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ORIGINAL ARTICLE

Molecular Detection of Virulence Associated Genes in Coagulase Negative Staphylococci Isolated from Blood Culture

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SUMMARY

Background: **Coagulase-negative staphylococci (CoNS) are one of the most important causes of infections. Unlike *Staphylococcus aureus*, less is known about their pathogenic mechanisms. In the present study, we aimed to evaluate the presence of virulence genes among 98 CoNS isolated from blood cultures of inpatients.**
Methods: **The isolates were identified by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). PCR was performed to detect 29 virulence factors using specific primers for *icaA, icaB, icaC, icaD, icaADB, aap, fbe, aae, sesI, atIE, hla, hlb, hld, gehC, gehD, sea, seb, sec, sed, see, seg, seh, sei, tst, eta, etb, etd, etx*, and *pvl* genes. The VITEK2 system (bio-Merieux, France) and the BD Phoenix™ System (Becton Dickinson, USA) were used for antimicrobial susceptibility testing.**
Results: ***Staphylococcus epidermidis*** **was found to be the most virulent CoNS species. All isolates were negative for *eta, etb, etd, sea, seb, sed, see, seg, sei,* and *pvl* virulence genes. We detected up to 15 virulence genes in a single isolate. The most common gene was *icaC* (73.5%), followed by *icaA* (57.1%), *icaD* (56.1%), *aap* (55.1%), *aae* (52.0%), *sesl* (51.0%), *gehC* (50.0%), *hld* (50.0%), *hlb* (49.0%), *fbe* (44.9%), *atIE* (37.8%), *icaADB* (37.8%), *gehD* (34.7%), *icaB* (31.6%), *hla* (30.6%), *etx* (2.0%), *sec* (1.0%), *seh* (1.0%), and *tst* (1.0%).**
Conclusions: **We determined high rates of genes encoding biofilm formation. Only four isolates did not possess either the *ica* operon or *aap* gene. Although we found low rates of toxin-related genes, our data indicates that apart from biofilm formation, the CoNS isolates could express various virulence genes similar to those of *Staphylococcus aureus*.**
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INTRODUCTION

Coagulase-negative staphylococci (CoNS) form a heterogeneous group of Gram-positive bacteria found in the environment and are members of the indigenous skin microbiota and mucous membranes of humans. Historically, CoNS were thought to be contaminant or less pathogenic than *Staphylococcus aureus* (*S. aureus*)

until the 1970s [1,2]. However, an increasing number of nosocomial infections due to CoNS has led researchers to reevaluate this bacterial group. *S. epidermidis*, *S. saprophyticus* subsp. *saprophyticus*, *S. haemolyticus*, and *S. lugdunensis* are the most significant species that cause various nosocomial infections, such as device-associated infections (DAIs), bloodstream infections (BSIs), prosthetic valve endocarditis, urinary tract infections (UTIs), and surgical site infections. *S. saprophyticus* is associated mainly with UTIs [1,3,4].

Coagulase negative staphylococci are the most problematic isolates of blood and circulatory system infections. They often reflect true bacteremia in patients with clinical signs of infection. Combinations of various criteria such as clinical data of patients, time interval of positive blood cultures, number of positive blood cultures, identification of the isolate to the level of species, time to initial positivity, antimicrobial susceptibility pattern of isolates are used for determining the significance of CoNS. Several approaches have been applied to decide whether an isolate of CoNS represents contamination or infection. Some of these require at least two blood cultures positive for CoNS. [5,6]. Unfortunately, none of them can fully solve this problem.

S. epidermidis is the most studied species of CoNS. S. *epidermidis* has 11 surface proteins (Ses) that stimulate bacterial adherence to the host or host tissue coated materials and promote escape from the host immune response. The role of some Ses proteins, such as Aap (SesF), SdrF, Bap/Bhp, and SdrG (Fbe), in biofilm formation has been well established [7-9]. Slime or biofilm formation is by far the main pathogenic and the most studied virulence factor of CoNS. Biofilm formation is a complex, multifactorial interaction among host, artificial device, and bacteria [10-12]. The process of biofilm formation can be divided into four steps - attachment, accumulation, maturation, dispersion, and detachment - and each step is regulated by several specific genetic mechanisms [13,14]. The autolysin/adhesion AtlE (autolysin of *S. epidermidis*; 148 kDa protein) and the Bhp protein (also referred to as Bap protein) manage the initial adhesion. In addition to AtIE, adherence to biotic surfaces requires specific interactions and additional bacterial virulence factors, such as a group of microbial proteins named microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). SdrF, SdrG (also known as Fbe), and Embp (extracellular matrix-binding protein) are considered MSCRAMMs, and these proteins can specifically bind to collagen, fibrinogen, and fibronectin [9,13,15,16]. In the second and third phases of biofilm formation, the production of polysaccharide molecules, such as polysaccharide intercellular adhesin (PIA) and polyglutamate, occurs, and bacteria accumulate in the multilayered biofilm construction. PIA, encoded by the *ica*ADBC gene locus, is the crucial component of staphylococcal biofilm related infections [4,7,13,17]. Lastly, extracellular enzymes (hydrolases, nucleases, proteases) and production of phenol-soluble modulin (PSM) peptides facilitate the dispersal and detachment of the biofilm by cytolytic activities [3,7,12].

CoNS possess various enzymes, such as lipases, proteases, esterases, and phospholipases, as well as hemolysins and other toxins, which assist in their survival and invasion of the host and in avoidance of host immune response. CoNS may also produce pyrogenic toxin superantigen, containing enterotoxins (SEA through SEM), exfoliative toxins (ETA, ETB, ETC, ETD), and toxic shock syndrome toxin 1 (TSST-1) [18-20]. However, the expression of such toxins by CoNS is rare. During the last few decades, CoNS have emerged as a major pathogen, especially in nosocomial infections. Compared to *S. aureus*, less is known about the pathogenic mechanisms in CoNS, except biofilm formation in *S. epidermidis*. Recent studies indicate that the pathogenicity of CoNS can be attributed to a variety of virulence factors as established in *S. aureus* [3,11]. Thus, the objective of the current study was to evaluate the presence of various virulence-related genes encoding biofilm formation (*icaA, icaB, icaC, icaADB, atlE, aap, sdrG/fbe, aae*), enterotoxins (*sea* through *sei*), toxic shock syndrome toxin-1 (*tsst-1*), haemolysins (*hla*, *hlb*, *hld*), lipases (*gehC, gehD*), for toxins (*eta*, *etb*, *etx*, *etd*), Panton-Valentine leukocidin (*pvl*), and *sesI* in 98 CoNS strains isolated from blood cultures of inpatients.

MATERIALS AND METHODS

Bacterial strains and identification

A total of 98 CoNS isolated from blood cultures of inpatients in intensive care units (ICUs) and various clinics at two teaching hospitals during a two-month period in 2018 were studied. Of these, 50 (51%) isolates were from Gulhane Training and Research Hospital in Ankara, and 48 (49%) were from Izmir Katip Celebi University Ataturk Education and Research Hospital in Izmir. Duplicate isolates were excluded from the study, and only one isolate per patient infection episode was included. CoNS isolates that were positive for at least two blood cultures were evaluated in the present study. Isolates grown on sheep blood agar were identified by MALDI-TOF MS (Bruker, Germany). The strains were stored in brain-heart infusion broth supplemented with 20% glycerol. All isolates were stored at -20°C until further analysis.

Antimicrobial susceptibilities

The VITEK2 system (bio-Merieux, France) and the BD Phoenix™ 100 System (Becton Dickinson, USA) were used for antimicrobial susceptibility testing (AST) of the isolates according to the European Committee of Antimicrobial Susceptibility Testing (EUCAST) criteria [21]. The isolates were tested for cefoxitin, ciprofloxacin, levofloxacin, gentamicin, clindamycin, daptomycin, fusidic acid, fosfomycin, trimethoprim/sulfamethoxazole, tetracycline, tigecycline, vancomycin, and linezolide in both laboratories. The minimum inhibitory con-

centration (MIC) values of cefoxitin were used as a surrogate marker for methicillin resistance.

DNA extraction

For all isolates, a single colony was inoculated into tryptic soy broth, and cultures were incubated aerobically at 37°C for 18 - 24 hours. After incubation, 1 mL suspension was centrifuged at 12,000 g for 5 minutes. Genomic DNA was extracted from the pellet by using DNA4PCR extraction kit (R Tech, Turkey) according to the manufacturer's recommendations.

Detection of virulence-associated genes by PCR

The presence of 29 virulence genes related to the pathogenesis of CoNS was investigated by PCR. The presence of biofilm-associated genes (*icaA, icaB, icaC, icaD, icaADB, aap, fbe*), genes encoding adhesins and adhesin-related proteins (*aae, sesI, atIE*), hemolysins (*hla, hlb, hld*), lipase (*gehC, gehD*), toxins such as enterotoxin (*sea, seb, sec, sed, see, seg, seh, sei*), *tsst*, exfoliative toxins (*eta, etb, etd, etx*), and *pvl* were screened. The pairs of specific oligonucleotide primers used for detection of each virulence gene are listed in the Supplemental Table 1 (available as supplementary data). PCR assays were performed in 50 µL mixtures containing 1 µL of dNTPs (10 mM), 0.4 µL of each primer (100 pmol), 5 µL Taq buffer with Mg^{++} (10X), 0.6 µL Taq DNA polymerase (5 U/µL) (ABM, Canada), and template DNA. Each gene was amplified separately. The virulence genes were amplified using the following protocol: temperature held at 94°C for 5 minutes, followed by 35 cycles of denaturation (94°C for 30 seconds), annealing (50 - 60°C for 30 seconds), and extension (72°C for 1 minute), with a single final extension of 7 minutes at 72°C. Using a 1% agarose gel, run for 30 minutes at 100 V and stained with Safe View (ABM, Canada), PCR products were visualized by electrophoresis and identified on the basis of fragment size, as shown in the Supplemental Table 1.

Statistical Analysis

The chi-squared test and Fisher's exact test were used to compare the prevalence of virulence-associated genes among study isolates by using SPSS statistical software (version 16, SPSS for Windows; SPSS Inc Chicago, IL, USA). A p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 98 CNS blood isolates from two clinical centers were studied. *S. epidermidis* was the most frequently isolated CoNS species (n = 39; 39.8%), followed by *S. hominis* (n = 23; 23.5%), *S. haemolyticus* (n = 17; 17.3%), *S. capitis* (n = 11; 11.2%), and other species (*S. warneri* (1), *S. simulans* (1), *S. intermedius* (1), *S. cohnii spp. urealyticum* (2), *S. xylosus* (1), *S. sciuri* (1), *S. vitulinus* (1), n = 8; 8%). The distribution of the isolates according to the centers is shown in Table 1.

Isolates were obtained from blood cultures of patients from ICUs (anesthesia, cardiology, internal medicine, pediatrics), internal medicine units (IMUs), and surgical medicine units (SMUs). Of the 98 isolates, 58 (59.2%), 34 (34.7%), and 6 (6.1%) were from patients in ICUs, IMUs, and SMUs, respectively. Except for *ses*I, no statistically significant difference was found among isolates from the clinics. *sesI* was positive in 35 (60.3%) of 58 ICU isolates and was detected in 15 (37.5%) isolates of IMUs and SMUs (p = 0.039).

Nearly similar results were obtained for the various CoNS among the centers in the study. *icaC* (80%) and *icaD* (68%) were the most detected genes among the 50 CoNS strains from Ankara Hospital, whereas *icaC* (66.7%) and *aae* (64.6%) were the most detected genes in isolates from Izmir Hospital. Of the detected virulence encoding genes in the isolates, significant differences in *icaD, aap, atIE, aae,* and *sesI* genes were observed among the centers. The distribution of virulence encoding genes in the isolates to the centers is presented in Table 2.

eta, etb, etd, sea, seb, sed, see, seg, sei, and *pvl* virulence genes were negative in all isolates tested in the study. The most common gene was *icaC* (73.5%), followed by *icaA* (57.1%), *icaD* (56.1%), *aap* (55.1%), *aae* (52.0%), *sesl* (51.0%), *gehC* (50.0%), *hld* (50.0%), *hlb* (49.0%), *fbe* (44.9%), *atIE* (37.8%), *icaADB* (37.8%), *gehD* (34.7%), *icaB* (31.6%), *hla* (30.6%), *etx* (2.0%), *sec* (1.0%), *seh* (1.0%), and *tst* (1.0%). Except for three isolates, all tested positive for at least two virulence genes. One *S. haemolyticus* isolate was only positive for *icaC*. One *S. hominis* isolate was only positive for *icaD*, and one *S. scuiri* was only positive for *aap*. We detected up to 15 virulence-related genes in a single isolate in the study. Only two isolates (one *S. vitulinis* and one *S. warneri*) were positive for *etx*. The *sec* gene was found in only one *S. haemolyticus* isolate, and *seh* was found in one other *S. haemolyticus* isolate. Further, *tst* was detected in only one *S. warneri* isolate, which was also positive for *etx*.

In particular, *S. epidermidis* was found to be the most virulent species in the study. A comparison of *S. epidermidis* and non-*S. epidermidis* species revealed statistically significant differences in the distribution of *icaB, icaD, icaADB, aap, fbe, aae, sesI, atIE, gehC, gehD, hlb,* and *hld* genes. The *ica* operon, suggested as an indicator for biofilm production, was detected in all the isolates except for 10 (four *S. epidermidis*, two *S. hominis*, one *S. capitis*, one *S. cohnii* spp. *urealyticum*, one *S. sciuri*, one *S. vitulinus*). Table 3 shows the distribution of virulence encoding genes according to CoNS species.

Antimicrobial susceptibility testing of clinical isolates was achieved by using automated systems. Among the 98 CoNS, 82 (83.7%) strains were resistant to methicillin, and all were susceptible to vancomycin. Susceptibility to vancomycin (100%) was the highest susceptibility

Table 1. Distribution of the isolates according to centers.

	S. epidermidis n (%)	*S. hominis* n (%)	*S. haemolyticus* n (%)	*S. capitis* n (%)	Others n (%)	Total n (%)
Center 1	**26 (26.5)**	**13 (13.3)**	**7 (7.1)**	**4 (4.1)**	**-**	**50 (51)**
Center 2	**13 (13.3)**	**10 (10.2)**	**10 (10.2)**	**7 (7.1)**	**8 (8.2)**	**48 (49)**
Total	**39 (39.8)**	**23 (23.5)**	**17 (17.3)**	**11 (11.2)**	**8 (8.2)**	**98 (100)**

Center 1: Gulhane Training and Research Hospital, Ankara, Turkey.
Center 2: Izmir Katip Celebi University Ataturk Education and Research Hospital, Izmir, Turkey.

Table 2. Distribution of CoNS virulence encoding genes according to centers.

Target Gene	Center 1 n (%)	Center 2 n (%)	p-value
icaA	**27 (54.0)**	**29 (60.4)**	**Ns**
icaB	**17 (34.0)**	**14 (29.2)**	**Ns**
icaC	**40 (80.0)**	**32 (66.7)**	**Ns**
icaD	**34 (68.0)**	**21 (43.8)**	**0.025**
icaADB	**18 (36.0)**	**19 (39.6)**	**Ns**
aap	**33 (66.0)**	**21 (43.8)**	**0.042**
sec	**-**	**1 (2.1)**	**Ns**
seh	**1 (2.0)**	**-**	**Ns**
tst	**-**	**1 (2.1)**	**Ns**
atlE	**25 (50.0)**	**12 (25.0)**	**0.013**
fbe	**25 (50.0)**	**19 (39.6)**	**Ns**
aae	**20 (40.0)**	**31 (64.6)**	**0.017**
gehC	**28 (56.0)**	**21 (43.8)**	**Ns**
gehD	**20 (40.0)**	**14 (29.2)**	**Ns**
sesl	**20 (40.0)**	**30 (62.5)**	**0.029**
etx	**-**	**2 (4.2)**	**Ns**
hla	**16 (32.0)**	**14 (29.2)**	**Ns**
hlb	**28 (56.0)**	**20 (41.7)**	**Ns**
hld	**29 (58.0)**	**20 (41.7)**	**Ns**

Center 1: Gulhane Training and Research Hospital, Ankara.
Center 2: Izmir Katip Celebi University Ataturk Education and Research Hospital, Izmir.
Abbreviations: Ns - Not significant statistically.

rate among isolates, followed by daptomycin (96.9%), tigecycline (92.8%), linezolide (91.7%), trimethoprim/sulfamethoxazole (81.6%), fosfomycin (56.1%), tetracycline (54.6%), gentamicin (48.0%), clindamycin (46.9%), levofloxacin (33.7%), ciprofloxacin (32.7%), fusidic acid (28.6%), and methicillin (16.3%). The antimicrobial susceptibility of the isolates is presented in Table 4.

DISCUSSION

CoNS are members of normal skin and mucous membrane microbiota. Over the last few decades, the use of invasive medical devices resulting in an increased incidence of infections caused by CoNS has emerged as a serious problem, especially in healthcare settings [1,2, 29]. Moreover, limited data is available about the pathogenic mechanisms of clinical CoNS, except on biofilm formation, which is the most studied pathogenic mechanism. Therefore, we conducted the present study to in-

Table 3. Distribution of virulence encoding genes according to CoNS species.

Virulence genes	*S. epidermidis* (n = 39) n (%)	*S. hominis* (n = 23) n (%)	*S. haemolyticus* (n = 17) n (%)	*S. capitis* (n = 11) n (%)	Others (n = 8) n (%)	Total (n = 98) n (%)
icaA	24 (61.5)	13 (56.5)	8 (47.1)	6 (54.5)	5 (62.5)	56 (57.1)
icaB	25 (64.1) *	4 (17.4)	2 (11.8)	-	-	31 (31.6)
icaC	31 (79.5)	17 (73.9)	13 (76.5)	9 (81.8)	2 (25.0)	72 (73.5)
icaD	35 (89.7) *	10 (43.5)	7 (41.2)	2 (18.2)	1 (12.5)	55 (56.1)
icaADB	23 (59.0) *	5 (21.7)	1 (5.9)	8 (72.7)	-	37 (37.8)
atIE	35 (89.7) *	1 (4.3)	1 (5.9)		-	37 (37.8)
fbe	32 (82.1) *	6 (26.1)	-	3 (27.3)	3 (37.5)	44 (44.9)
aap	28 (71.8) *	18 (78.3)	4 (23.5)	2 (18.2)	2 (25.0)	54 (55.1)
aae	30 (76.9)*	6 (26.1)	5 (29.4)	8 (72.7)	2 (25.0)	51 (52.0)
gehD	26 (66.7) *	5 (21.7)	1 (5.9)	1 (9.1)	1 (12.5)	34 (34.7)
gehC	36 (92.3) *	4 (17.4)	2 (11.8)	3 (27.3)	4 (50.0)	49 (50.0)
sesI	28 (71.8)*	8 (34.8)	4 (23.5)	7 (63.6)	3 (37.5)	50 (51.0)
hla	5 (12.8)	11 (47.8)	13 (76.5)*	-	1 (12.5)	30 (30.6)
hlb	37 (94.9) *	6 (26.1)	1 (5.9)	4 (36.4)	-	48 (49.0)
hld	36 (92.3) *	7 (30.4)	3 (17.6)	3 (27.3)	-	49 (50.0)
sec	-	-	1 (5.9)	-	-	1 (1.0)
seh	-	-	1 (5.9)	-	-	1 (1.0)
etx	-	-	-	-	2 (25.0)	2 (2.0)
tst	-	-	-	-	1 (12.5)	1 (1.0)

*** Indicates a statistically significant difference (p < 0.05). Comparison of *S. epidermidis* and non-*S. epidermidis* species revealed statistically significant differences in the distribution of *icaB, icaD, icaADB, aap, fbe, aae, sesI, atIE, gehC, gehD, hlb,* and *hld* genes.**

Table 4. Antibiotic susceptibility rates of CoNS.

	Antibiotics S (%)												
Isolates	MET	CIP	LEV	CN	DA	DAP	FA	FOT	SXT	TET	TIG	VA	LNZ
S. epidermidis (n = 39)	12.8	30.8	33.3	48.7	41	94.9	30.8	87.2	70.6	42.1	100	100	92.3
S. hominis (n = 23)	21.7	47.8	47.8	65.2	60.9	100	34.8	34.8	94.1	73.9	95.7	100	86.4
S. haemolyticus (n = 17)	11.8	17.6	17.6	35.3	47.1	100	11.8	41.2	72.7	41.2	88.2	100	94.1
S. capitis (n = 11)	27.3	36.4	36.4	36.4	63.6	90.9	36.4	9.1	75	72.7	90.9	100	100
Others (n = 8)	12.5	25	25	37.5	12.5	100	25	62.5	50.0	62.5	87.5	100	85.7
Total (n = 98)	16.3	32.7	33.7	48.0	46.9	96.9	28.6	56.1	81.6	54.6	92.8	100	91.7

Abbreviations: S - Susceptibility, MET - Methicillin, CIP - Ciprofloxacin, LEV - Levofloxacin, CN - Gentamicin, DA - Clindamycin, DAP - Daptomycin, FA - Fusidic acid, FOT - Fosfomycin, SXT - Trimethoprim/sulfamethoxazole, TET - Tetracycline, TIG - Tigecycline, VA - Vancomycin, LNZ - Linezolide.

vestigate the presence of virulence genes among 98 CoNS isolated as pathogens in patients with bloodstream infections.

In this study, we detected high prevalence of methicillin resistance in the isolates. Among 98 CoNS, 82 (83.7%) were resistant to methicillin. In general, nosocomial isolates of CoNS have high resistance rates for methicillin, and methicillin resistance is emerging due to its association with multidrug resistance. The detected resistance rates to ciprofloxacin, levofloxacin, gentamicin, tetracycline, clindamycin, fosfomycin, and fusidic acid were very high among isolates studied. Multidrug resistance accompanying virulence associated genes will undoubtedly cause difficulties in the treatment of infections. Resistance to vancomycin is another emerging problem in CoNS. Although isolates resistant to linezolide, which is another last choice therapeutic agent, were found in our study, we did not detect any vancomycin-resistant isolate.

Biofilm is considered the major pathogenic factor of CoNS, and the presence of *ica* operon is thought to be a good predictor of biofilm capacity. Also, the *aap* gene could regulate intercellular adhesion and biofilm formation in CoNS lacking the *ica* operon [23,30,31]. Various studies have shown that the *app* gene and *ica* operon can regulate intercellular adhesion and biofilm formation. Arciola et al. [31] identified the presence of *icaA* and *icaD* genes as important virulence markers in *S. epidermidis*. They found that 49% (33/68) of *S. epidermidis* strains were *icaA* and *ica*D positive. Frebourg et al. [32] showed that the *icaAB* gene was significantly detected in invasive *S. epidermidis* isolates. The authors determined that the rates of *ica*AB were 76.9% in sepsis strains and 68.2% in strains of colonizing intravascular devices. However, a significant correlation was not reported between *ica* operon and biofilm proliferation *in vivo* in some studies. Pedroso et al. [17] could not find a direct correlation between the *ica* locus and biofilm formation in 59 CoNS obtained from blood cultures. Eftekhar et al. [33] reported failure to find a direct correlation between the presence of the *ica*ADBC operon and biofilm formation, although *icaADBC* operon was present in 110 (68.3%) isolates. Cahieb et al. [34] indicated that the ability of *S. epidermidis* to produce slime is not associated with the presence of *icaA* and *icaD* genes. In our study, 56 (57.1%) isolates were positive for *icaA*, 31 (31.6%) for *icaB*, 72 (73.5%) for *icaC*, 55 (56.1%) for *icaD*, and 37 (37.8%) for *icaADB*. *S. epidermidis* was the most virulent species isolated in the present study. The percentages of *atlE*, *fbe*, and *aap* detected were 37.8%, 44.9%, and 55.1%, respectively. We found considerable presence of genes encoding biofilm formation in bacteremia isolates. *ica* operon was found in all except for 10 isolates. Moreover, only four isolates lacking either the *ica* operon or *aap* gene were found in our study. Thus, we suggest that the detection of only one of the *ica* genes may not be enough to determine if the bacteria have the potential to produce biofilm.

Recent studies have demonstrated that *S. epidermidis* surface proteins may play an important role in *S. epidermidis* adhesion and invasion. Pena et al. [35] suggested that *ses* genes may be useful tools to differentiate commensal and invasive isolates. Qi et al. [8] found that the prevalence of *sesI* was 20.8% (26/125) in *S. epidermidis* invasive isolates and 3.8% (4/106) in colonizing isolates. In our study, the *sesI* gene was detected in 50 (51%) isolates, 28 of which were in *S. epidermidis* isolates, and the prevalence of *hlb*, *hld*, *gehC*, *gehD*, *icaADB*, *fbe*, *aae*, and *atlE* genes was determined to be significantly higher among *sesI*-positive isolates than among *sesI*-negative isolates.

Few studies have investigated the presence of lipases in CoNS. For instance, Salgueiro et al. [9] found that the percentage of the *gehD* gene was 74% among BSI isolates and 58% among nasal isolates in their study. Among our isolates, 49 (50.0%) harbored *gehC* and 34 (34.7%) harbored *gehD*. Notably, we found higher rates of these lipases - 92.3% and 66.7% of our *S. epidermidis* isolates harbored *gehC* and *gehD*, respectively.

Toxins may act as super antigens that trigger immune response by activating T cells and can cause moderate to severe illnesses. We evaluated the presence of the cytotoxin-encoding genes in CoNS isolates by using *hla*, *hlb*, and *hld* species-specific primers. Although the presence of toxigenic CoNS has been reported in the literature, the toxigenic potential of CoNS is controversial in contrast to that of *S. aureus* [19,36]. Pinheiro et al. [25] detected *hla* gene in 91.7% of *S. haemolyticus* isolates; *hlb* and *hld* were found in 92.9% and 95.3% of the *S. epidermidis* isolates, respectively. Okee et al. [37] detected *hld* gene in all *S. epidermidis* isolates from ICUs, whereas *hla* gene was detected only in 20%, *tsst*1 gene in 7%, and *sea* in 3% of their isolates. We found the *hla* gene in 76.5% of *S. haemolyticus* isolates; *hlb* and *hld* were found in 94.9% and 92.3% of *S. epidermidis* isolates, respectively. Different rates of genes encoding enterotoxins among CoNS have been reported in the literature. However, the production of such toxins in *S. epidermidis* is uncommon. Vasconcelos et al. [18] reported the presence of *seg*, *seh*, and *sei* genes in 32 of 90 *S. aureus* isolates, whereas the presence of some of these genes was observed in 22 of the 63 (34.9%) CoNS blood isolates. Pinheiro et al. [25] detected *sea*, *seg*, and *sei* genes in 53.3%, 64.5%, and 67.5% among the 169 CoNS (85 *S. epidermidis* and 84 *S. haemolyticus*) blood culture isolates, respectively. Similarly, Pedroso et al. [15] found higher rates for enterotoxin genes in 59 CoNS isolated from blood cultures. They reported that 76.2% of the strains were positive for *sea*, 23.7% for *sec*, 1.6% for *sed*, and 30.5% for *tsst*-1. Cunha et al. [19] reported that 26.7% of blood culture CoNS isolates produced some type of toxin (*sea*, *seb*, *sec*, *sed*, *tsst*), and one or more toxin genes were found in 50% (32/64) of the isolates. Only two enterotoxin genes were detected in two *S. haemolyticus* isolates in our study; the *sec* gene was found in one and the *seh* gene in the other. The *etx* gene was also detected among two of our iso-

lates - one *S. vitulinis a*nd one *S. warneri* - and the former isolate was also positive for *tsst*. The isolates were negative for the *pvl* gene. PVL is a cytotoxin which is found especially in community acquired *S. aureus* isolates causing skin and soft tissue infections, necrotizing pneumonia, necrotizing fasciitis, and severe sepsis syndrome. The isolates with the *pvl* gene are considered to have high morbidity and mortality rates, as well as more virulent and more resistant to antibiotics [38,39]. *pvl* gene positivity is not common among coagulase negative species isolated from humans. One reason for this situation is that studies for the *pvl* gene are mostly screened in *S. aureus* isolates. More studies are needed to screen toxin genes in coagulase-negative staphylococci, which are probably underestimated.

S. epidermidis was the most frequently isolated CoNS species in the present study, and *eta, etb, etd, sea, seb, sed, see, seg, sei, pvl* were negative in all tested isolates. Except for three, all tested isolates were positive for at least two virulence genes. We detected up to 15 virulence-related genes in a single isolate in our study. We found a higher percentage of *S. epidermidis* isolates producing significant biofilm-associated genes compared to other species in this study. In general, we determined high rates of genes encoding biofilm formation. Only four isolates lacked either the *ica* operon or *aap* gene in our study. Although we found low rates of toxin-related virulence genes, the current study revealed the presence of various virulence factors, such as biofilm formation, lipases, hemolysins, enterotoxins, exfoliative toxins, and toxic shock syndrome toxin, in CoNS strains isolated from blood cultures. The greatest limitation of our study is that it was conducted with the limited number of isolates and species distribution. However, our data shows that CoNS isolated from blood culture could express a variety of virulence-related genes similar to those of *S. aureus*. Consequently, further studies are needed to investigate the toxin-related genes in CoNS by using sensitive and reliable techniques such as PCR to differentiate species and their clinical features.

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