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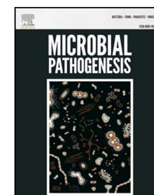

Expression of superantigens and the *agr* system in *Staphylococcus epidermidis*

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ABSTRACT

Infections with coagulase-negative staphylococci (CoNS) can involve the production of toxins such as superantigens, which contribute to tissue degradation and inflammatory immune responses. The accessory gene regulator (*agr*) quorum sensing system coordinates the expression of most *S. aureus* virulence factors. Therefore, the aim of this study was to investigate the expression of these superantigens and the presence of the *agr* locus in CoNS strains isolated from blood cultures. PCR was used to detect enterotoxin and *agr* genes and expression was analyzed by RT-PCR. Expression of the *sea* gene was observed in one *S. epidermidis* isolate andof *sec-1* in two, *seg* and *sei* were expressed concomitantly in one isolate, and *sei* was expressed in another isolate. The *agr* group I was detected in *S. epidermidis* expressing the *sea*, *seg* and *sei* genes, whereas *agr* group II was detected in isolates expressing the*sec-1* gene. The *agr* groups were only expressed in strains expressing the*sec-1* gene. The results show that enterotoxin genes are highly frequent in CoNS isolated from clinical specimens and confirm the toxin-producing ability of these strains. The *agr* group II may be associated with enterotoxin C production by *S. epidermidis*, increasing the virulence of strains isolated from blood cultures and consequently the severity of sepsis caused by these organisms.

1. Introduction

Most species belonging to the genus *Staphylococcus* are coagulase-negative staphylococci (CoNS), i.e., they cannot produce the coagulase enzyme. Although CoNS are part of the human microbiota, they are considered opportunistic microorganisms because they take advantage of situations such as post-trauma tissue damage and the presence of foreign bodies to proliferate and spread to other tissues, developing a pathogenic behavior [1]. CoNS are the main cause of bacteremia in hospitals and their pathogenesis is complex, involving the production of a variety of virulence factors such as toxins [2,3].

Staphylococcal toxins can contribute to tissue degradation and elicit anti-inflammatory immune responses [4]. Staphylococcal enterotoxins and toxic shock syndrome toxin 1 (TSST-1) are superantigens whose toxic effects can trigger the nonspecific proliferation of T cells through direct binding to major histocompatibility complex class II molecules and to the Vβ region of the T cell antigen receptor. Unlike normal antigen processing, they stimulate many T cells that overproduce cytokines such as interleukin 1 (IL-1), IL-2, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) [5].

During infection, toxin production by staphylococci is a multi-stage process that is coordinated by a complex system of communication between bacterial cells. This mechanism, called quorum sensing, allows bacteria to share information about cell density and to establish a phenotypic reaction according to the growth stage of the culture [6,7]. Quorum sensing via the accessory gene regulator (*agr*) system is one of the main systems that coordinate staphylococcal virulence factors [8]. This system comprises promoters P2 and P3, which operate in opposite directions and produce transcripts RNAII and RNAIII, respectively. RNAIII is responsible for the gene transcription of a number of virulence factors, including extracellular toxins and enzymes and surface proteins [9]. The *agr* system is composed of four genes (*agrA*, *agrB*, *agrC*, and *agrD*) arranged in an operon. The products of these genes are proteins AgrA, AgrB, AgrC and AgrD, which are necessary for the function of the system. Proteins AgrB and AgrD combine to produce the autoinducing polypeptide (AIP), which is released into the extracellular medium. When the transmembrane protein AgrC detects AIP in the external environment, it phosphorylates AgrA that induces expression from P2 and P3. The final product of the *agr* locus is RNAIII, an mRNA that induces or inhibits toxin genes [7].

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Three polymorphisms of the *agr* locus (*agr* group I, group II and group III) have been described in *Staphylococcus epidermidis* [10]. These groups show variations in the *agrB*, *agrC* and *agrD* genes and thus in AIP and AgrC, with AIP binding to a specific receptor for each allelic group. When AIP of one allele group binds to the AgrC receptor of another group, it does not produce intrinsic factors and thereby behaves as an antagonist. AIP is the only agonist to its own allele group and bacteria of one *agr* group can therefore affect the regulation of accessory proteins of bacteria from another *agr* group [7].

In view of the increasing incidence of CoNS infection, studies on associated virulence factors are important to better understand the toxigenic potential of these microorganisms. Therefore, the present study evaluated the occurrence and expression of superantigens and the role of the *agr* system in the production of virulence factors by CoNS species isolated from blood cultures of patients hospitalized in the University Hospital of the Botucatu Medical School.

2. Material and methods

2.1. Strains

Three hundred CoNS strains isolated from blood cultures of patients admitted to the University Hospital of the Botucatu Medical School, State University of São Paulo (UNESP), were evaluated. The blood samples were collected between 1990 and 2009 and stored in the Culture Collection of the Department of Microbiology and Immunology, Biosciences Institute of Botucatu (UNESP). An average prevalence of 35%, with a 5% error and 95% confidence interval, were defined as criteria for selection of each CoNS species.

The strains were isolated on blood agar plates as described by Koneman et al. [9] and suspected colonies were submitted to Gram staining. After confirmation of morphology and specific staining, catalase and coagulase tests were used to identify the isolates. Biochemical tests were performed for phenotypic identification of the CoNS species according to the simplified method of Cunha et al. [10]. Genotypic identification was carried out by PCR-based determination of internal transcribed spacer (ITS) regions according to Couto et al. [11].

2.2. Extraction of DNA

Total DNA was extracted from the CoNS strains cultured on blood agar, individually inoculated into BHI broth, and incubated for 24 h at 37 °C. In brief, staphylococcal cells were digested with lysozyme (10 mg/mL) and proteinase K(20 mg/mL). Next, 500 μL of the extraction solution was added and the mixture was centrifuged at 5000 × *g* for 1 min. The supernatant was transferred to a GFX column and centrifuged at 5000 × *g* for 1 min. The fluid collected was discarded and 500 μL of the extraction solution was again added to the column. After centrifugation and disposal of the collected fluid, 500 μL of the washing solution was added and the column was centrifuged at 14,000 rpm for 3 min. The column was transferred to a 1.5-ml tube and 200 μL Milli-Q water heated to70°Cwas used for elution. The samples were centrifuged at 5000 × *g* for 1 min and the GFX column was discarded. The extracted DNA was stored in a freezer at 4 °C.

2.3. Detection of enterotoxin and TSST-1 genes

PCR was carried out in 0.5-ml centrifuge tubes containing10 pmol of each primer (Table 2), 2.5 U Taq DNA polymerase, 200 μmol/L deoxyribonucleotide triphosphates (dNTP), 20 mmol/L Tris-HCl, pH 8.4, 0.75 mmol/L $MgCl_2$, and 3 μL of the sample in a final volume of 25 μL. A negative control in which the nucleic acid was replaced with water was included in all reactions. Amplification was carried out in an MJ Research PTC-100 thermocycler using the following parameters: one cycle at 94 °C for 4 min, denaturation at 94 °C for 2 min, primer annealing at 55 °C and extension at 72 °C for 1 min 30 s, followed by a second cycle of denaturation at 94 °C for 2 min, annealing at 53 °C and extension at 72 °C for 1 min 30 s. In the third cycle, the annealing temperature was reduced to 51 °C and 37 cycles were carried out using the last parameters. After completing40 cycles, the tubes were incubated at 72 °C for 7 min and then cooled to 4 °C.

2.4. Extraction of RNA and cDNA synthesis

Total RNA was extracted using the Illustra RNA spin Mini kit according to manufacturer recommendations. After treatment with DNAse, the mRNA samples were converted into cDNA. For that purpose, 12 μL mRNA treated with DNase was added to 1 μL of random primer(75 ng/μL), 6 μL nuclease-free water, and 1 μL dNTP (200 μM). The mixture was heated for 5 min at 65 °C for RNA denaturation and primer binding and 4 μL reverse transcription buffer, 1 μL dithiothreitol and 1 μL SuperScript™ III (200 U/μL) were added. cDNA was synthesized in a PTC-100 thermocycler using one cycles at 25 °C for 5 min, 50 °C for 60 min, and 70 °C for 15 min, followed by cooling at 4 °C. As internal control, the expression of 16S rRNA using 16S1 and 16S2 primers (Table 2) was analyzed, which correspond to gene regions that are conserved in staphylococci and specific to the genus. The cDNA obtained was amplified by PCR and the resulting products were visualized by electrophoresis.

2.5. Determination of the agr group

The CoNS strains that tested positive for superantigen mRNA by the reverse transcription polymerase chain reaction (RT-PCR) were subjected to *agr* group typing by PCR as described by Li et al. [12]. The reactions were performed with primers targeting *agrA*, *agrB* and *agrC* (Table 1).

2.6. Staphylococcus epidermidis typing by PFGE

The modified protocol of McDougal et al. [13] was used to determine the clonal profile of the *S. epidermidis* spp. isolates. The strains were inoculated into BHI broth and incubated for 24 h at37 °C.The isolates were centrifuged in microtubes at 15,294 × *g* for 1 min. The supernatant was discarded and 300 μLTE solution (10 mM Tris, 1 mM EDTA, pH 8.0) was added. The strains were incubated in a water bath for 10 min at 37 °C. The cells were lysed by adding 5 μL lysostaphin and vortexed, and 300 μL of 1.8% low-melt agarose was added at 37 °C. Plugs were prepared from the strains and the agarose was allowed to solidify. The plugs were then transferred to a 24-well plate containing 2 mL EC solution (6 mM Tris-HCl, 1 M NaCl, 100 mM EDTA, 0.5% Brij-58, 0.2% sodium deoxycholate, 0.5% sodium lauroyl sarcosinate) and incubated for 4 h at 37 °C. The EC solution was removed and the plugs were washed four times in 2 mL TE solution for 30 min at 21 °C.

One-third of the plug and 2 μL SmaI were used for the restriction of genomic DNA. For restriction, buffer without the enzyme (45 μL Milli-Q water and 5 μL of the enzyme buffer) was added to a 96-well plate and the plate was stored in a refrigerator for 30 min at 4 °C. The buffer without the enzyme was removed and buffer containing the enzyme (43 μL Milli-Q water, 5 μL enzyme buffer, and 2 μL of the enzyme) was added. The plate was incubated in an oven for 6 min at 37 °C. Electrophoresis was carried out in a CHEF-DR III System using 1% agarose gel prepared in 0.5 M TBE (0.1 M Tris, 0.08 M boric acid, 1 mM EDTA) under the following conditions: pulse times of 5–40 s for 21 h on a linear ramp; 6 V/cm; angle of 120°; 14 °C; 0.5 M TBE as running buffer. The Lambda Ladder PFG Marker was used as a molecular marker. The gels were stained with GelRed (400 mL distilled water and 30 μL GelRed) for 1 h and photographed under UV transillumination.

The BioNumerics 6.1 software was used for analysis of similarity, calculation of the Dice correlation coefficient, and construction of the dendrogram by the UPGMA method. Band position tolerance and optimization were set at 1.25 and 0.5%, respectively. A similarity

Table 1
Primers used for PCR detection of staphylococcal enterotoxin genes, *agr* locus and 16S and 23S genes.

Primer	5' to 3' nucleotide sequence	Size (bp)	Product
sea1	TTGGAAACGGTTAAAACGAA	123	Enterotoxin A
sea2	GAACCTTCCCATCAAAAACA		
seb1	TCGCATCAAACTGACAAACG	478	Enterotoxin B
seb2	GCAGGTACTCTATAAGTGCC		
sec-11	GACATAAAAGCTAGGAATTT	257	Enterotoxin C
sec-12	AAATCGGATTAACATTATCC		
sed1	CTAGTTTGGTAATATCTCCT	317	Enterotoxin D
sed2	TAATGCTATATCTTATAGGG		
see1	CAAAGAAATGCTTTAAGCAATCTTAGGCCAC	482	Enterotoxin E
see2	CTTACCGCCAAAGCTG		
seg1	AATTATGTGAATGCTCAACCCGATC	642	Enterotoxin G
seg2	AAACTTATATGGAACAAAAGGTACTAGTTC		
seh1	CAATCACATCATATGCGAAAGCAG	375	Enterotoxin H
seh2	CATCTACCCAAACATTAGCACC		
sei1	CTCAAGGTGATATTGGTGTAGG	576	Enterotoxin I
sei2	AAAAAACTTACAGGCAGTCCATCTC		
tsst1	ATGGCAGCATCAGCTTGATA	350	TSST-1
tsst2	TTTCCAATAACCACCCGTTT		
agrA1	GCTGCAACCAAGAAACAACC	1022	*agrI, II, III*
agrA2	CGTGTATTCATAATATGCTTCGATT		
agrB1	TATGCAAGCCAAGCACTTGT	453	*agrIII*
agrB2	GTGCGAAAGCCGATAACAAT		
agrC1	CCTTGGCTAGTACTACACCTTC	615	*agrII*
agrC2	GTGCTTGGCTTGCATAAACA		
G1	GAAGTCGTAACAAGG		16S
L1	CAAGGCATCCACCGT	-	23S

References: Jarraud et al.[27], Li et al. [12], and Couto et al. [11].

coefficient of 80% was chosen for the definition of clusters.

The following international clones, kindly provided by Dr. Antonio Carlos Campos Pignatari, Laboratório Especial de Microbiologia Clínica, Disciplina de Infectologia, Universidade Federal de São Paulo/Escola Paulista de Medicina, and by Dr. Agnes Marie Sá Figueiredo, Universidade Federal do Rio de Janeiro, Instituto de Microbiologia Prof. Paulo de Góes, Brazil, were used as controls: USA800 (SCC*mec* IVa), JCSC 1968/CA05 (SCC*mec* IVa), JCSC 978/8/6-3P (SCC*mec* IVb), MR108 (SCC*mec* IVc), JCSC 4469 (SCC*mec* IVd), WB72/USA300 (SCC*mec* IV), USA400 (SCC*mec* IV), USA500 (SCC*mec* IV), 0SPC (SCC*mec* IV), HAR24/EMRSA 15 (SCC*mec* IV), HU25 (SCC*mec* IIIa), 85/2082 (SCC*mec* III), and ANS 46 (SCC*mec* III).

3. Results

3.1. Species identification

The biochemical method for CoNS identification detected 223 (74.3%) *S. epidermidis*, 27 (9.0%) *S. haemolyticus*, 22 (7.3%) *S. hominis*, 14 (4.7%) *S. warneri*, 9 (3.0%) *S. lugdunensis*, and 5 (1.7%) *S. capitis*. The molecular method (ITS-PCR)identified 223 (74.3%) *S. epidermidis*, 29 (9.7%) *S. haemolyticus*, 23 (7.7%) *S. hominis*, 11 (3.7%) *S. warneri*, 9 (3.0%) *S. lugdunensis*, and 5 (1.7%) *S. capitis.* The agreement between methods was 98%.

3.2. Detection and expression of enterotoxin genes

TSST-1 and enterotoxin genes were analyzed in the 300 CoNS isolates and 90.7% carried at least one of the genes. The *sea* gene was detected in 172 isolates (57.3%), *seb* in 70 (23.3%), *sec-1* in 105 (35.0%), *sed* in 7 (2.3%), *see* in 8 (2.7%), *seg* in 188 (62.7%), *seh* in 39 (13.0%), *sei* in 200 (66.7%), and *tst* in 15 (5.0%) (Table 2; Fig. 1).

The isolates that were positive for superantigen genes were subjected to RT-PCR for mRNA detection. Five *S. epidermidis* isolates were positive for enterotoxin mRNA (1 for s*ea*, 1 for *sei*, 2 for *sec-1*, and 1 for both *seg* and *sei*).

3.3. Detection and expression of the agr locus

Agr groups I, II and III were studied in all *S. epidermidis* strains and their expression was analyzed in those producing enterotoxins. Of the 223 *S. epidermidis* studied, 143 (64.1%) carried *agr* group I, 53 (23.7%)

Table 2
Determination of enterotoxin and TSST-1 genes in coagulase-negative staphylococci isolated from blood cultures.

Gene (N)		*S. epidermidis* (223)		*S. haemolyticus* (29)		*S. hominis* (23)		*S. warneri* (11)		*S. lugdunensis* (9)		*S. capitis* (5)	
		N	%	N	%	N	%	N	%	N	%	N	%
sea	(172)	120	69.8	22	12.8	17	9.9	7	4.1	2	1.2	4	2.3
seb	(70)	45	64.3	11	15.7	10	14.3	4	5.7	0	0	0	0
sec-1	(105)	73	69.5	9	8.6	11	10.5	4	3.8	6	5.7	2	1.9
sed	(7)	5	71.4	1	14.3	0	0	1	14,3	0	0	0	0
see	(8)	4	50.0	2	25.0	1	12.5	1	12.5	0	0	0	0
seg	(188)	131	69.7	22	11.7	19	10.1	7	3.7	4	2.1	5	2.7
seh	(39)	24	61.5	7	17.9	5	12.8	1	2.6	2	5.1	0	0
sei	(200)	144	72.0	18	9.0	20	10.0	7	3.5	7	3.5	4	2.0
tst	(15)	11	73.3	1	6.7	1	6.7	0	0	1	6.7	1	6.7

N: number of isolates.

sea	**172**								
seb	50	**70**							
sec-1	62	27	**105**						
sed	7	3	2	**7**					
see	7	2	2	0	**8**				
seg	134	59	69	6	6	**146**			
seh	28	11	13	0	0	24	**39**		
sei	134	54	71	6	6	122	32	**200**	
tst	5	0	10	0	0	2	2	9	**15**
	sea	*seb*	*sec-1*	*sed*	*see*	*seg*	*seh*	*sei*	*tst*

Fig. 1. Combinations of staphylococcal enterotoxin and TSST-1 genes detected in CoNS strains isolated from blood cultures. The shaded squares indicate the total number of genes detected in the isolates.

agr group II, and 4 (1.8%) *agr* group III. The *agr* locus was not identified in 23 (10.3%) of the *S. epidermidis* isolates analyzed.

The *S. epidermidis* isolates expressing *sea*, *sec-1* and *sei* carried *agr* group I and those expressing *sec-1* carried *agr* group II. Of the 5 *S. epidermidis* isolates expressing enterotoxin mRNA, only 2 expressed *agr* group II.

3.4. *Staphylococcus epidermidis* typing by PFGE

The similarity rate between the *S. epidermidis* isolates that expressed the enterotoxin genes was less than 80%, which is the thre shold value for clustering. Therefore, the *S. epidermidis* isolates studied did not exhibit a clonal profile (Fig. 2).

4. Discussion

Coagulase-negative staphylococci are the main microorganisms isolated from clinical materials and the primary cause of bacteremia in hospitals, especially in immunosuppressed patients. The ability of these bacteria to colonize the skin and to spread through the body during infections is due to the production of virulence factors such as enterotoxins. The present study investigated 300 CoNS isolated from blood cultures of patients seen at the University Hospital of Botucatu. The isolates were collected over a period of 20 years to study staphylococcal superantigen genes, the expression of virulence factors, and detection of the *agr* locus.

Staphylococcus epidermidis was the most frequent species detected, accounting for 74.3% of all CoNS isolated. Despite their lower prevalence, the other species isolated are also of clinical importance since they can cause serious infections. A number of studies have shown that *S. epidermidis* is isolated from 74 to 92% of patients with nosocomial infections [14] and is the main species isolated from infections, particularly those originating from invasive procedures such as implantation of peripheral and central catheters [15]. Similar to our findings, other studies identified *S. haemolyticus* as the second most common CoNS species isolated from blood cultures, which can cause different infections such as sepsis, peritonitis, otitis, and urinary infection [16]. *Staphylococcus hominis* has been identified in cases of bacteremia [17] and *S. warneri*, *S. capitis* and *S. lugdunensis* have been associated with serious infections such as endocarditis [18–20].

Among the CoNS strains studied, 90.7% exhibited enterotoxin genes (*sea* to *sei*) or TSST-1 (*tst*). *Staphylococcus epidermidis* carried the highest proportion of these genes, followed by *S. haemolyticus* and *S. hominis*. The higher frequency of superantigen genes in *S. epidermidis* supports the importance of this species, which has been implicated in the etiology of serious infections. In addition to being the main member of the human microbiota, the selective pressure in the hospital environment and use of antimicrobials and disinfectants may favor the persistence of more resistant and virulent strains. Cunha et al. [21] investigated the presence of enterotoxin A and D and TSST-1 genes in *Staphylococcus* strains isolated from clinical samples of newborns seen at the same hospital as the present study by PCR and found that 40% of the CoNS isolates were positive for at least one of the genes. The authors also observed that 32.2% of the same CoNS isolates carried at least one of the following genes: *see*, *seg*, *seh*, and *sei* [22].

Among the classical enterotoxin genes (*sea* to *see*), *sea* was the most frequent and was also expressed at the mRNA level in one of the *S. epidermidis* isolates. Calsolari et al. [23] detected classical enterotoxin genes in 49 of 90 CoNS strains and *sea* was identified in 18.6%. In the present study, *sea* was detected along with other genes in a number of combinations. The *sea* gene is carried on a prophage [24], facilitating its dispersal among *Staphylococcus* strains. Its product, enterotoxin A, is one of the main toxins associated with food poisoning, exhibiting toxic effects at low levels [25].

The *sec-1* was the second most frequent classical enterotoxin gene and was detected by RT-PCR in two *S. epidermidis* isolates. *Sec* is a chromosomal gene located on pathogenicity islands. Its three subtypes (*sec-1*, *sec-2* and *sec-3*) are classified according to their specific antigens

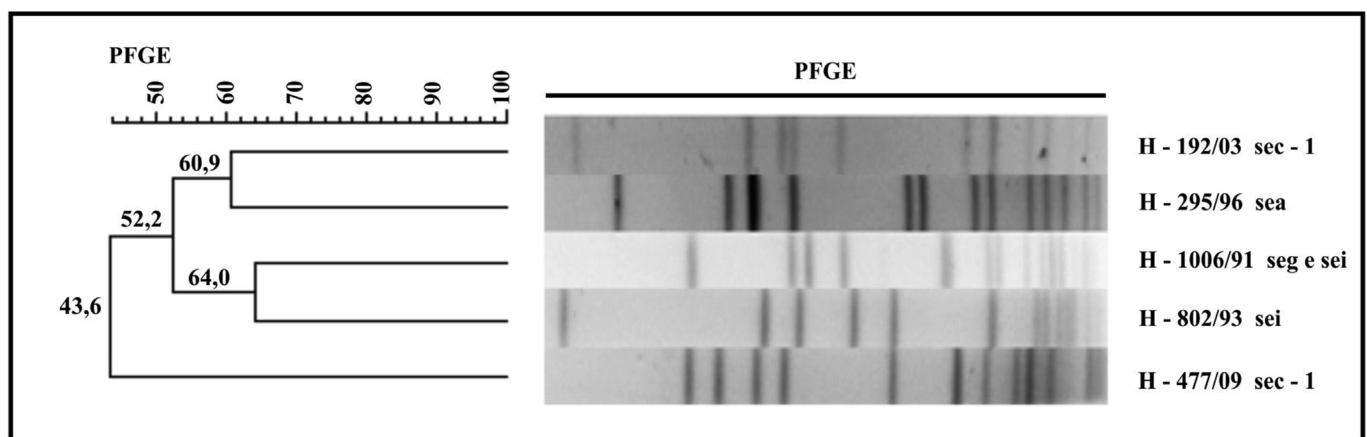

Fig. 2. Determination of the clonal profileof enterotoxin-producing *Staphylococcus epidermidis* strains isolated from blood cultures.

and the respective host. Some studies suggest that the heterogeneity of enterotoxin C is related to the selection of *sec* sequences, which can enhance staphylococcal survival in the corresponding hosts [25,26].

The most frequent staphylococcal enterotoxin genes were *seg* and *sei*, which were identified in 62.7% and 66.7% of the CoNS isolates, respectively. These genes showed a high correlation and were associated with other genes in several combinations. Vasconcelos et al. [22] found lower proportions of *seg* and *sei* in CoNS strains(12.2% and 22.2%, respectively) and described their co-occurrence. Jarraud et al. [27] reported the co-occurrence of *seg* and *sei*, which are arranged in tandem orientation in an enterotoxin gene cluster (*egc*), and their expression along with *sem*, *sen* and *seo*. Varshney et al. [28] detected *seg* and *sei* (44% and 46%, respectively) in *S. aureus* isolated from blood cultures and found the complete gene cluster in 10% of the strains. The *seg* and *sei* mRNAs were simultaneously detected in one *S. epidermidis* isolate and *sei* mRNA alone in another isolate. The other enterotoxin genes (*seb*, *sed*, *see*, *seh*, and *tst*) were detected at lower levels, but their expression was not confirmed.

The *agr* locus was investigated in the *S. epidermidis* isolates and three groups were identified. Corroborating earlier studies [12,29], there was a predominance of *agr* group I, followed by *agr* group II and group III. According to Li et al. [12], polymorphisms in the *agr* locus may be associated with pathogenicity. In this respect, *agr* group I is associated with pathogenic *S. epidermidis* isolated from blood cultures and catheters, while *agr* group II and group III are found in *S. epidermidis* isolated from healthy individuals [29]. Some authors suggest that variations in the *agr* locus were selected during evolution and represent a fundamental aspect of strain divergence that permits adaptation to the microenvironment of specific infection sites [6,29].

The *agr* group I and group II were found in the strains that expressed the enterotoxin genes. The isolate expressing *sea* carried *agr* group I, but mRNA was not transcribed from this locus. Enterotoxin A is produced at the beginning of the exponential phase of bacterial proliferation and the expression of its gene is not regulated by *agr*, in contrast to enterotoxins B, C and D that depend on the *agr* system for total expression [30–34]. The strains expressing *seg* and *sei* also carried *agr* group I, but mRNA was not transcribed from this locus. The *agr* group II was detected in the two strains expressing *sec-1* which were positive for mRNA, suggesting that this locus is associated with the expression of the enterotoxin C gene in *S. epidermidis*. Regassa et al. [31] suggested the *agr* locus to be one of the determinants of maximum *sec* expression in *S. aureus* and found mRNA levels to be 2 to 3 times lower in *agr*-negative strains. *Staphylococcus epidermidis* is a versatile microorganism that can undergo rapid metabolic adaptations through sophisticated gene regulation mechanisms to cope with external conditions, to escape the host immune response, and to communicate with surrounding cells [32].

The *S. epidermidis* strains that expressed the enterotoxin genes were subjected to PFGE to determine their clonal profile. However, the results showed no similarities, indicating diversity among strains isolated in the same hospital that express toxin genes. The detection of *S. epidermidis* strains from blood cultures is important because they can produce superantigens similar to those produced by *S. aureus*, aggravating infections. Studies have suggested enterotoxins to play a role in the development of septic arthritis, in which the activation of an exaggerated inflammatory response triggers the release of significant amounts of cytokines that activate a large number of T cells and macrophages, causing tissue damage [33,34]. Enterotoxins have also been described to be involved in sepsis, osteomyelitis, and sinusitis [35]. Thus, attention must be paid to the bacteria that produce these toxins and to potential pathogen dissemination in the hospital environment.

A small number of isolates expressed toxin genes at a higher frequency, which are adaptive to *S. epidermidis* given that low virulence is positive for its relationship with the host. According to Massey et al. [36], evolution can favor species that cause little or no damage to the host because the infection can persist for a long period of time, favoring transmission from one host to another.

5. Conclusions

The present study showed that the clinical CoNS strains tested are reservoirs of enterotoxin genes. The genes are mainly associated with *S. epidermidis*, which carries a regulatory system that allows their expression. The findings on toxigenicity and gene regulation of *S. epidermidis* isolated from blood samples highlight the importance of correct identification of CoNS associated with nosocomial bacteremias in order to improve the accuracy of positive blood culture results and to implement strategies for the treatment and control of infections caused by this microorganism.

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Author contributions

Valéria Pereira: Conceived and designed the study, performed the microbiological tests, developed the molecular techniques, analyzed the data, and wrote the article.

Luiza Pinheiro: Participated in the identification and detection of enterotoxin genes.

Katheryne Martins: Participated in RNA extraction.

Adilson Oliveira: Participated in RNA extraction.

Danilo Riboli: Participated in the PFGE analysis.

Maria de Lourdes da Cunha: Responsible for the conception and design of the study, coordination of laboratory work, data analysis, and manuscript writing. All authors read and approved the final version of the manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

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