Toxigenicity in *Staphylococcus* with emphasis on coagulase-negative staphylococci

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Representatives of the *Staphylococcus* genus are the most common pathogens found in hospital environments, and they are the etiological agents for a large variety of infections. Various virulence factors are responsible for the symptoms and severity of infections caused by *Staphylococcus aureus*. Among them are staphylococcal enterotoxins (SEs), which cause staphylococcal food poisoning, and Toxin-1 of the Toxic Shock Syndrome (TSST-1). Some reports indicate the production of TSST-1 and of staphylococcal enterotoxins also by coagulase-negative staphylococci (CNS). This review mainly aims at approaching aspects related to the toxigenic capacity in *Staphylococcus*, with emphasis on coagulase-negative staphylococci, since such microorganisms are becoming more and more frequent in nosocomial infections.

Keywords Toxins; *Staphylococcus aureus*; Coagulase-negative staphylococci

1. Staphylococcal toxins

1.1 General aspects

Staphylococcal toxins can be divided into two groups, the hemolysins or cytotoxins according to their capacity of lysing cells, which are capable of producing lesion directly to the outer membrane of target cells [1], and the so-called superantigenic toxins, which do not have direct lytic action, but can produce lesion through the overproduction of cytotoxins from activated T-cells and from the monocytes/macrophages [2].

There are presently 18 serologically distinct enterotoxins designated by letters SEA, SEB , SEC, SED, SEE, SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER, SEU [3] and toxin TSST-1, denominated Toxin 1 of the Toxic Shock Syndrome [4].

Staphylococcal enterotoxins (SEs) are monomeric, globular water-soluble proteins with molecular weight from 26,000 to 29,000 daltons which are rich in lysine, aspartic and glutamic and with cysteines forming the disulfide bridge [5]. They are relatively resistant to heat and to proteolytic enzymes trypsin, pepsin, renin, which enables their passage through the gastrointestinal tract without activity loss [5]. Thermostable enterotoxins and their inactivation depend on temperature as well as on the medium's purity, composition and pH. Studies have shown that SEC is the most thermostable of enterotoxins, followed by SEB and SEA [6].

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1.2 Genetic aspects

Many of the genes responsible for virulence factors are located in strain-specific genetic elements. Genetic elements such as plasmids, transposons, bacteriophages and, more recently, pathogenicity islands have been described for *S. aureus* (Table 1) [7].

Various regulation systems are involved in the determination of *S. aureus* virulence, such as the accessory gene regulator (*agr)*, staphylococcal accessory regulator (*sar*) and various homologous *sar*s, as for instance the repressor of toxins (*rot*) [8-10]. Of all these existing regulation systems, those which have been best characterized are *agr* and *sar* [11].

Table 1 – Genetic support of some staphylococcal enterotoxin genes.

Gene	Genetic location	References
sea	prophage	Betley e Makalanos, 1985; Betley,1994
seb	chromosome, plasmid, transposon	Shafer and Iandolo, 1978; Shalita et al,1977;
seb	transposon	Altboum et al.,1985
sec_{bov}	pathogenicity islands	Fitzgerald et al.,2001
sed	plasmid (pIB485)	Bayles and Iandolo,1989
see	defective phage	Couch et al.,1988
seg	egc, chromosome	Jarraud et al.,2001
sei	egc, chromosome	Jarraud et al.,2001
sej	plasmid (pIB485)	Zhang et al., 1998
sek	pathogenicity island	Orwin et al.,2001
sel	pathogenicity island	Fitzgerald et al., 2001
sem	egc, chromosome	Jarraud et al., 2001
sen	egc, chromosome	Jarraud et al., 2001
seo	egc, chromosome	Jarraud et al., 2001

sea: enterotoxin gene A; *seb*: enterotoxin gene B; sec_{bov}: enterotoxin gene C; *sed*: enterotoxin gene D; *see*: enterotoxin gene E; s*eg*: enterotoxin gene G; *sei*: enterotoxin gene I; *sej*: enterotoxin gene J; *sek*: enterotoxin gene K; *sel*: enterotoxin gene L; *sem*: enterotoxin gene M; *sen*: enterotoxin gene N; *seo*: enterotoxin gene O; *egc*: enteroxin gene cluster.
Source: Loir et al., 2003 [7].

On the *agr* locus, two promoters with opposite directions, P_2 and P_3, produce two transcripts, RNAII and RNAIII, respectively. RNAIII is formed by 510 nucleotides responsible for the transcription of genes for a large number of virulence factors, such as extracellular toxin, enzymes and cellular-surface proteins in *Staphylococcus aureus* [12].

An *agr* operon has also been shown in some coagulase-negative staphylococcus species. In a study [13], RNAIII was detected in *S. lugdunensis* species and other authors [14] described an *agr* locus in *S. epidermidis* with a homology of 68% as compared with the *agr* locus of *S. aureus*. *S. epidermidis* RNAII, similarly to that of *S. aureus*, also codifies proteins *agr A*, *B*, *C*, and *D* involved in the regulation system of genes responsible for toxin production.

1.3 Staphylococcal intoxication

Staphylococcal intoxication occurs after the ingestion of food contaminated with enterotoxin produced by bacteria of the *Staphylococcus* genus, and *S. aureus* is the main agent. However, some authors have reported the isolation of other enterotoxigenic coagulase-positive species, such as *S. intermedius* and *S. hyicus* [15-16].

Studies have also reported the enterotoxigenicity of coagulase-negative staphylococcus, including *S. cohnii, S. epidermidis, S. saprophyticus, S. sciuri, S. warneri, S. chromogenes* and *S. lentus* [17-19].

The major symptoms, which occur from 1 to 6 hours after toxin ingestion, are nausea, vomiting, diarrhea, abdominal pain, headache and muscle cramps. Clinical conditions are relatively mild, with duration of few hours to one day. It may be severe at times, thus requiring hospitalization. This occurs as a result of the ingested amount and of the individual's susceptibility, including dehydration, cephalea, sudoresis and body temperature alteration. Deaths are rare; however, they have been observed among children and elderly individuals [20].

The necessary amount of enterotoxin responsible for causing food intoxication is not exactly known. In a study on human volunteers to whom enterotoxin was administered, it was estimated that only 1-5μg / 70Kg would be capable of triggering symptoms [21]. Evenson et al. [22] described an outbreak involving children after the ingestion of contaminated chocolate milk. The concentration of enterotoxin found was only 0.5 ng of SEA/ml.

1.4 Superantigenicity

In addition to its important role in staphylococcal food intoxication, enterotoxins can also activate the unspecific proliferation of T-cells; therefore they are denominated superantigens.

Bacterial superantigens are capable of unspecifically activate T-cells by externally bonding to the Vβ domain of the T-cell receptor and to the α chain of the class-II MHC molecule with previously unprocessed antigens. Such bond produces a signal that induces the proliferation and polyclonal activation of approximately 10 to 30% of the T-cell repertoire, and CD4 T-cells comprise the prevailing responsive population. Hence, a large production of post-inflammatory cytokines from T-cells, such as IFN-γ and TNF-α, as well as IL-2 from monocytes, such as IL-1 e TNF-α, is observed. The massive production of post-inflammatory cytokines triggers an intense inflammatory response, causing damage to the host's tissues [23].

TSST-1 [4] is responsible for the Toxic Shock Syndrome (TSS), an acute systemic disease characterized by fever, arterial hypotension, cutaneous rash and skin scaling. On the other hand, occasionally, isolated lineages of patients with TSS do not produce TSST-1. As indicated, enterotoxins A, B and C may be related to clinical manifestations of the disease [21]. TSS, which was previously associated with the use of vaginal tampons, may occur after any staphylococcal infection caused by a TSST-1-producing lineage, and *S. aureus* is the major species involved [24]. Nevertheless, some reports indicate TSST-1 production also by CNS [18-19].

Staphylococcal enterotoxins also play a role in other serious pathological processes, and they are related to sepses, osteomyelitis and the respiratory distress syndrome, which is characterized by a pulmonary dysfunction almost always associated with a septic process [25].

2. Toxigenicity in coagulase-negative staphylococci

The existing disagreement concerning the enterotoxigenicity of CNS and their capacity to cause food intoxication and/or other associated diseases point out the need for further studies using reliable techniques which can confirm the capacity of such staphylococci to produce toxins.

In Brazil, when investigating the presence of toxigenic genes in CNS samples isolated from food by the PCR technique, Cunha [26] found 10% of positive samples, of which 25% were for gene *sec-1* and 75% for gene *sea*. Some authors have also reported the production of enterotoxins and TSST-1 by *S. epidermidis, S. haemolyticus* and *S. warneri* by using the RPLA method [19]. When investigating food handlers, Udo et al. [18] also found, by using the RPLA method, samples of CNS and *S. aureus* which produced enterotoxins and TSST-1. Of the total CNS investigated, 14.1% were enterotoxins or TSST-1 producers, and samples of species *S. hominis, S. warneri, S. saprophyticus, S. epidermidis, S. xylosus, S. haemolyticus* and *S. schleiferi* were positive for toxins SEA, SEB, SEC and/or TSST-1. Marín et al. [17] describe a study performed on enterotoxin-producing staphylococci in ham samples by the RPLA

method. Of the 135 isolated staphylococci, two belonged to the *S. epidermidis* species and one was an SEC producer.

When studying the virulence factors of CNS isolated from RN, Cunha et al. [27] found, by the RPLA method, a percentage of 37.6% of CNS producing SEA, SEB or SEC. Species *S. epidermidis,* and *S. lugdunensis* produced SEA, SEB and SEC whereas *S. haemolyticus*, *S. hominis* and *S. simulans* produced only SEC.

Cunha [28] used the PCR technique to detect the genes responsible for enterotoxins in lineages of staphylococcus isolated from newborns. The results obtained for PCR were compared to those by the Reverse Passive Latex Agglutination (RPLA) method. From the total number of 120 *S. aureus* samples isolated, 38.3% were enterotoxin producers, according to RPLA, whereas PCR detected 46.6% of positive samples. Coagulase-negative staphylococcus presented 40.0% of positive lineages by PCR as compared to 26.7% by the RPLA method.

Although reports of toxigenic genes in CNS species can be found in the literature, many authors still question their toxigenic potential; therefore, genotypic identification is important for the confirmation of CNS species. Lotter & Genigeorgis [29] reported not to believe that coagulase-negative staphylococci could produce enterotoxins and suggested the possibility of error in species identification. Later, Fox et al. [30] reported the isolation of *S. aureus* lineages which produced little coagulase or did not express that enzyme, being identified as coagulase-negative staphylococci. Hence, genotypic identification confirms phenotypic identification by excluding the possibility of error.

With the purpose to reject this possibility, Cunha et al. [31] performed genotypic identification by the Internal Transcribed Spacer-PCR (ITS-PCR) technique for species confirmation in CNS samples presenting toxigenic genes. Those authors observed that all the samples identified as CNS by the phenotypic method were confirmed to be CNS by the genotypic technique.

The PCR technique, however, enables the detection of genes contained in the lineages independently of their expression, since although the gene is present in the microorganism, it may not be active. The development of genotypic methods also provides the possibility of detecting the RNAm sequence which is responsible for the target-enterotoxin expression, by using the RT-PCR technique. The presence of the RNAm sequence which codifies toxin synthesis leaves no doubts as regards the microorganism's toxic potential. RNAm research has become a practical tool, since when the presence of genes for certain virulence factors is investigated by the PCR technique utilizing specific primers, such very primers can be used to confirm the expression of genes by the cDNA of the mRNA.

In a study on CNS samples isolated from newborns in Brazil which used the RT-PCR technique, Cunha et al. [31] observed that from 14 CNS samples presenting toxigenic genes for SEA, three confirmed the RNAm expression of such toxin whereas from the 33 positive samples for SEC by PCR, only three confirmed its expression. None of the thirteen samples which showed to be toxigenic for TSST-1 presented toxin production when investigated by the RT-PCR technique. *S. epidermidis* was the most toxigenic species among CNS, and five samples were positive for RNAm expression which codifies SEA and SEC. Among other CNS species, only *S. lugdunensis* showed positive results for SEC by the RT-PCR technique.

Although various authors have questioned the toxigenic potential of CNS [29-30], operon *agr*, which plays an important role in regulating the expression of staphylococcal toxins, has also been found in other staphylococci species, such as *S. intermedius* [32], *S. lugdunensis* [13] and *S. epidermidis* [14].
The exact mechanism by which these systems are activated or inhibited has not yet been fully clarified. The presence of an *agr locus* in *S. epidermidis* species and its capacity to produce delta toxin associated with this *agr* system [14] makes it evident that the system is acting and does not exclude the possibility of enterotoxin production at any moment. Therefore, the prevalence of *S. epidermidis* among the toxigenic CNS species may be associated with such staphylococcal regulation systems that are also present in this species.

The RT-PCR method is a rapid and efficient technique, and it clearly shows that CNS are capable of expressing RNAm which codifies staphylococcal enterotoxins; however, as it depends on gene activation, further investigation on environmental factors and clarification of the regulation mechanisms that may interfere with its expression must be evaluated.

Various studies distinguish CNS as important pathogens and point out their toxigenic potential by emphasizing that greater attention must be given to such microorganisms, which are still often considered to be only contaminants.

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