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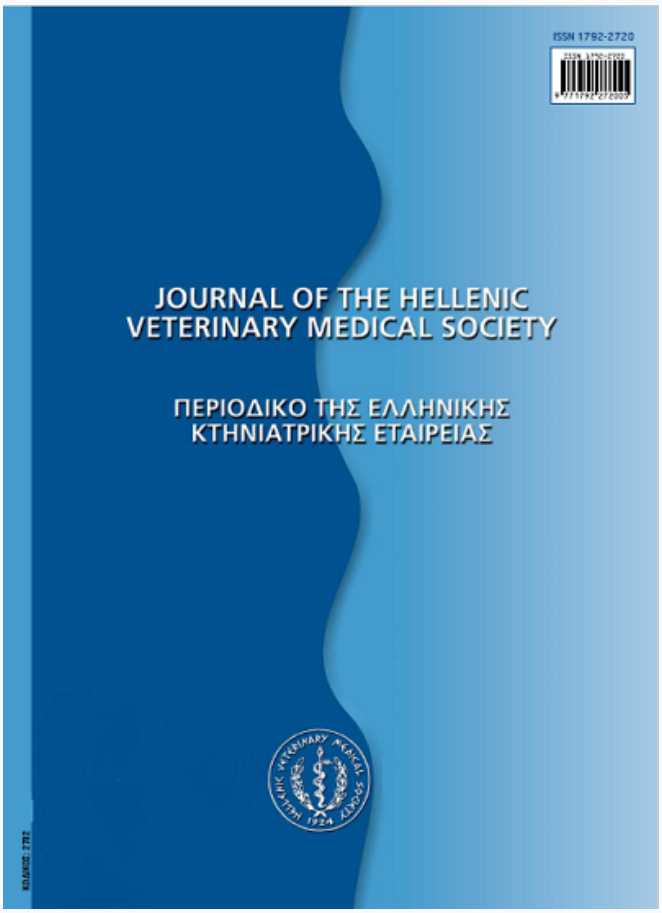

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A PEXARA, A BOURRIEL, A GOVARIS

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Review article
Ανασκόπηση

Staphylococcus aureus and Staphylococcal enterotoxins in foodborne diseases

Pexara A.[1], DVM, PhD, **Burriel A.**[2], DVM, MSc, MSc, PhD,
Govaris A.[1], DVM, PhD, DipECVPH

[1] *Laboratory of Hygiene of Foods of Animal Origin,* [2] *Laboratory of Microbiology and Parasitology*

Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece

Staphylococcus aureus και Σταφυλοκοκκικές εντεροτοξίνες σε τροφιμογενείς νόσους

Α. Πεξαρά[1], DVM, PhD, **Burriel A.**[2], DVM, MSc, MSc, PhD,
Α. Γκόβαρης[1], DVM, PhD, DipECVPH

[1] *Εργαστήριο Υγιεινής Τροφίμων Ζωικής Προέλευσης,* [2] *Εργαστήριο Μικροβιολογίας και Παρασιτολογίας*

Τμήμα Κτηνιατρικής, Πανεπιστήμιο Θεσσαλίας, Καρδίτσα

ABSTRACT. Staphylococcal food poisoning (SFP) is one of the most significant foodborne diseases. It is a mild intoxication, which is caused by the ingestion of food containing one or more preformed staphylococcal enterotoxins (SEs). The toxic dose of SEs in human is usually ranged from 20 ng to 1 μg. SEs are proteins produced by *Staphylococcus* spp in various parts of the environment, including foods. Although several staphylococci can produce SEs, the majority of SFP cases is attributed to S. aureus. Traditionally, five antigenic SE types have been recognized: SEA, SEB, SEC, SED and SEE. During the 1990's, new SEs (SEG, SEH, SEI and SEJ) were reported and their genes were described. Several studies revealed that SEH, SEG and SEI were, also, involved in the gastroenteric syndrome. More recent data have indicated the presence of "new" SE genes and new SEs, designated as "staphylococcal enterotoxin-like" (SEl). The role of SEl in food poisoning has not yet been clarified. In contrast to *S. aureus*, SEs are remarkably heat resistant (D-values of 3–8 min at 121°C). They may be present in foods even when viable cells of *S. aureus* are absent. The enterotoxins are, also, resistant to proteolytic enzymes. It is generally accepted that toxic levels of SEs are produced in foods when *S. aureus* concentration exceeds 10^5 cfu/ml. *S. aureus* can grow at a temperature range of 7–48.5°C, with optimum 30–37°C, a pH range of 4.2–9.3, with optimum 7–7.5, minimum a_w of 0.86, and up to 15% NaCl. SEs can be produced at a temperature range of 10–46°C, with optimum 40–45°C, a pH range of 4.8–9.0, with optimum 5.3–7.0, and a_w range of 0.86–0.99, with optimum 0.90. Among the foods implicated in SFP are usually milk, dairy products and meat. SEA and SED are usually identified in foodborne outbreaks, while SEC is an important cause of SFP associated with the consumption of dairy products. The European regulation has set criteria for presence of SEs in cheeses, milk powder and whey powder (Regulation EC, 2073/2005). If population of coagulase-positive staphylococci in samples exceeds 10^5 cfu/g, these samples should be further tested for the presence of SEs. In this case, SEs must not be detected in 25 g of the products.

Keywords: *Staphylococcus aureus*, enterotoxin, foodborne pathogens.

Correspondence: Govaris A.
Laboratory of Hygiene of Foods of Animal Origin, Faculty of Veterinary Medicine, University of Thessaly, 224 Trikalon Str., 431 00 Karditsa, Greece
Tel.: 0030 24410 66086, Fax: 0030 24410 66087, E-mail: agovaris@vet.uth.gr

Αλληλογραφία: Α. Γκόβαρης
Εργαστήριο Υγιεινής Τροφίμων Ζωικής Προέλευσης, Τμήμα Κτηνιατρικής, Πανεπιστήμιο Θεσσαλίας, Τρικάλλων 224, 431 00 Καρδίτσα
Τηλ.: 24410 66086, Fax: 24410 66087, E-mail: agovaris@vet.uth.gr

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ΠΕΡΙΛΗΨΗ. Η σταφυλοκοκκική τοξίνωση (ΣΤ) είναι ένα από τα πιο συχνά αίτια τροφιμογενούς νόσου. Είναι μια τοξίνωση που προκαλείται από την πρόσληψη με τα τρόφιμα μίας ή περισσοτέρων προσχηματισμένων σταφυλοκοκκικών εντεροτοξινών (ΣΕ). Οι τοξικές δόσεις των ΣΕ για τον άνθρωπο κυμαίνονται από 20 ng έως 1 μg. Οι ΣΕ είναι πρωτεΐνες που παράγονται από ορισμένα είδη σταφυλόκοκκων τόσο στο περιβάλλον όσο και στα τρόφιμα. Παρόλο που διάφορα είδη σταφυλόκοκκου μπορούν να προκαλέσουν τροφιμογενείς λοιμώξεις, σχεδόν όλα τα περιστατικά ΣΤ αποδίδονται στο *S. aureus*. Παραδοσιακά έχουν αναγνωριστεί οι κλασικοί τύποι σταφυλοκοκκικών εντεροτοξινών SEA, SEB, SEC, SED και SEE. Στη δεκαετία του 1990 ανιχνεύτηκαν νέες τοξίνες (SEG, SEH, SEI, SEJ) και προσδιορίστηκαν τα γονίδια που ελέγχουν την παραγωγή τους. Πιο πρόσφατα στοιχεία οδήγησαν στον προσδιορισμό επιπλέον «νέων» γονιδίων για την παραγωγή ΣΕ. Οι νέες αυτές ΣΕ αναφέρονται ως σταφυλοκοκκικές εντεροτοξίνες του τύπου ΣΕ "staphylococcal enterotoxins-like" (SEl) και ο ρόλος τους στην πρόκληση τροφιμογενούς νόσου δεν έχει ακόμη πλήρως διευκρινιστεί. Αντίθετα με το μικροοργανισμό που τις παράγει, οι ΣΕ είναι ιδιαίτερα ανθεκτικές στην επίδραση της θερμικής επεξεργασίας (D_{121} τιμές 3–8 min) και μπορεί να βρίσκονται στα τρόφιμα, ακόμη και αν δεν υπάρχουν ζωντανά κύτταρα του *S. aureus*. Οι ΣΕ είναι επίσης ανθεκτικές στη δράση των πρωτεολυτικών ενζύμων. Είναι γενικά αποδεκτό ότι οι ΣΕ παράγονται σε τοξικές δόσεις για τον άνθρωπο, όταν ο πληθυσμός του *S. aureus* στο τρόφιμο υπερβαίνει τα 10^5 cfu/ml. Ο *S. aureus* αναπτύσσεται σε εύρος θερμοκρασιών 7–48,5°C, με βέλτιστη 30–37°C, σε εύρος τιμών pH 4,2–9,3, με βέλτιστη 7–7,5, σε ελάχιστη τιμή a_w 0, 86 και σε συγκέντρωση NaCl έως 15%. Οι ΣΕ παράγονται σε θερμοκρασίες μεταξύ 10–46°C, με βέλτιστη 40–45°C, pH 4,8–9,0 με βέλτιστη 5,3–7,0, και σε τιμές a_w μεταξύ 0,86–0,99 με βέλτιστη 0,90. Μεταξύ των τροφίμων που αναφέρονται συχνότερα στην πρόκληση ΣΤ είναι το γάλα, τα γαλακτοκομικά προϊόντα και το κρέας. Οι τοξίνες που συχνότερα ανιχνεύονται σε τροφιμογενείς λοιμώξεις από την κατανάλωση διαφόρων τροφίμων είναι οι SEA και SED, ενώ από την κατανάλωση γαλακτοκομικών προϊόντων είναι η SEC. Η Ευρωπαϊκή Ενωση έχει θεσπίσει κριτήρια για την παρουσία των ΣΕ στα τυριά, γάλα σε σκόνη και σκόνη ορού γάλακτος (Κανονισμός 2073/2005). Δείγματα που έχουν πληθυσμό σταφυλόκοκκων θετικών στην πηκτάση μεγαλύτερο από 10^5 cfu/g πρέπει να ελέγχονται για την παρουσία ΣΕ, που δεν θα πρέπει να ανιχνεύονται στα 25 g δείγματος.

Λέξεις ευρετηρίασης: *Staphylococcus aureus*, εντεροτοξίνες, τροφιμογενή παθογόνα.

INTRODUCTION

Staphylococcal food poisoning (SFP) is one of the most significant foodborne diseases worldwide (Tirado and Schimdt 2001). SFP is considered an intoxication, which is caused by the ingestion of contaminated food with one or more preformed staphylococcal enterotoxins (SEs) (Seo and Bohach 2007).

SEs are proteins produced by certain *Staphylococcus* spp. in various parts of the environment, including food. Although enterotoxin production is primarily associated with coagulase and thermonuclease positive strains of Staphylococcus aureus, many coagulase positive or negative *Staphylococcus* spp. can, also, produce SEs. Certain coagulase positive strains of *S. intermedius* have been found enterotoxigenic (Becker et al. 2001) and have been clearly involved in SFP outbreaks (Khambaty et al. 1994). Among the coagulase negative *Staphylococcus* spp., *S. cohnii, S. epidermis, S. xylosus* and *S. haemolyticus* can, also, produce one or more SEs (Bautista et al. 1988). Particularly, *S. epidermidis* was, also, implicated as the aetiological agent in a foodborne outbreak (Breckinridge and Bergdoll 1971). However, the majority of the SFP cases are almost attributed to *S. aureus* (Seo and Bohach 2007).

Staphylococcus aureus

S. aureus is a major foodborne pathogen. It is usually found in humans and animals and can cause various diseases ranging in severity, from slight skin infection to complicated diseases, such as pneumonia and septicaemia (Lowy 1998). It is usually harboured in the nares of 20–30% of healthy individuals, while about 60% of the human population can carry the microorganism intermittently (Kluytsman et al. 1997). *S. aureus* is, also, present on the skin and mucosae of food-producing animals, such as ruminants, and it is frequently associated to mastitis leading to contamination of milk and dairy products (Jablonski and Bohach 1997). The organism has been identified as the aetiological agent of 30% to 40% of all mastitis cases (Asperger and Zangerl 2003).

S. aureus is, also, a ubiquitous organism. It may be found in many parts of our environment, including dust, water, air, clothes or equipments.

— Characteristics of *S. aureus*

S. aureus is a non-motile, cluster forming spherical Gram-positive bacterium (Huong et al. 2010). It is a facultative anaerobic bacterium and can grow at a temperature range of 7-48.5°C, and optimum 30-37°C

(Schmitt et al. 1990), a pH range of 4.2-9.3, and optimum 7-7.5 (Bergdoll 1989), or up to 15% NaCl. Several studies indicate a minimum a_w (water activity) of 0.86 (Le Loir et al. 2003). Due to these characteristics, *S. aureus* can grow in a wide variety of foods.

S. aureus is quite sensitive to microbial competition. Antagonistic bacteria to *S. aureus* growth are several bacterial species, such as *Acinetobacter, Aeromonas, Bacillus, Pseudomonas, S. epidermidis*, the Enterobacteriaceae, the Lactobacillaceae and enterococci (Mossel 1975).

The organism is usually readily killed at pasteurisation temperatures. D60 (values at 60°C) were found between 2 to 50 min depending on the type of the food. During heating, the cells of S. aureus are quite sensitive in Ringer's solution at pH 7.2 (D_{60} = 0.11 min), but resistant in milk at pH 6.9 (D_{60} = 10.0 min). In frankfurters, heating at 71.1°C was found to be destructive to several strains of *S. aureus* (Palumbo et al. 1977).

S. aureus strains can also be classified into biotypes according to their human or animal origin (Le Loir et al. 2003).

Staphylococcal Enterotoxins (SEs)

— Classification

SEs are exoproteins forming a single chain with a molecular weight ranging from 26,000 to 29,600 Da and belong to a large family of pyrogenic toxin, known as superantigens (Normanno et al. 2005). Traditionally, five classic antigenic SE types have been recognized: SEA, SEB, SEC, SED and SEE. SECs furthermore are distinguished into three subtypes SEC1, SEC2 and SEC3 (Balaban and Rasooly 2000). During the 1990's, new SEs (SEG, SEH, SEI and SEJ) were reported and their genes were described. More recent data resulting from partial or complete genome sequence analyses have led to the description of further "new" SE genes (Morandi et al. 2007). The role of these new SEs in food poisoning has not yet been elucidated (Boerema et al. 2006, Vernozy-Rozand et al. 2004). Studies revealed that the new SEs lack emetic activity, while their toxic properties were not investigated (Vernozy-Rozand et al. 2004). Thus, it has been proposed to designate them "staphylococcal enterotoxin-like" SEl (Lina et al. 2004). The classical SEs (SEA–SEE), as well as the new SEs, SEH (Su and Wong 1995) SEG and SEI (Omoe et al. 2002) can, also, induce the gastroenteric syndrome.

S. aureus can, also, produce the toxic shock syndrome toxin 1 (TSST-1). This toxin was initially recognized as the causative agent of the toxic shock syndrome in humans and animals (Akineden et al. 2001). TSST-1 was once referred to as enterotoxin F (Bergdoll et al. 1991, 1982). Although this toxin shares many biological activities with SEs, it did not cause emesis.

Production of SEs is encoded by phage (SEA), chromosome (SEC) or plasmid genes (SED, SEJ) (Proft and Fraser 2003, Balaban and Rasooly 2000, Zhang et al. 1998, Johnson et al. 1991). The gene for SEB is chromosomal in clinical isolates of S. aureus. However, the same gene is carried by a plasmid in other strains of S. aureus (Balaban and Rasooly 2000). DNA sequence identity indicates that SEA, SED and SEE are closely related (Van den Bussche et al. 1993).

SEA and SEB are often produced by the human biovars, while SEC and SED are predominantly produced by the ovine biovars and the Non-Host-Specific (NHS) biovars, respectively. With the exception of SEE, all the rest SEs can be synthesized by animal biovar strains. Thus, the synthesis of SEs supports the potential zoonotic role S. aureus in SFP (Olsvik et al. 1981).

— Properties

Although the various SEs differ in certain physicochemical properties, they are also sharing basic characteristics. Unlike the producing organism, SEs are remarkably heat resistant and they may be present in foods even when viable *S. aureus* are absent (Jorgensen et al. 2005, Jablonski and Bohach 1997). Heat stability seems to be dependent on the growth media or foods, the toxin type, the pH, salt concentration and other environmental factors related to the level of toxin denaturation. SEs usually show D121 values of 3 min to 8 min (Asperger and Zangerl 2003). SEB was found active even after heating for 16 hours at 60°C and pH 7.3 (Schantz et al.1965). The $D_{121.1}$ value of SEB in laboratory media was estimated 9.9-11.4 min (Genigeorgis 1989). Heating of SEC at 60°C for 30 min showed no change in toxin activity. The heating of SEA at 80°C for 3 min or at 100°C for 1 min resulted in its inactivation (Bergdoll 1967). In general, the heat resistant of SEB has been found

higher than that of SEA, but lower than that of SEC (Tibana et al. 1987).

The enterotoxins are, also, resistant to proteolytic enzymes, such as trypsin, chymotrypsin, rennin and papain (Bergdoll 1967).

— Production

It is well established that staphylococci can, also, grow with no production of any enterotoxin. The number of *S. aureus* cells required to produce the minimum toxic dose of SEs in humans, depends on the food and the enterotoxin type. SEA was detected, when population of S. aureus were low (10^4 cfu/g) in laboratory media (Hirooka et al. 1987), or high (10^7 cfu/g) in meat products (Notermans et al. 1983). It is generally accepted that SEs are produced in foods at toxic doses for human, when S. aureus concentration exceeds 10^5 cfu/g (Le Loir et al. 2003, Tranter 1996).

SEs can be produced at a temperature range of 10-46o C, with optimum 40- 45 °C. SEs were produced in meat products under anaerobic conditions during storage at 10 °C for several weeks (Genigeorgis et al. 1969; Tatini 1973). In unpasteurized milk at 10 °C, SE production was observed after 3 weeks (Schmitt et al. 1990). It is well known that refrigerated storage of foods does not favour production of SEs at sufficient levels for initiating the SFP (Schmitt et al. 1990). A risk of SE production is only evident, when foods are stored at 14 to 15 °C for several days or at room temperatures of 18 to 25 °C for many hours (Schmitt et al. 1990).

SEs can, also, be produced at a pH range of 4.8 - 9.0 and optimum 5.3-7.0, a_w range of 0.86-0.99 and optimum 0.90 (Smith et al. 1983). SEs were produced in broths with 10% NaCl and a pH value of 5.45 or higher, but not in broths with 12% NaCl (Genigeorgis et al. 1971). SEB was produced in broths with 10% NaCl and pH 6.9, but not in broths with 4% NaCl and pH 5.1 (Genigeorgis et al. 1971). Microbial competition can, also, affect SEs production. Growth of several lactic acid bacteria (LAB) resulted in a decrease of SEA production because of the specific enzymes and other metabolites of the LAB (Chordash and Potter 1976).

— Mode of Action

SEs belong to a large family of staphylococcal and streptococcal pyrogenic exotoxins. These toxins were implicated in foodborne diseases, several allergic and autoimmune diseases, as well as in toxic-like shock syndromes. In addition to SEs, two forms of the toxic shock syndrome toxin (TSST) and a group of streptococci pyrogenic exotoxins were, also, included within this group (Balaban and Rasooly 2000).

SEs can act as potential gastrointestinal toxins, as well as superantigens that stimulate non-specific T-cell proliferation. Although these two separate functions are localized on separate domains of the proteins, there is a high correlation between these activities (Harris et al. 1993).

The SEs action as superantigens is well understood, but little is known about SEs action in SFP. It was found that intestinal cells can facilitate the transcytosis of SEs (Shupp et al. 2002). Ingested SEB may be found in the blood faster than ingested SEA (Hamad et al. 1997). Emetic activity of SEs has, also, been attributed to a direct effect on intestinal epithelium or the stimulation of the emetic center via the vagus nerve (Le Loir et al. 2003). Data from recent works revealed that SEA and SEB induced massive intestinal cytokine production, which was the key factor in the initiation of the inflammatory response and the diarrheal disease (Pinchuk et al. 2007).

— Detection of SEs

Several methods for SEs' detection in foods have been developed throughout the recent years, including biological, immunological, chromatographic and molecular assays (Normanno et al. 2007). Among the immunological methods, enzyme-linked immunosorbent assay (ELISA), enzyme-linked fluorescent assay (ELFA) and reverse passive latex agglutination (RPLA) are the most common methods for the detection of the classic SEs.

The polymerase chain reaction (PCR) has been introduced as a simple technique for the detection of enterotoxigenic strains (Peles et al. 2007). Although the PCR-based approach is specific, highly sensitive and rapid, it can only demonstrate the presence of enterotoxin genes in S. aureus isolates rather than the production of the SE protein (Boerema et al. 2006).

Characteristics of Staphylococcal food poisoning (SFP)

SFP is a mild intoxication, which is caused by eating foods contaminated with SEs (Berdgoll 1989). The toxic doses of SEs in humans are not known. However,

information from food poisoning outbreaks (Bergdoll 1990, Evenson et al. 1988) and human challenge studies (Dangerfield 1973) indicate that individuals experiencing illness probably consumed 20 ng to 1 μg of SEs. Despite the differences among various enterotoxins, each SE has about the same potency of inducing SFP.

SFP symptoms develop within a few hours (1–6 h) after the ingestion of the contaminated food. The symptoms include nausea, abdominal cramps, diarrhoea and a characteristic projectile vomiting (Le Loir et al. 2003). Clinical signs generally disappear within 24–48 h, while deaths occur rarely, and particularly in the very young, elderly and severely debilitated persons (Martin and Iandolo 2000).

Since *S. aureus* can grow and produce SEs under a wide range of conditions, a variety of foods has, also, been implicated in SFP (Le Loir et al. 2003). Due to the low antagonistic activity of the *S. aureus* against other microorganisms, SFP from consumption of raw foods is rare (Bergdoll 1989). Foodstuff can be contaminated directly from infected food-producing animals or from poor hygiene during the production processes, storage and transportation of foods, since humans may, also, carry *S. aureus* (Kusumaningrum et al. 2003).

Among the foods implicated in SFP are usually milk, dairy products and meat (De Buyser et al. 2001, Normanno et al. 2005, Normanno et al. 2007). Implicated foods are often those that require considerable handling during the production process and are stored at slightly elevated temperatures than the refrigerated ones. *S. aureus* grow in the food and produce one or more SEs. The relative heat resistance of the SEs contributes to the prevalence of the disease.

SFP was often ranked third among the reported annual foodborne diseases in the world (Boerema et al. 2006). In U.S., the annual count of SFP cases was 185,000 with about 1,750 hospitalizations in 1998 (Mead et al. 1999), and in Europe *S. aureus* caused 5.1% of the foodborne outbreaks between 1993 and 1998 (Tirado and Schimdt 2001). *S. aureus* was indicated as the causative agent in 182 verified foodborne outbreaks in the European Union in 2007. A total of 1,945 people were affected, 204 were hospitalised and 3 persons died (EFSA 2009).

However, the real incidence of SFP is underestimated due to various reasons, such as unreported sporadic cases because of the mild symptoms, misdiagnosis of the illness, similar symptoms to other types of foodborne diseases (such as vomiting caused by *Bacillus cereus* toxin), inadequate sampling for laboratory analyses and improper laboratory examination (Smyth et al. 2004).

SEA and SED are the most common SEs and are usually identified in foodborne outbreaks (Manfreda et al. 2005, Balaban and Rasooly 2000). The lowest number of outbreaks is associated with SEE. SEC has been recognized as an important source of SFP associated with the consumption of dairy products (Manfreda et al. 2005, Tamarapu et al. 2001).

Control measures and Legislation

Since *S. aureus* is widespread in the environment, application of control measures to prevent the contamination of the foods with the pathogen and SEs is important for the consumers' safety. Application of control measures such as good manufacturing practices (GMP) and hazard analysis critical control points system (HACCP) in food processing lines can prevent contamination of the foods with pathogens like *S. aureus*. The food industry should apply control measures to avoid post production contamination of food products with this pathogen.

Among microbiological criteria for foodstuffs, the European Union has set criteria for the presence of SEs in cheeses, milk powder and whey powder. In accordance with the Commission Regulation No. 2073/2005, samples with coagulase-positive staphylococci counts exceeding 10^5 cfu/g should be further tested for the presence of SEs. In this case, SEs must not be detected in 25 g of the products.

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