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Research article

Biofilm synthesis and its relationship with genetic characteristics in clinical methicillin-resistant staphylococci

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Abstract: *Staphylococcus aureus* can cause a broad range of infections, including skin infections, pneumonia and bacteraemia. Coagulase-negative staphylococci (CNS), mainly *S. epidermidis*, have also emerged as important pathogens, especially in immunocompromised patients or those with prosthetic devices, such as intravascular catheters or biomaterials. Of great importance in the initiation of these infections is the ability of staphylococci to adhere to various surfaces, such as host tissues and prosthetic devices and to form biofilm. The staphylococcal adhesins are encoded by a number of genes such as *fnbA* (*S. aureus* fibronectin binding protein A), *sasG* (*S. aureus* surface protein G), *aap* (*S. epidermidis* accumulation associated protein), *bhp* (Bap homologue protein) and *fbe* (fibrinogen binding protein epidermidis). In this study, 106 methicillin-resistant *S. aureus* (MRSA), 145 methicillin-resistant *S. epidermidis* (MRSE) and 70 non-epidermidis methicillin-resistant CNS (MR-CNS; 58 *S. haemolyticus*, 10 *S. hominis* and two *S. lugdunensis*) were compared in terms of biofilm formation, antimicrobial resistance, clonal distribution and adhesin genes carriage. Isolates were classified into pulsotypes by PFGE and assigned to sequence types by MLST. In total, 121/321 isolates (37.7%) produced biofilm and 219 (68.2%) carried *ica* operon. The majority was multidrug resistant (94.7%) and carried one or more adhesin genes. MRSE and all other MR-CNS prevailed in biofilm formation ($P < 0.001$) and antimicrobial resistance ($P < 0.05$) as compared to MRSA. MRSE also prevailed in *ica* carriage compared to the other methicillin-resistant

staphylococci ($P \leq 0.007$) Among MRSE, isolates from bacteraemias prevailed in biofilm formation ($P = 0.031$), whereas, strains from prosthetic device-associated infections carried more frequently *aap* ($P = 0.003$). Even though PFGE showed genetic diversity among MRSE, MLST revealed three major clones (ST2, ST5, ST16). MRSA isolates were less diverse, with five PFGE types and, among them, one major PFGE type (C) consisting of 77/106 strains (72.6%). MLST identified five sequence types: ST5, ST30, ST80, ST225 and ST239. One major PFGE type (h) was identified in *S. haemolyticus*. A clonal relationship was found concerning *fnbA* carriage in MRSA, *ica* carriage in MRSE, and antimicrobial susceptibility in both groups reinforcing the aspect of clonal expansion in hospital settings.

Keywords: methicillin-resistant staphylococci; biofilm, adhesins; bacteraemia; device-associated infections; clones

1. Introduction

Bacterial infections caused by the genus *Staphylococcus* are of great importance for human health. Coagulase-positive staphylococci are mainly represented by *S. aureus*, a pathogen that can cause a broad range of infections, including skin infections, pneumonia and bacteraemia [1]. Coagulase-negative staphylococci (CNS), especially *S. epidermidis*, have emerged as a significant health problem in hospital settings during the past decades. CNS are part of the normal skin flora but can cause severe infections, especially in immunocompromised patients or those with prosthetic devices, such as intravascular catheters or biomaterials [2].

Staphylococci express resistance to many antimicrobials used for infection treatment, an increasing problem around the globe, especially among nosocomial pathogens [3]. The introduction of methicillin and other semi-synthetic penicillins such as oxacillin and penicillinase-resistant methicillin in 1959 represented a significant step in antistaphylococcal therapy. However, the first report on methicillin resistance was published shortly after, in 1961 [4]. Today, methicillin-resistant staphylococci represent a major health problem around the globe.

Of great importance in the initiation of staphylococcal infections is the ability of these bacteria to adhere to various surfaces, such as host tissues and prosthetic devices and, subsequently, to form biofilm, which is a microbial-derived sessile community with cells attached to a substratum, interface, or to each other [5]. Bacterial cells in biofilms are embedded in a matrix of extracellular polymeric substances they produced and exhibit an altered phenotype with respect to growth rate and gene transcription [5]. In staphylococci, biofilm formation is often mediated by the production of a polysaccharide intercellular adhesin (PIA), encoded by the *ica* operon [6].

The first step in biofilm formation is bacterial attachment to a surface. Initial attachment is promoted by adhesins grouped into a single family, named Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) [7]. Staphylococcal adhesins are encoded by a number of genes such as *fnbA* (fibronectin binding protein A) [8], *sasG* (*S. aureus* surface protein G) [9], *aap* (accumulation associated protein) [10], *fbe* (fibrinogen binding protein epidermidis) [11] and *bhp* (Bap homologue protein of *S. epidermidis*) [12]. *S. aureus* fibronectin-binding protein A (FnbA) possesses multiple regions capable of conferring adherence to both soluble

and immobilized forms of fibronectin. Thus, *S. aureus* is able to invade endothelial cells both in vivo and in vitro. FnbA also promotes bacterial attachment to fibrinogen and adherence and aggregation of activated platelets [8]. SasG has been identified as another adherence factor for nasal epithelium cells [13]. However, it does not exhibit adherence ability for major extracellular matrices such as fibronectin or fibrinogen, suggesting that this protein is a unique adhesin involved in the intercellular aggregation of *S. aureus*.

A highly homologous to SasG protein has also been identified in *S. epidermidis*. Aap is a 220 kDa protein which acts as a polysaccharide-independent mechanism of *S. epidermidis* biofilm accumulation and intercellular adhesion [10]. Fbe, another member of the MSCRAMM family found in *S. epidermidis*, is similar to the clumping factor of *S. aureus* [14]. Moreover, *bhp* in *S. epidermidis* encodes a cell-wall associated protein, similar to the biofilm-associated protein Bap of *S. aureus* [12]. This protein is implicated in biofilm formation, even in *ica*-negative staphylococci [15].

The aim of the present study was to investigate possible differences in biofilm forming ability, antimicrobial resistance patterns and genetic background of methicillin-resistant *S. aureus* and *S. epidermidis* isolated in a University Hospital in Greece. Clonal distribution and the frequency of *ica* and adhesin-encoding genes, as well as, their contribution to biofilm formation were also determined.

2. Materials and Methods

2.1. Patients and hospital

A total of 321 staphylococci from different patients hospitalized in a tertiary-care teaching hospital in Greece (University General Hospital of Patras, UGHP), during an one-year period (1st July 2010 till 30th June 2011) were selected to be further analyzed. One hundred and six *S. aureus* strains were recovered from skin and soft tissue infections (SSTIs), broncheal aspirations (BAs) nasal carriage (NC), and bloodstream infections (BSIs). One hundred and forty-five *S. epidermidis*, 58 *S. haemolyticus*, ten *S. hominis* and two *S. lugdunensis* were recovered from patients with BSIs defined by established criteria (clinical symptoms and two or more positive blood cultures within two days apart) [16] or prosthetic device-associated infections (PDAIs, patients with intravascular catheters, local signs of infection and $\geq$15 cfu in semi-quantitative catheter culture).

2.2. Phenotypic identification and antibiotic susceptibility testing

Staphylococci were identified to species level by the Vitek 2 Advanced Expert System (bioMerieux, Marcy l'Etoile, France) and by restriction fragment length polymorphism analysis of the amplified *tuf* gene [17]. Susceptibility to cefoxitin (FOX), erythromycin (E), clindamycin (CC), kanamycin (KAN), tobramycin (NN), gentamicin (GM), ciprofloxacin (CIP), fusidic acid (FA) and sulfamethoxazole/ trimethoprim (SXT) was tested by the disk diffusion method according to EUCAST guidelines [18]. MICs of oxacillin (OX), vancomycin (VA), teicoplanin (TEC), linezolid (LNZ) and daptomycin (DAP) were determined by E-test (bioMerieux). Isolates resistant to at least three different classes of antimicrobials were considered multidrug resistant. Biofilm formation was tested by the quantitative assay in microtiter plates using the reference *S. epidermidis* ATCC35984 (RP62A, biofilm-positive /*ica*-positive) and ATCC12228 (biofilm-negative/*ica*-negative) strains, as

positive and negative controls respectively [19]. Beta-lactamase production was tested by nitrocefin assay (Becton Dickinson, Franklin Lakes, New Jersey, USA).

2.3. Molecular analysis

Amplification of *mecA*, two genes of the *ica* operon (*icaA, icaD*) in all staphylococci and the adhesin-encoding genes *sasG* and *fnbA* in *S. aureus*, as well as, *aap*, *fbe* and *bhp in S. epidermidis*, was performed by PCR with specific primers as previously described [13,20,21,22,23,24,25,26]. PCR products were analyzed by electrophoresis into 1% agarose gels.

2.4. Clonal identification

Staphylococci were classified into pulsotypes by Pulsed-Field Gel Electrophoresis (PFGE) of chromosomal DNA after digestion with the restriction enzyme *SmaI* [27]. A dendrogram comparing molecular weights of DNA fragments was performed by FPQuest software version 4.5 (Bio-Rad Laboratories Inc). Patterns differing by less than 79% (corresponding to a difference of less than seven bands) were considered to belong to the same PFGE type [28]. Ninety selected strains of the main *S. aureus* and *S. epidermidis* PFGE types were characterized by Multilocus Sequence Typing (MLST) (http://mlst.net). Results were analyzed by the application of eBURST algorithm. Clonal complexes were defined by using the default setting, in which all STs within a clonal complex differed by no more than one allele from at least one other ST in the clonal complex.

2.5. Statistical analysis

Pearson's chi-square test and Fisher's exact test were used to evaluate differences in the frequencies of variables among tested strains, conducted by IBM SPSS Statistics version 20 (SPSS, Inc., Chicago, IL). Isolates were assorted according to species, origin, biofilm formation and clone distribution. Results were considered statistically significant at a P-value < 0.05.

3. Results

Studied isolates (321) comprised five species: *S. aureus* (106 strains), *S. epidermidis* (145), *S. haemolyticus* (58), *S. hominis* (10) and *S. lugdunensis* (2). The majority of *S. aureus* strains (75/106, 70.8%) derived from SSTIs (wounds and abscesses), eighteen (17%) from BSIs, five (4.7%) from NC and eight (7.5%) from BAs. *S. epidermidis* isolates were recovered from BSIs (70/145, 48.3%) and PDAIs (75/145, 51.7%). Twenty nine *S. haemolyticus*, five *S. hominis* and one *S. lugdunensis* were also recovered from BSIs, whereas, the remaining isolates derived from PDAIs.Twelve *S. aureus*, 82 *S. epidermidis*, 30 *S. haemolyticus*, five *S. hominis* and one *S. lugdunensis* were recovered from children.

All staphylococci were cefoxitin- and oxacillin-resistant carrying *mecA* gene. All isolates were susceptible to daptomycin (MICs 0.064–1 mg/L), teicoplanin and vancomycin (MICs 0.25–2 mg/L for *S. aureus* and 0.25–4 mg/L for CNS). Eight *S. epidermidis* were resistant to linezolid (MICs 16–256 mg/L). The majority of tested staphylococci (304/321, 94.7%) were multi-resistant. MRSE and the other MR-CNS expressed higher resistance rates to antimicrobials than MRSA and were more

frequently multi-resistant (Table 1). The non-epidermidis MR-CNS expressed higher resistance rates to kanamycin, gentamicin, ciprofloxacin and SXT as compared to MRSE. However, no significant difference in beta lactamase production was identified (Table 1).

Table 1. Comparison of antibiotic resistance characteristics and biofilm formation among methicillin-resistant *S.aureus* (MRSA), *S. epidermidis* (MRSE) and other CNS (MR-CNS) isolates.

	MRSA N=106 (%)	**MRSE** N=145 (%)	**MR-CNS** N=70 (%)	***P*[a]-value**	***P*[b]-value**	***P*[c]-value**
Beta lactamase	102 (96.2)	134 (92.4)	66 (94.3)	0.284	0.199	0.778
Multi-drug resistance	93 (87.7)	142 (97.9)	69 (98.6)	0.001	0.009	1.000
Clindamycin resistance	32 (30.2)	127 (87.6)	64 (91.4)	<0.001	<0.001	0.493
Erythromycin resistance	61 (57.5)	137 (94.5)	69 (98.6)	<0.001	<0.001	0.277
Kanamycin resistance	96 (90.6)	123 (84.8)	67 (95.7)	0.250	0.249	0.022
Tobramycin resistance	51 (48.1)	131 (90.3)	67 (95.7)	<0.001	<0.001	0.280
Gentamicin resistance	22 (20.8)	106 (73.1)	64 (91.4)	<0.001	<0.001	0.002
Ciprofloxacin resistance	17 (16)	98 (67.6)	65 (92.9)	<0.001	<0.001	<0.001
Fusidic acid resistance	76 (71.7)	133 (91.7)	66 (94.3)	<0.001	<0.001	0.590
Sulfamethoxazole/ trimethoprim resistance	24 (22.6)	108 (74.5)	64 (91.4)	<0.001	<0.001	0.003
Biofilm formation	19 (17.9)	71 (49)	31 (44.3)	<0.001	<0.001	0.562
ica in total	72 (67.9)	120 (82.8)	27 (38.6)	0.007	<0.001	<0.001
ica in biofilm (+)	13/19 (68.4)	65/71 (91.5)	12/31 (38.7)	0.017	0.079	<0.001
ica in biofilm (-)	59/87 (67.8)	55/74 (74.3)	15/39 (38.5)	0.389	0.003	<0.001

[a]Comparison between MRSA and MRSE,

[b]Comparison between MRSA and MR-CNS, [c]Comparison between MRSE and MR-CNS.

S. aureus isolates were less PFGE diverse, with five pulsotypes and, among them, one major PFGE type (C) consisting of 77/106 strains (72.6%). MLST data revealed five sequence types: ST5, ST30, ST80, ST225 and ST239. The main type, ST80, included the majority (97.4%) of PFGE type (C) isolates. Analysis with eBURST algorithm showed that identified STs belonged to three clonal groups (CC1 includes ST5, ST225 and ST239, whereas, CC2 and CC14 include ST30 and ST80, respectively). According to susceptibility results, type (C) strains were less resistant to clindamycin, erythromycin, gentamicin and sulfamethoxazole/trimethoprim as compared to the other pulsotypes.

Table 2. Characteristics of the main *S. aureus*, *S. haemolyticus* and the two major *S. epidermidis* PFGE types.

	S. aureus			*S. epidermidis*			*S. haemolyticus*		
PFGE types	**Type C** N=77 (%)	**Others** N=29 (%)	***P*-value**	**Type a** N=48 (%)	**Type b** N=34 (%)	***P*-value**	**Type h** N=44 (%)	**Others** N=14 (%)	***P*-value**
Beta lactamase	75 (97.4)	27 (93.1)	0.301	45 (93.8)	30 (88.2)	0.441	44 (100)	14 (100)	-
Clindamycin resistance	11 (14.3)	21 (72.4)	<0.001	46 (95.8)	26 (76.5)	0.014	42 (95.5)	12 (85.7)	0.243
Erythromycin resistance	22 (28.6)	23 (79.3)	<0.001	46 (95.8)	32 (94.1)	1.000	43 (97.7)	14 (100)	1.000
Kanamycin resistance	73 (94.8)	23 (79.3)	0.024	47 (97.9)	23 (67.6)	<0.001	43 (97.7)	14 (100)	1.000
Tobramycin resistance	38 (49.4)	13 (44.8)	0.828	47 (97.9)	28 (82.4)	0.018	43 (97.7)	13 (92.9)	0.428
Gentamicin resistance	6 (7.8)	16 (55.2)	<0.001	45 (93.8)	16 (47.1)	<0.001	42 (95.5)	13 (92.9)	1.000
Ciprofloxacin resistance	12 (15.6)	5 (17.2)	1.000	48 (100)	20 (58.8)	<0.001	43 (97.7)	12 (85.7)	0.142
Fusidic acid resistance	57 (74)	19 (65.5)	0.469	47 (97.9)	28 (82.4)	0.018	42 (95.5)	12 (85.7)	0.243
Sulfamethoxazole / trimethoprim resistance	5 (6.5)	19 (65.5)	<0.001	47 (97.9)	17 (50)	<0.001	43 (97.7)	11 (78.6)	0.040
Biofilm formation	15 (19.5)	4 (13.8)	0.582	22 (45.8)	16 (47.1)	1.000	20 (45.5)	8 (57.1)	0.545
ica	52 (67.5)	20 (69)	1.000	46 (95.8)	25 (73.5)	0.006	17 (38.6)	3 (21.4)	0.338
sasG	51 (66.2)	20 (69)	1.000	-	-	-	-	-	-
fnbA	68 (88.3)	19 (65.5)	0.010	-	-	-	-	-	-
aap	-	-	-	22 (45.8)	23 (67.6)	0.072	-	-	-
fbe	-	-	-	42 (87.5)	32 (94.1)	0.459	-	-	-

PFGE typing revealed a diverse MRSE population; one hundred and forty five *S. epidermidis* were grouped in 52 PFGE types including two main pulsotypes (type a included 48, whereas, type b 34 strains) that comprised 56.6% of the studied *S. epidermidis* population (82 out of 145 strains). Among the *S. epidermidis* strains, three major sequence types were identified: ST2, ST5 and ST16. The main PFGE pulsotype (a) was characterized as ST2, whereas, type (b) strains belonged to ST5 and ST16 (59.6% and 40.4%, respectively). Analysis with eBURST software showed that all three STs belonged to the same clonal complex (CC2), with ST2 being the primary group founder. Type (a) strains displayed a higher resistance rate to clindamycin, kanamycin, tobramycin, gentamicin, ciprofloxacin, fusidic acid and sulfamethoxazole/trimethoprim as compared to pulsotype (b) ($P < 0.05$, Table 2). Among the non-epidermidis MR-CNS a variety of PFGE types was characterized; 12 clones were identified in *S. haemolyticus*, eight in *S. hominis* and two in *S. lugdunensis*. One major PFGE type (h) prevailed in *S. haemolyticus* population, including 44/58 isolates. No difference in the antimicrobial resistance rates between type h and the other PFGE types was identified, except for sulfamethoxazole/ trimethoprim ($P = 0.040$, Table 2).

In total, 121 out of 321 staphylococcal isolates (37.7%) produced biofilm (19 *S. aureus*, 71 *S. epidermidis*, 28 *S. haemolyticus* and three *S. hominis*) whereas, 219 (68.2%) carried *ica* operon. MRSE prevailed in biofilm formation and *ica* carriage. In particular, 19/106 (17.9%) MRSA, 71/145 (49%) MRSE and 31/70 (44.3%) MR-CNS produced biofilm. The presence of *ica* operon was more frequent in MRSE ($P^a = 0.007$ and $P^c < 0.001$, Table 1). The majority of MRSA and MRSE carried at least one adhesin gene. In total, 96/106 (90.6%) MRSA carried *sasG*, *fnbA* or both genes and 131/145 (90.3%) MRSE carried at least one of the genes *aap*, *fbe*, or *bhp*. No difference in the adhesin gene carriage between MRSA and MRSE was identified, in biofilm-positive (P = 0.674) or biofilm-negative (P = 0.795) isolates. In MRSA, 56/72 (77.8%) *ica*-positive isolates carried *sasG*, whereas 66/120 (55%) *ica*-positive MRSE carried *aap*.

There is a statistically significant difference between biofilm producers and non-producers with regards to *ica* operon carriage, in favor of the biofilm-positive isolates, among the *S. epidermidis* population (91.5% vs 74.3%, P = 0.008, Table 3). *S. epidermidis* belonging to pulsotype (a) also showed a higher rate of *ica* operon carriage as compared to strains of type (b) (95.8% vs 73.5%, P = 0.006). Among MRSE isolates, *fbe* and *aap* were detected in the majority of strains tested (121/145, 83.4% and 79/145, 54.5%, respectively). Ten isolates carried *aap* but not *fbe* and only two produced biofilm. Three MRSE that were *aap*-positive and did not carry *ica* or *fbe* did not produce biofilm either. On the contrary, *bhp* was detected in only 38/145 (26.2%) MRSE. No significant difference between biofilm-positive and biofilm-negative isolates was found concerning the adhesin genes carriage (P > 0.05, Table 3). In MRSE, isolates from BSIs prevailed in biofilm formation (58.6% vs 40%, P = 0.031), whereas, strains from PDAIs carried more frequently *aap* (66.7% vs 41.4%, P = 0.003).

FnbA was the predominant adhesin among MRSA and specifically in type (C) (P = 0.010, Table 2), whereas, *sasG* was also detected in the majority of isolates. Nine MRSA carried *sasG* without *fnbA* and only one was biofilm-positive. One isolate was *sasG*-positive but did not carry *ica* or *fnbA* and it did not produce biofilm. No difference related to the origin of MRSA isolates was identified regarding biofilm formation, *ica* and adhesin genes carriage.

Table 3. Characteristics of MRSA and MRSE isolates in relation to biofilm formation.

	MRSA				MRSE		
	Biofilm (+)	**Biofilm (-)**	***P*-value**		**Biofilm (+)**	**Biofilm (-)**	***P*-value**
	N=19 (%)	N=87 (%)			N=71 (%)	N=74 (%)	
ica	13 (68.4)	59 (67.8)	1.000	*ica*	65 (91.5)	55 (74.3)	0.008
sasG	11 (57.9)	60 (69)	0.422	*aap*	40 (56.3)	39 (52.7)	0.739
fnbA	16 (84.2)	71 (81.6)	1.000	*fbe*	63 (88.7)	58 (78.4)	0.119
				bhp	14 (19.7)	24 (32.4)	0.092

4. Discussion

S. aureus is an important aetiological agent of human infections including skin and soft tissue infections, endocarditis, osteomyelitis and septic arthritis. It colonises the skin and mucosa of humans and several animal species, especially the anterior nares of the nose [29]. Coagulase-negative staphylococci, especially *S. epidermidis*, are also frequent part of the human flora, but can emerge as pathogens in patients with low immune response or foreign bodies, particularly prosthetic cardiac valves, cerebrospinal fluid shunts, intravascular catheters and orthopaedic implants [2]. In our collection of methicillin-resistant staphylococci, MRSA were isolated mainly from skin and soft tissue infections and bacteraemias, whereas, MR-CNS were recovered from BSIs and PDAIs.

The increasing resistance rate of staphylococci to antimicrobials has been frequently reported [3]. In our study, all isolates were methicillin-resistant, but a high prevalence of multidrug resistance was also identified. MRSE and MR-CNS were more frequently multi-resistant as compared to MRSA. MR-CNS isolates were also associated with higher resistance rates to all antimicrobials tested, in accordance with previously published data [3]. Among MRSE, resistance was associated with clone distribution. In particular, PFGE type (a) (ST2) expressed higher resistance rates to clindamycin, aminoglycosides, ciprofloxacin, fusidic acid and sulfamethoxazole/trimethoprim, as compared to type (b) (ST5 and ST16). In *S. aureus*, the main PFGE type C was less resistant as compared to other pulsotypes, with the exception of kanamycin. In spite of the high resistance level, all staphylococci were susceptible to vancomycin, teicoplanin and daptomycin. However, identification of vancomycin MICs of 2 mg/L in *S. aureus* and 4 mg/L in *S. epidermidis* often renders the use of this antibiotic inefficient.

S. aureus isolates showed a comparatively low level of genetic diversity, with one major PFGE type (C) consisting of 77/106 strains (72.6%). MLST analysis concluded that there was one major sequence type, ST80, which included the majority (97.4%) of PFGE type (C) isolates. In a 12-year survey of MRSA infections in six hospitals in Greece, ST80 predominated and infiltrated the hospital settings in the period 2001-2012, successfully replacing other clones [1]. Predominance of ST239 was also reported [1]. ST239 was also one of the five clones identified in our *S. aureus* collection.

Although polyclonality was observed among *S. epidermidis*, sequence types ST2, ST5 and ST16 predominated in this study. All three clones belong to the same clonal complex, CC2. ST2 has been identified as a major clone in previous epidemiologic studies [30]. A collection of *S. epidermidis*

isolates from various sources including blood cultures and catheter tips from patients in Germany was analyzed by MLST by Mertens *et al* and ST2 was found to be the predominant one [31]. One major PFGE type (h) prevailed in the *S. haemolyticus* population, including 44/58 isolates. This clone was associated with high resistance rate to sulfamethoxazole/ trimethoprim. PFGE type h has also been identified in previous epidemiologic studies in Greece [24].

A major factor in the pathogenesis of staphylococcal infections is biofilm formation [5]. Both *S. aureus* and *S. epidermidis* display a strong capacity to form biofilms [32]. Biofilm development involves the initial attachment, accumulation/maturation and detachment. The prevalent mechanism of *Staphylococcus* biofilm accumulation is linked to the synthesis of PIA, encoded by the *ica* operon [6]. In our study, biofilm formation was directly associated with *ica* operon carriage in MRSE, since biofilm-positive isolates carried *ica* operon in a statistically higher percentage (91.5%) as compared to biofilm-negative MRSE (74.3%, $P = 0.008$).

To become a pathogen, staphylococci have to gain access to the human host usually by adhering to biotic surfaces, such as components of the extracellular matrix or host tissue, or to abiotic surfaces, such as medical devices. Upon adherence, bacteria colonize and proliferate on the respective biotic or abiotic surface by forming a biofilm [33]. Primary attachment to a biotic surface in host tissues and synthetic surfaces coated with plasma proteins, such as fibronectin, fibrinogen and vitronectin is mediated by adhesins like *S. aureus*' fibronectin-binding protein A (FnbA) and *S. epidermidis*' fibrinogen-binding protein Fbe. The Bhp protein promotes the bacterial primary attachment to abiotic surfaces, as well as intercellular adhesion during biofilm formation [34]. In our collection of staphylococci, *fnbA* and *fbe* were identified in the majority of isolates, whereas, *sasG*, *aap* and *bhp* were detected to a lesser extent. No relation between the origin of the isolate and gene carriage was found. However, *fnbA* carriage was related with clonal distribution, as it was mainly found in the major PFGE type C.

S. aureus colonizes the moist squamous epithelium of the anterior nares. One of the adhesins likely to be responsible is its surface protein G (SasG), which has sequence similarity with the protein Aap (accumulation associated protein) of *S. epidermidis* [35]. Aap can promote either the primary attachment or accumulation phase of biofilm formation. Most isolates in our study carried *sasG* or *aap*. In MRSE, *aap* was more prevalent in strains from PDAIs. Patel *et al* used an in vitro model to demonstrate that enhanced expression of *aap* and *ica* genes plays an important role in initial foreign body colonization and potentially in the establishment of a device-associated *S. epidermidis* infection [36].

Even though no clonal relationship was found concerning biofilm formation, a statistically significant difference for *ica* and *fnbA* gene carriage in favour of specific clones was identified. The main *S. aureus* clone ST80 was significantly related with *fnbA* carriage.

5. Conclusion

Certain *S. aureus*, *S. epidermidis* and *S. haemolyticus* clones predominate in patients with various staphylococcal infections. The majority of methicillin-resistant isolates in our study was multidrug resistant and carried *ica* and adhesin-encoding genes, whereas, biofilm formation was mainly identified in *S. epidermidis* and non-epidermidis CNS isolates. Specific staphylococcal phenotypic and genotypic characteristics combined with successful clonal expansion render these bacteria important pathogens in hospital settings.

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Conflict of Interest

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