the clinical strains, resistance to erythromycin, clindamycin, and cefoxitin was higher than in the other strains, and more than 50% of the strains showed resistance to these three antibiotics. Moreover, among *S. epidermidis* strains isolated from healthy people, resistance to all antibiotics was less than 50%, and the highest resistance was observed to erythromycin. The rate of resistance to antibiotics among the clinical strains was significantly higher than in the strains isolated from healthy people (Table 4). Among the biofilm-producing strains of both sources, resistance to erythromycin was higher compared to other antibiotics, and the rate of resistance to clindamycin and trimethoprim-sulfamethoxazole among the strains isolated from healthy people was significantly higher than in clinical strains (Table 4). Moreover, in this study, 89% (n=25) and 60% (n=9) of biofilm producing *S. epidermidis* strains isolated from patients and healthy people, showed resistance to cefoxitin and were classified as methicillin resistant *S. epidermidis* (MRSE), respectively. On the other hand, it was found that among all 56 MRSE strains, 20% (n=11) belonged to outpatients, and out of the 25 biofilm producing MRSE strains, 18 (72%) strains were isolated from hospitalized patients.

Typing of biofilm producing strains: Typing of 43 biofilm-positive *S. epidermidis* strains isolated from healthy people and clinical samples showed the presence of 10 diverse PhP types consisting of 3 common types (CTs) (n=36, 84%) and 7 single types (STs) (16%) (Table 5 and Accessory File 1); among which the strains isolated from healthy people belonged to 2 CTs and 1 ST, and clinical strains were more diverse and belonged to 3 CTs and 6 STs. In this study, CT1 and CT2 were the most common types among the isolates of both sources, and CT1 was the dominant CT type (49%, n=21 strains). On the other hand, CT3 (n=4, 9%) was only found among the clinical isolates.

SCC*mec* typing: All 34 cefoxitin-resistant *S. epidermidis* strains carried *mec*A gene, and the results of SCC*mec* typing of biofilm-producing MRSE strains revealed that out of 25 clinical strains, 19 (76%) strains were positive for SCC*mec* Type III and classified as hospital-acquired MRSE (HA-MRSE) strains (Table 5). Furthermore, 5 (20%) strains harbored SCC*mec* Type IV, and one strain (4%) also carried SCC*mec* Type V and was regarded as community acquired MRSE (CA-MRSE). On the other hand, among 9 MRSE strains isolated from healthy people, SCC*mec* Type III was prevalent in 8 (89%) strains, and the presence of SCC*mec* Type IV was limited to 1 (11%) strain.

Discussion

According to the results of MTP, as a quantitative gold standard method for the detection of biofilm formation, 28% of *S. epidermidis* clinical isolates were found to form biofilm, which is lower than those reported in some studies in Iran, France, Thailand, and Egypt [8, 21-27]. The present study results showed a higher biofilm formation capacity in clinical isolates (28%) in comparison to strains isolated from healthy people (12%). In a study in France, the

prevalence of *ica* gene among *S. epidermidis* strains isolated from clinical samples and healthy people was 5.6 and 11.4%, respectively [28]. Patient's conditions such as gender, age, and duration of hospitalization may affect the biofilm formation capacity of bacteria [29]. No significant difference was found in the frequency of *S. epidermidis* strains isolated from men and women (p=.6715). Moreover, the isolation rate of *S. epidermidis* strains was higher among older and younger men and women, respectively, which is almost consistent with the result of another study by Pinheiro et al. (2014) [30]. In general, these findings could be due to the weakened immune system in infants and the elderly patients, which are at higher risk of contamination with *S. epidermidis* in these age ranges. The type of biological specimen is another factor that affects the amount of biofilm production.

The qualitative Congo-red agar test may be influenced by medium composition and culture conditions, which results in the variation in the interpretation of the test results, the low efficiency of this test for screening biofilm-producing strains has been reported previously [31], In a study in Shiraz, Iran, 81.9% of *S. epidermidis* strains were positive for the presence of *ica*A/D genes [23]. Furthermore, in another study in southwest Iran, *ica*A and *ica*D genes were found in 40 and 19% of isolates. On the other hand, some studies have shown that "the ability of *S. epidermidis* to form biofilm is not always dependent on the presence of *ica* genes" [31].

In the present study, the binding ability of clinical strains carrying *ica* genes to polystyrene microtiter plate was more than that of the strains isolated from healthy people, as the prevalence of these genes in clinical specimens was more than in the strains isolated from healthy people. According to the results of this study, the role of *ica*A and *ica*D genes in biofilm formation among *S. epidermidis* strains is very important, and their presence and expression are necessary for biofilm formation in this bacterium. In line with the present study, in a study in China, it was shown that biofilm formation was significantly higher among clinical isolates carrying an *ica*A gene, which indicates the important role of *ica*A gene in biofilm production [32].

The rate of resistance to a variety of antibiotics was higher among clinical isolates than in the strains isolated from healthy people, which could be due to the high rate of hospitalization in different hospitals for the treatment of various bacterial infections. Furthermore, all of the *S. epidermidis* strains isolated from healthy people and patients were susceptible to chloramphenicol, vancomycin, teicoplanin, quinupristin-dalfopristin, and linezolid. The rate of resistance to vancomycin among *S. aureus* and *S. epidermidis* strains is very low in Iran, and linezolid and quinupristin-dalfopristin are new antibiotics used for the treatment of infections caused by vancomycin resistant isolates; thus, low resistance to these antibiotics was expected. The present study findings revealed that 89 and 60% of biofilm-positive strains isolated from patients and healthy people showed resistance to cefoxitin and were classified as MRSE, respectively. A lower rate of resistance to this antibiotic has been reported in southwest Iran [21].

In this study, the clonal dissemination of 43 biofilm producing *S. epidermidis* strains was investigated using a combination of high-resolution PhP, *agr*, and SCC*mec* typing method, of which only nine strains showed susceptibility to cefoxitin and were not typeable for SCC*mec*. The distribution of SCC*mec* types was only limited to Type I, III, and V, and SCC*mec* Type III was the dominant one among the clinical and healthy samples,

which indicated the hospital origin of these isolates only found in CTs, this finding is consistent with the finding of our previous study [8]. On the other hand, all CA-MRSE strains, which harbored SCC*mec* Type IV and V, were only classified as STs. In contrast to MRSE strains isolated from healthy people, the results showed the higher distribution of SCC*mec* Type IV in clinical samples, suggesting the community origin of such MRSE isolates in this hospital in Isfahan. These findings were not expected and could be due to the recent dissemination of these clone types from the community into the studied hospital. Several structural differences in SCC*mec* elements have identified in CoNs in different countries [33]. Consistent with the present study findings, Du et al. (2013) reported Type III as the dominant SCC*mec* type (97.5%) in hospitals in China [32]. In contrast to the present study results, a study in Iran showed the presence of SCC*mec* Types II-VI among clinical MRSE isolates in Isfahan hospitals with Type V as the dominant one [34]. Najar-Peerayeh et al. (2016) reported another type of SCC*mec* (VII) among clinical isolates of *S. epidermidis* isolated from patients in ICU in Tehran, Iran [35]. According to their study results, the difference in SCC*mec* types might be due to the differences in some factors such as geographical items, place of sampling, and source of samples [34].

All the 43 biofilm-producing *S. epidermidis* strains were typeable using *agr* typing method, and all 3 *agr* types were detected in this study, which is in contrast to other studies indicating that some strains were not typeable [11,20,36]. Moreover, *agr* Type I was the most frequent *agr* type among *S. epidermidis* strains isolated from hospitalized patients and healthy people, which is consistent with other studies results [11, 36-37].

The usefulness and capability of PhenePlate system for typing of staphylococci has been reported previously [15, 19, 38-40]. In this study, diverse PhP types consisting of 3 CTs and 7 STs were identified; CT1 and CT2 were the most common types among the strains isolated from patients and healthy people, indicating their common origin. Moreover, CT3 was only found among the strains isolated from clinical samples, suggesting the unique origin of this clone and its limited prevalence only in the hospital and not in the community. Among 7 CTs identified, 6 CTs belonged to the strains isolated from clinical samples, and only one strain isolated from healthy people was classified as ST. The presence of common PhP types among healthy and clinical samples suggests the dissemination of biofilm-positive *S. epidermidis* strains from hospitals to communities and the circulation of the same clones in the communities as well as in the hospital environments [15].

Conclusion

The results of this study showed the presence and persistence of diverse clone types of biofilm-producing *S. epidermidis* strains among patients and healthy people, harboring common PhP, *agr*, and SCC*mec* types; also, antibiotic resistance patterns of these strains were determined. Moreover, the high prevalence of antibiotic-resistant biofilm-producing *S. epidermidis* strains is an important public health problem that makes the treating management so difficult. Vancomycin, linezolid, and quinupristin-dalfopristin could be considered as the best choice for antibiotic treatment of antibiotic-resistant biofilm-producing MRSE strains in Isfahan, Iran.

Acknowledgment: Not applicable.

Ethical Permission: Not applicable.

Conflict of Interest: The authors declare that there is no conflict of interest with the organization that sponsored this research and publications arising from this research.

Authors' Contributions: Conceptualization: FR; Data curation and formal analysis: MD and FR; Investigation: MD and FR; Methodology and project administration: FR; Supervision: FR; Validation: FR; Writing of original draft: MD; Writing, reviewing, and editing: FR.

Fundings: The results presented in this paper were part of Maryam Danesh's MSc thesis supported by a research budget from University of Isfahan.

Consent to participate: Consent form was obtained from all healthy people .

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