Study of virulence factors in coagulase-negative staphylococci isolated from newborns

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Coagulase-negative staphylococci (CNS) have been identified as the etiological agent in various infections and are currently the microorganisms most frequently isolated in nosocomial infections. However, little is known about the virulence factors produced by CNS that contribute to the pathogenesis of infections caused by these microorganisms. The study of CNS isolated from infectious processes of newborns hospitalized in the Neonatal Unit of the Hospital of the Botucatu Medical School, Unesp, indicated Staphylococcus epidermidis *as the most frequently isolated species (77.8%), which was also associated with clinically significant situations. The analysis of virulence factors revealed the production of slime in 20 (17.1%) of all CNS samples isolated and the synthesis of a broad spectrum of enzymes and toxins, including hemolysins (19.6%), lipase (17.1%), lecithinase (3.4%), DNAse (15.4%), thermonuclease (7.7%), and enterotoxin A, B or C (37.6%). Taking into consideration that the etiological importance of CNS has often been neglected, the present investigation confirmed that these microorganisms should not be ignored or classified as mere contaminants.*

Key words: coagulase-negative staphylococci - virulence factors - slime - enzymes - toxins

Few reports of infections with coagulase-negative staphylococci (CNS) were published before the 1970s; clinicians and microbiologists considered them to be contaminants of clinical samples, with *Staphylococcus aureus* being the only pathogenic species within the genus *Staphylococcus* (Kloos & Bannerman 1995). This distinction, which has been widely used for clinical diagnosis, represents a challenge in relation to the role of these microorganisms in infectious processes.

At present, CNS are considered to be basically opportunistic microorganisms that prevail in numerous organic conditions, producing serious infections (Kloos & Bannerman 1995, Lark et al. 2000). The recognition of CNS as etiological agents may also be due to the valorization of this group of organisms as opportunistic pathogens and to the increasing use of invasive procedures such as intravascular catheters and to prosthetic interference. Data obtained between 1995 and 1998 by the Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE) program have demonstrated that CNS are the etiologic agents most frequently found in nosocomial bacteremias in the United States (Edmond et al. 1999).

The virulence factors produced by CNS and how they contribute to the pathogenicity of infections associated with foreign bodies are currently under investigation. Evidence indicates that pathogenicity might be related to the production of an extracellular polysaccharide, known as slime, that permits these microorganisms to adhere to smooth plastic surfaces, colonizing catheters, prosthetic heart valves, pacemakers, and joint prostheses (Vogel et al. 2000).

Differentiation between virulent and non-virulent strains has been difficult since the virulence factors of these microorganisms are still not well defined (Gemmell 1987). According to this author and to Koneman et al. (1997), CNS produce other virulence factors, such as hemolysins, lipases, proteases, and toxins.

Based on the above considerations, we decided to evaluate this question in our institution, with the main objectives being the identification of CNS species isolated from clinical cases of newborns hospitalized in the Neonatal Unit of the Hospital of the Botucatu Medical School, Unesp, Botucatu, Brazil, and the determination of the production of slime, enzymes, and toxins by the different isolates.

MATERIALS AND METHODS

Organisms - CNS isolates were obtained from 107 newborns hospitalized in the Neonatal Unit of the University Teaching Hospital, Unesp, Botucatu, between 1990 and 1996. The procedures used were approved by the Ethics Committee of the School.

Identification of CNS - CNS were isolated on blood agar. Bacterial colonies were stained by the Gram method and submitted to catalase and coagulase tests (Koneman et al. 1997). The genus *Staphylococcus* was differentiated from *Micrococcus* by the glucose oxidation and fermentation test and by resistance to bacitracin (0.04 U) and sensitivity to furazolidone (100 μg) as described by Koneman et al. (1997).

CNS species were identified as described by Kloos and Schleifer (1975), Kloos and Bannerman (1995), and Cunha et al. (2004). The following tests were used: utiliza-

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tion of xylose, arabinose, sucrose, trehalose, mannitol, maltose, lactose, xylitol, ribose and fructose, characterization of hemolysis, nitrate reduction, urease, decarboxylase ornithine, and resistance to novobiocin.

The following international CNS reference strains were used as controls: *S. epidermidis* (ATCC 12228), *S. simulans* (ATCC 27851), *S. warneri* (ATCC 10209), *S. xylosus* (ATCC 29979), and *S. saprophyticus* (ATCC 15305).

Clinical relevance - Data were obtained by analysis of the patients' medical records. Data regarding perinatal infection risk factors were reviewed, including prolonged membrane rupture (> 24 h), gestational age, birthweight, and invasive procedures such as arterial or venous umbilical catheterization, central venous or peripheral catheterization, mechanical ventilation, surgical procedures, peritoneal dialysis, parenteral nutrition, chest drainage, and ventriculoperitoneal shunts. The possibility that foreign bodies had been removed during CNS infection was also examined.

The progression of the clinical situation of the newborn during the week before and after CNS isolation was analyzed, with emphasis on diagnosis and clinical charts suggestive of CNS infection, characterized by insidious and non-specific symptoms, more frequently affecting general health, thermal instability, and apnea (Hall et al. 1987).

With progression of the clinical situation, alterations in the blood count and/or positivity for C-reactive protein were determined at the time of CNS isolation. Normal hematological parameters were those proposed by Manroe et al. (1979). The deaths observed were attributed to CNS infection occurring within the first 72 h after agent isolation, and possible association with CNS between days 4 and 7 after CNS isolation.

Another aspect investigated and considered to be of clinical relevance was the previous use of antibiotic therapy, including antibiotics adequate for CNS after bacteriological diagnosis, as well as the use of specific antibiotics such as vancomycin, oxacillin, or teicoplanin.

The CNS isolated in the present study were classified as "significant" and "contaminant" according to modified CDC criteria (Garner et al. 1996), as follows:

"Significant" - CNS isolated from newborns who presented three or more of the following features: risk factors for infection, clinical or hematological alterations, and adequate antibiotic therapy. Isolates were also considered to be significant in patients who presented only two of these features and who died without receiving adequate antibiotic therapy.

"Contaminant" - CNS isolated from newborns who presented only risk factors for infection and/or only one of the other features (clinical or hematological alterations, or adequate antibiotic therapy). Isolates from newborns who presented all three features, but showed a satisfactory course of the infection without the administration of adequate antibiotics were also considered to be contaminants. The isolation of another etiological agent from internal fluids and foreign bodies at the time of CNS isolation was also used as a criterion for the classification of contamination.

Study of slime production - Slime production was analyzed as described by Christensen et al. (1982).

Determination of hemolysin production - The production of hemolysins and cytolytic toxins was determined on plates containing blood agar base consisting of 5% rabbit blood and 5% sheep blood incubated at 37°C for 24 h. A positive result was indicated by the formation of hemolysis zones around the isolated colonies.

Determination of lipase and lecithinase - Lipolytic activity (Jessen et al. 1959) was determined on plates containing blood agar base enriched with 0.01% $CaCl_2.2H_2O$ and 1% Tween 80. A positive result was defined as the formation of opacity around the colonies after incubation at 37°C for 18 h, followed by incubation for 24 h at room temperature. The production of lecithinase (Owens 1974) was studied in Baird-Parker medium. A positive result was indicated by the formation of an opaque halo around the colonies.

Determination of DNAse and TNAse - Nuclease (DNAse) and thermonuclease (TNAse) were determined by the metachromatic Toluidine blue O-DNA agar diffusion technique according to Lachica et al. (1971). Positive results were interpreted by comparing the halos obtained with the *S. aureus* reference strain (ATCC 25923), DNAse, and positive TNAse.

Culture supernatants obtained by the sac culture method (Donnelly et al. 1967), as described below, were also tested for DNAse and TNAse production.

Production of toxins - The toxigenic profile of the isolates was determined using the sac culture method of toxin production (Donnelly et al. 1967). Culture supernatants obtained were stored at –20°C until the time of use.

Detection of enterotoxins and TSST-1 - Enterotoxins and toxic shock syndrome toxin 1 (TSST-1) were detected by the reversed passive latex agglutination (RPLA) assay as described by Shingaki et al. (1981). The SET-RPLA-T900 and TST-RPLA-TD940 (Oxoid Diagnostic Reagents) kits were used for the detection of enterotoxin A (SEA), enterotoxin B (SEB), enterotoxin C (SEC) and enterotoxin D (SED), and of TSST-1, respectively. The culture supernatant was previously treated with 5% (v/v) normal rabbit serum or 5% purified rabbit IgG to prevent the occurrence of nonspecific reactions (Pereira et al. 1997). Samples that presented nonspecific reactions even after these procedures were filtered through a Millipore membrane (8 µm) and, if necessary, diluted 1:10 with 0.02 M phosphate buffer in 0.9% NaCl, pH 7.4.

The following international CNS reference strains were used as controls: *S. aureus* (ATCC 13565, SEA producer), *S. aureus* (ATCC 14458, SEB producer), *S. aureus* (ATCC 19095, SEC producer), and *S. aureus* (ATCC 23235, SED producer).

Statistical analysis - The data were analyzed by the χ^2 test or Fisher's exact test, with $n < 20$. The nonparametric Mann-Whitney test was used for the analysis of newborn birthweight and age. The level of significance was set at $p < 0.05$ for all tests.

RESULTS

Organisms - A total of 117 CNS were isolated from different materials collected from 107 newborns. Sixty isolates were obtained from blood cultures collected between 1990 and 1996, 41 isolates were from foreign bodies (30 from catheter tips, 10 from cannula tips, 1 from a chest drain tip), 13 from secretions (2 drain secretions, 5 gastric secretions, 6 tracheal secretions), and three from urine, all obtained between 1994 and June 1996.

Identification of CNS - *S. epidermidis* was the CNS species most frequently isolated, corresponding to 91 (77.8%) isolates. The remaining species were distributed among *S. haemolyticus* (7 isolates, 6%), *S. lugdunensis* (7 isolates, 6%), *S. hominis* (5 isolates, 4.3%), *S. simulans* (4 isolates, 3.4%), *S. warneri* (2 isolates, 1.7%), and *S. xylosus* (1 isolate, 0.8%).

Clinical relevance - Of the 60 blood culture isolates, 35 (58.3%) were interpreted to be significant and 25 (41.7%) to be contaminants. Of the 41 CNS isolated from foreign bodies, 21 (51.2%) were considered to be significant, including 14 isolates from catheter tips (66.7%), six from cannula tips (28.6%), and one from a chest drain tip (4.7%). Of the 13 isolates from secretions, four were considered to be significant, including one from chest drain secretion and three from tracheal secretion.

Of the 107 newborns, 54 had CNS infection and 53 were infection-free. Table I shows that 27 (50%) of the infected newborns presented a birthweight < 1500 g, significantly different from infection-free newborns (20.8%). Median birthweight also showed a significant difference between the CNS-infected group (1495 g) and the infection-free group (2270 g). Median age at CNS isolation differed significantly between the infection group (10 days old) and the infection-free group (4 days old). Most CNS-infected newborns (42, 77.8%) were submitted to two or more invasive procedures, including the use of a catheter in 48 (88.9%), parenteral nutrition in 35 (64.8%), and mechanical ventilation in 33 (61.1%).

The results showed a higher frequency of *S. epidermidis* associated with infection (86.7%) than with contamination (68.4%) ($p < 0.05$). No significant differences were observed for the other species.

Clinically significant CNS were isolated at a higher proportion from blood than secretions ($p < 0.05$), but there was no statistically significant difference when compared to foreign bodies.

Slime production - Fig. 1 shows that 20 (17.1%) of the 117 CNS isolates were positive for slime production, including 18.7% of *S. epidermidis* strains, 28.6% of *S. lugdunensis* strains, 20% of *S. hominis* strains. *S. haemolyticus*, *S. simulans*, *S. warneri* or *S. xylosus* strains were negative for slime production.

Table II shows that 12 (23.1%) of the 52 significant *S. epidermidis* isolates were slime producers compared to five (12.8%) of the 39 contaminant isolates, with no significant difference between groups. Slime production was not observed in any of the other significant species. Among contaminant samples, two of the four *S. lugdunensis* samples and one of the five *S. hominis* samples produced slime.

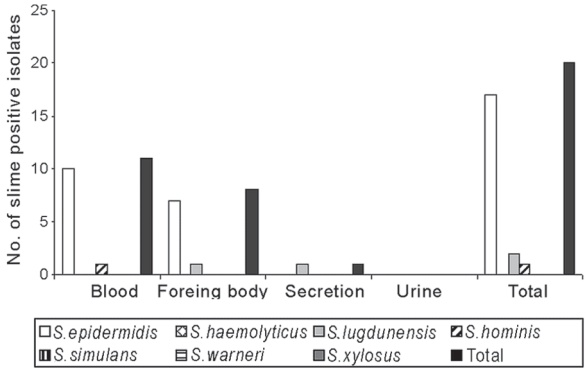

Fig. 1: production of slime by coagulase-negative staphylococci according to species and clinical material. *S: Staphylococcus.*

TABLE I

Perinatal risk factors of coagulase-negative staphylococci infection

Risk factors	With infection		Infection free		Total		
	N	%	N	%	N	%	p value
Median BW (g)	1495		2270		1985		0.0011
BW < 1500 g	27	50	11	20.8	39	36.4	0.0002
Median age (days)	10		4		7		0.0042
Catheter	48	88.9	33	62.3	81	75.7	0.0004
Chest drainage	10	18.5	5	9.4	15	14	ns
Mechanical ventilation	33	61.1	18	34	51	47.7	0.0138
Parenteral nutrition	35	64.8	22	41.5	57	53.3	0.0040
Ventriculoperitoneal shunt	3	5.5	2	3.8	5	4.7	ns
Non-removal of foreign body	31	54.7	17	32.1	48	44.8	0.0010
Membrane rupture > 24 h	18	33.3	17	32.1	35	32.7	ns
Two or more foreign bodies	42	77.8	20	37.7	62	57.9	0.0001
Total of neonates	54	50.5	53	49.5	107	100	

BW: birthweight; ns: $P > 0.05$ (values did not differ significantly at the 5% level of significance).

TABLE II

Frequency of enzyme- and slime-producing coagulase-negative staphylococci isolates according to species and clinical relevance

	Species											
	S. epidermidis		*S. haemolyticus*		*S. lugdunensis*		*S. hominis*		*S. simulans*		*S. warneri*	
	S^{ns}	C^{ns}	S	C	S	C	S	C	S	C	S	C
Enzymes	52[a]	39	2	5	3	4	0	5	1	3	1	1
Hemolysin	7	4	2	3	1	4	0	0	0	0	1	0
Lipase	6	8	1	1	0	1	0	0	0	2	0	1
Lecithinase	1	1	0	1	0	0	0	0	0	0	1	0
DNAse	3	6	1	1	3	2	0	0	0	2	0	0
TNAse	2	3	0	0	1	2	0	0	0	1	0	0
Slime	12	5	0	0	0	2	0	1	0	0	0	0

S: Staphylococcus; *a*: total number of strains; S: clinically significant strains ; C: contaminant strains; ns: $P > 0.05$ (values did not differ significantly at the 5% level of significance).

Enzyme production - The distribution of significant *S. epidermidis* isolates according to enzyme production is shown in Table II. Enzyme concentration is important for the identification of CNS producers of DNAse and TNAse. Direct detection of DNAse and TNAse in supernatants from overnight cultures in BHI broth only revealed production by one *S. lugdunensis* strain. However, when the culture supernatants were concentrated by the sac culture method (Donnelly et al. 1967), production was observed in various species.

Of the 52 clinically significant *S. epidermidis* isolates, seven produced hemolysin, six lipase, three DNAse, and two TNAse. No significant difference in enzyme production was observed between the *S. epidermidis* contaminant isolates and the clinically significant isolates.

The two clinically significant enzyme-producing *S. haemolyticus* isolates produced hemolysin, one produced lipase, and one produced DNAse (Table II). Among the three clinically significant *S. lugdunensis* isolates, one produced hemolysin, all three produced DNAse, and one produced TNAse (Table II).

Production of enterotoxins and TSST-1 - Fig. 2 shows that 44 (37.6%) isolates produced one or a combination of two or more enterotoxins. The distribution of CNS toxin producers according to clinical relevance is shown in Table III. Of the 52 clinically significant *S. epidermidis* isolates, 18 (34.6%) produced enterotoxins, 14 of them producing only SEC, one concomitantly producing SEA and SEB, two producing SEB and SEC, and one simultaneously producing SEA, SEB, and SEC. There was no significant difference in enterotoxin production between clinically significant and contaminant *S. epidermidis* isolates.

Simultaneous production of SEA and SEB and SEB and SEC was also observed in one clinically significant *S. lugdunensis* strain (Table III). Isolated production of SEC was observed in one isolate each of contaminant *S. haemolyticus*, *S. lugdunensis*, *S. hominis*, and *S. simulans* (Table III). The only *S. simulans* strain considered to be significant also produced SEC, whereas *S. warneri* and *S. xylosus* produced no enterotoxins. None of the 117 CNS studied produced SED or TSST-1.

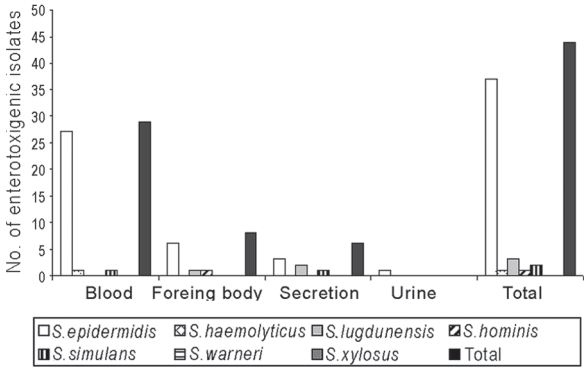

Fig. 2: production of enterotoxins by coagulase-negative staphylococci according to species and clinical material. *S: Staphylococcus*.

DISCUSSION

CNS are important microorganisms indigenous to humans and have emerged over recent years as etiologic agents in a series of infections (Hall et al. 1987, Kloos & Bannerman 1995). In a study carried out by the Centers for Disease Control and Prevention (Atlanta, GA) between 1986 and 1994, involving 99 hospitals, 13,179 cases of infection in newborns were confirmed, with CNS being the agents most frequently isolated in hospital infections (Gaynes et al. 1996). In the 1990s, CNS appeared as the main cause of sepsis in neonatal intensive care units, with an incidence of 33 to 55% among very low birthweight infants (< 1500 g) (Kacica et al. 1994). The occurrence of CNS in neonatal intensive care units has been attributed to the higher survival rates of premature low birthweight newborns and to procedures such as the use of vascular catheters, parenteral nutrition, mechanical ventilation, and prolonged hospital stays (Fleer & Verhoef 1984).

In the present study, *S. epidermidis* was the most frequently isolated species (77.8%), in agreement with other investigators (Hall et al. 1987, Neumeister et al. 1995). It was also the most frequently isolated species in clinically significant situations, such as newborns with infection (88.9%). The predominance of *S. epidermidis* among CNS

TABLE III

Frequency of toxin-producing coagulase-negative staphylococci strains according to species and clinical relevance

	Species									
	S. epidermidis		*S. haemolyticus*		*S. lugdunensis*		*S. hominis*		*S. simulans*	
	S^{ns}	C^{ns}	S	C	S	C	S	C	S	C
Toxins	52^{a}	39	2	5	3	4	0	5	1	3
SEA	0	0	0	0	0	0	0	0	0	0
SEB	0	0	0	0	0	0	0	0	0	0
SEC	14	17	0	1	0	1	0	1	1	1
SED	0	0	0	0	0	0	0	0	0	0
TSST-1	0	0	0	0	0	0	0	0	0	0
SEA+SEB	1	0	0	0	1	0	0	0	0	0
SEB+SEC	2	2	0	0	1	0	0	0	0	0
SEA+SEB+SEC	1	0	0	0	0	0	0	0	0	0
Total	18^{ns}	19^{ns}	0	1	2	1	0	1	1	1

S: Staphylococcus; *a*: total number of strains; S: clinically significant strains; C: contaminant strains; SEA: enterotoxin A; SEB: enterotoxin B; SEC: enterotoxin C; SED: enterotoxin D; TSST-1: toxic shock syndrome toxin-1; ns: $P > 0.05$ (values did not differ significantly at the 5% level of significance).

species causing infection in newborns has been recognized by other investigators, with its frequency ranging from 60 to 90% (Hall et al. 1987, Neumeister et al. 1995).

Although *S. epidermidis* is the most frequent etiological agent, other pathogenic CNS species have been isolated from various clinical sources (Kloos & Bannerman 1995). In the present study, other species were found to be associated with infection, including two *S. haemolyticus* strains, three *S. lugdunensis* strains, and one strain each of *S. simulans*, *S. warneri*, and *S. xylosus*. Hall et al. (1987) isolated three *S. haemolyticus* strains, two *S. hominis* strains, two *S. warneri* strains, and one *S. simulans* strain from children with clinical and laboratory evidence of sepsis and also pneumonia.

There are few reports of infection with *S. lugdunensis* in newborns, probably because this species has only been described recently (Freney et al. 1988). However, studies on adult patients have shown that this species is an opportunistic and significant pathogen (Fleurette et al. 1989, Lambe et al. 1990).

The mechanisms by which CNS provoke infections have not been completely elucidated. However, in opportunistic situations, these microorganisms cross protection barriers such as the skin and mucosa and colonize sites adjacent to the normal flora. Several authors have emphasized the production of exopolysaccharide or slime as an epidemiological marker of infection (Christensen et al. 1982, Hall et al. 1987, Vogel 2000). On the other hand, other investigators have found no association between slime-producing strains and the occurrence of infections caused by these microorganisms (Christensen et al. 1983, Riley & Schneider 1992).

The present study shows that a small proportion of the isolates which produce this exopolysaccharide (22.2%) are associated with infectious processes. Other authors also found no evidence of slime being a virulence factor (Christensen et al. 1982). Riley and Schneider (1992) also suggested that slime production does not seem to be an important virulence factor of *S. saprophyticus* isolated from women with urinary tract infection.

Various experiments using animal models have been carried out to determine the importance of slime as a virulence factor. Baddour et al. (1984), in an experimental study on catheter-induced endocarditis in rats, observed a difference in virulence between *S. epidermidis* and *S. hominis*. All animals inoculated with *S. epidermidis* developed endocarditis compared to only 12.5% of animals inoculated with *S. hominis* ($p < 0.001$). However, the authors found no association between slime production and the development of endocarditis, suggesting that this polysaccharide is not a critical determinant of virulence. Additionally, Patrick et al. (1992), using mice with subcutaneous implants, observed that slime-producing samples did not increase the risk of infection. The authors suggested that traumatized tissue, associated with the presence of catheters, might be a sufficient condition for the development of infections caused by CNS, and that factors other than slime-mediated colonization determine the pathogenicity of these microorganisms.

However, if slime production promotes adherence to prostheses, thus acting as a virulence factor, infection control becomes more difficult since it protects CNS cells from antimicrobial agents and the host's natural defense mechanisms. Gray et al. (1984) reported that the mucous substance produced by CNS can interfere with the cell-mediated immune response. Davenport et al. (1986) showed that only 32% of infections caused by slime-producing CNS were cured by antibiotics, whereas a 100% success rate was achieved for non-producing strains. These results suggest that the control of infections caused by slime-producing CNS requires the removal of the prosthesis, as well as conventional antibiotic therapy.

Slime production can also vary among different species. According to Christensen et al. (1983), this characteristic is more frequent in strains of *S. capitis*, *S. epidermidis*, *S. hominis*, and *S. saprophyticus*. Among

the CNS species isolated in the present study, slime production was observed in *S. epidermidis*, *S. lugdunensis*, and *S. hominis*. Slime production by *S. lugdunensis* has also been reported by Fleurette et al. (1989).

The study of CNS pathogenicity has also shown that various metabolites are produced by these microorganisms, including enzymes and toxins which may play a role in the pathogenicity of these microorganisms (Gemmell 1987). Other authors have observed the production of hemolysins or cytolytic toxins by CNS. In the present study, hemolysins were produced by isolates of *S. epidermidis*, *S. haemolyticus*, *S. lugdunensis*, and *S. warneri*, but not by strains of *S. hominis*, *S. simulans*, and *S. xylosus*. Similar results have been reported by Kloos and Schleifer (1975), Fleurette et al. (1989), Lambe et al. (1990), and Cunha et al. (2004).

The production of lipase, DNAse, and TNAse by these organisms has also been reported. Lambe et al. (1990) verified that most *S. epidermidis*, *S. warneri*, and *S. hominis* strains included in their study produced lipase and DNAse. In the present investigation, except for *S. hominis* and *S. xylosus*, all species produced lipase. With regard to DNAse and TNAse production, production of these enzymes was observed in strains of *S. epidermidis*, *S. lugdunensis*, and *S. simulans*, and production of DNAse by *S. haemolyticus*. Production of TNAse by *S. lugdunensis* has also been demonstrated by Fleurette et al. (1989). Gramoli and Wilkinson (1978) detected TNAse production in some strains of *S. xylosus*, *S. simulans*, *S. capitis*, and *S. sciuri*.

Among the species included in the present study, *S. epidermidis* was the only one that produced all of these exoenzymes, but when the isolates involved in the etiology of the infections were compared with the contaminant isolates, no significant difference was observed. These findings are similar to those reported by Nataro et al. (1994), and suggest that the infections caused by these microorganisms do not only depend on virulence factors but also on the conditions that predispose the host to infection, including factors innate to newborns and the use of invasive procedures. Analysis of risk factors in newborns indicated that a birthweight < 1500 g, the presence of foreign bodies – catheters, mechanical ventilation, parenteral nutrition etc. – and non-removal of foreign bodies were factors significantly predisposing to CNS infection.

In the present study, concomitant production of SEA + SEB and SEB + SEC and isolated production of SEC were observed in these organisms. Production of SEC was also detected in one *S. haemolyticus* strain, one *S. hominis* strain, and one *S. simulans* strain, but production of SED and TSST-1 was not observed in any of the CNS studied.

Valle et al. (1991) found a toxigenic capacity in 45 (16.5%) CNS isolated from goats, including *S. epidermidis*, *S. haemolyticus*, *S. warneri*, and *S. xylosus*, which simultaneously produced TSST-1 and TSST-1 + SEC. Crass and Bergdoll (1986) reported the isolated production of SEA and SEC, or in combination with TSST-1, by CNS isolated from patients with toxic shock syndrome or other infections. However, production of TSST-1 by CNS has been questioned by other investigators who did not confirm these findings (Parsonnet et al. 1987, Kreiswirth et al. 1987).

Although much controversy about the production of these toxins by CNS still exists, the present results and those reported by other authors demonstrate that their toxic ability cannot be ignored. According to Bergdoll and Chesney (1991), these microorganisms have been associated with the etiology of serious staphylococci infections, and there is no reason not to consider them as toxic.

The exact role of extracellular staphylococci products in the pathogenicity of a systemic infection is still unclear. Enterotoxins and TSST-1 have received renewed attention by researchers due to their "superantigen" properties. As "superantigens", enterotoxins bind directly to the class II major histocompatibility complex, without the typical process of normal antigens, resulting in the stimulation of many T cells and, therefore, an overproduction of cytokines such as interleukin-1 (IL-1), IL-2, gamma interferon, and tumor necrosis factor alpha (Marrack & Kappler 1990). Current evidence indicates that the physiological events that occur in neonatal sepsis are mediated by activated cytokines in response to the presence of bacterial components (Kilpatrick & Harris 1998). The fact that these toxins are superantigens and that the release of immunological mediators increases the inflammatory response observed during the pathogenesis of sepsis and neonatal shock suggests that enterotoxins and TSST-1 may play an important role in the progression of infections caused by toxigenic CNS strains.

The existing divergences regarding the toxigenicity of CNS emphasize the need for further studies using sensitive and reliable genotypic techniques to confirm the ability of these staphylococci to produce toxins.

Our study revealed the presence of one or more virulence factors in 77.8% of the CNS strains isolated, suggesting that CNS virulence factors provide a selective advantage for skin colonization of hospitalized newborns. Very low birthweight newborns submitted to invasive procedures show a higher risk of subsequent infection with these strains.

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