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Review

***Staphylococcus aureus* and food poisoning**

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ABSTRACT. Food-borne diseases are of major concern worldwide. To date, around 250 different food-borne diseases have been described, and bacteria are the causative agents of two thirds of food-borne disease outbreaks. Among the predominant bacteria involved in these diseases, *Staphylococcus aureus* is a leading cause of gastroenteritis resulting from the consumption of contaminated food. Staphylococcal food poisoning is due to the absorption of staphylococcal enterotoxins preformed in the food. Here, we briefly review the latest data on staphylococcal enterotoxins and some papers exemplifying the interactions between *S. aureus* and the food matrix; environmental factors affecting staphylococcal enterotoxin production are discussed.

Key words: *Staphylococcus aureus*, Food poisoning, Enterotoxins, Food matrix

INTRODUCTION

Food-borne diseases (FBD) are defined by the World Health Organization as “diseases of infectious or toxic nature caused by, or thought to be caused by the consumption of food or water”. More than 250 FBDs have been described. Symptoms vary widely, depending on the etiological agents. Diarrhea and vomiting are the most common. Among FBDs, food-borne infections are caused by many different disease-causing pathogens that can contaminate foods, while food-borne poisoning is caused by poisonous chemicals, or other harmful substances that are present in food. In many countries, national health care organizations record FBD outbreaks, defined as the occurrence of two or more cases of a similar illness resulting from the ingestion of a common food. True incidence of FBDs is difficult to evaluate, as many cases remain undeclared. Nevertheless, in the United States (Food and Drug Administration; Center for Food Safety & Applied Nutrition. <http://www.cfsan.fda.gov>), FBDs are suspected of ~76 million illnesses, 325,000 hospitalizations, and 5,200 deaths each year. Among these cases, known pathogens, clearly identified and involved in FBD, cause 14 million illnesses, 60,000 hospitalizations, and 1,800 deaths annually. In France, for the two-year period 1999-2000 (records from the Institut de Veille Sanitaire, <http://www.invs.sante.fr>; Haeghebaert et al., 2002), there were 1267 FBD outbreaks, involving 17,378 persons, and causing 1383 hospitalizations, and 10 deaths. In most countries (including the USA and France, whose statistical data are given here as an example, Table 1), bacteria are the leading cause of FBD and appear to be the causative agents of more than two thirds of the recorded FBD outbreaks. Bacteria causing food-borne infections have a pathogenesis centered on their ability to penetrate, survive and multiply in host cells. The pathogenesis of bacteria causing food-borne poisoning depends on their capacity to produce toxins after ingestion (in the digestive tract) or before (toxins preformed in foodstuff). Some Gram-positive bacteria involved in food-borne poisoning are described.

Table 1. Causative agents of food-borne disease outbreaks recorded in France between 1999 and 2000. Frequencies of each type of agent are given in percent.

Causative agents	Outbreaks (N = 530)	Cases (N = 6451)	Hospitalizations (N = 872)	Death (N = 7)
<i>Salmonella</i> sp. (Enteritidis, Typhimurium, Heidelberg, and other serotypes)	63.8	47.7	16.8	100
<i>Staphylococcus aureus</i>	16	25.6	17.1	0
<i>Clostridium perfringens</i>	5.1	12.3	0.5	0
<i>Bacillus cereus</i>	2.8	3.7	10.0	0
Histamine	3.8	1.4	30.4	0
Other pathogens (<i>Campylobacter</i> sp., <i>Dinophysis</i> , <i>C. botulinum</i> , <i>Shigella</i> sp., Calicivirus, HAV, <i>Vibrio</i> sp., <i>E. coli</i> , etc.)	8.5	9.2	7.6	0

After Haeghebaert et al., 2002.

BACTERIA CAUSING FOOD-BORNE POISONING

Among the bacteria that cause food-borne poisoning, some are particularly important in terms of frequency and/or of seriousness of the disease. Miscellaneous bacteria (including Gram positive and Gram negative ones) produce toxins that cause food-borne poisoning, resulting in symptoms ranging from gastrointestinal disorders to paralysis and death. We give here some examples of Gram-positive bacteria, other than *Staphylococcus aureus*, involved in food-borne poisoning.

Clostridium perfringens is the second most common causative agent of FBD in the US, after *Salmonella*. It is an anaerobic, Gram-positive, spore-forming rod (Brynstad and Granum, 2002) and is widely distributed in the environment (frequent in the intestines of humans and many domestic and feral animals). Spores persist in soil, sediments, and areas subject to human or animal fecal pollution. *Clostridium perfringens* causes perfringens food poisoning, the symptoms of which are intense abdominal cramps and diarrhea. Symptoms appear 8 to 22 h after consumption of contaminated foods and are over within 24 h (1 or 2 weeks in the elderly). A more serious but rare illness is caused by food contaminated with type C strains. The latter illness is known as necrotic enteritis or pig-bel disease and is often fatal. Deaths in pig-bel syndrome are caused by infection and necrosis of the intestines and from resulting septicemia. The infective dose is around 10^8 vegetative cells.

Clostridium botulinum is an anaerobic, Gram-positive, spore-forming rod. It produces a potent neurotoxin (Brown, 2000). The spores are heat resistant and can survive in foods that are incorrectly or minimally processed. Food-borne botulism is a severe type of food poisoning caused by the ingestion of foods containing the potent neurotoxin formed during growth of the organism. The toxin is heat labile and can be destroyed if heated at 80°C for 10 min or longer. The incidence of the disease is low, but the disease is of considerable concern because of its high mortality rate if not treated immediately and properly. The infective dose is very small (a few nanograms). The onset of symptoms occurs 18 to 36 h after ingestion. Botulinum toxin causes paralysis by blocking motor nerve terminals at the myoneural junction. The resulting asphyxia causes death.

Bacillus cereus is a Gram-positive, facultatively aerobic, spore-forming rod (McKillip, 2000). Two types of illness are caused by two distinct metabolites. Diarrheal type illness is caused by a large molecular weight heat-labile protein. This type of *B. cereus* poisoning mimics *C. perfringens* food poisoning: watery diarrhea, abdominal cramps, and pain (6 to 15 h after consumption of contaminated food, lasting 24 h). Vomiting (emetic) type of illness is caused by a low molecular weight, heat-stable peptide. This type causes nausea and vomiting (within 0.5 to 6 h after consumption of contaminated food, during less than 24 h), and occasionally, abdominal cramps and/or diarrhea. Symptoms of the emetic type are similar to those caused by staphylococcal food-borne poisoning.

STAPHYLOCOCCUS AUREUS AND STAPHYLOCOCCAL FOOD POISONING

Staphylococcus aureus is a facultative anaerobic Gram-positive coccus; it is non-motile and catalase and coagulase positive. Cells are spherical single or paired cocci, or form grape-like clusters (*staphylo* means grape in greek). The staphylococcal cell wall is resistant to lysozyme and sensitive to lysostaphin, which specifically cleaves the pentaglycin bridges of *Staphylococcus* spp. Some *S. aureus* strains are able to produce staphylococcal enterotoxins (SEs) and are the causative agents of staphylococcal food poisonings. Unlike *C. perfringens*,

C. botulinum, and *B. cereus*, mentioned above, *S. aureus* does not form spores. Thus, *S. aureus* contamination can be readily avoided by heat treatment of food. Nevertheless, it remains a major cause of FBD because it can contaminate food products during preparation and processing. *Staphylococcus aureus* is indeed found in the nostrils, and on the skin and hair of warm-blooded animals. Up to 30-50% of the human population are carriers. *Staphylococcus aureus* is able to grow in a wide range of temperatures (7° to 48.5°C with an optimum of 30 to 37°C; Schmitt et al., 1990), pH (4.2 to 9.3, with an optimum of 7 to 7.5; Bergdoll, 1989) and sodium chloride concentrations (up to 15% NaCl). These characteristics enable *S. aureus* to grow in a wide variety of foods. This, plus their ecological niche, can easily explain their incidence in foodstuffs that require manipulation during processing, including fermented food products, such as cheeses. Risk assessment in foodstuffs relies on classical microbial detection and quantification of coagulase positive staphylococci on a selective Baird-Parker medium, whose composition is standardized (for France, norms AFNOR V08-057/1 and 2, ISO 6888/1 and 2). Sensitivity of these routine tests is around 10² cfu/g for solid foodstuffs and 10 cfu/g for liquid samples. The different media used for the detection and quantification of *S. aureus* have been reviewed by Baird and Lee (1995). In many countries, low degree contaminations by *S. aureus* are tolerated in most foodstuffs (up to 10³ cfu/g in raw milk cheeses, in France), as they are not considered a risk for public health.

Staphylococcus aureus strains can be classified into biotypes according to their human or animal origin. Devriese (1984) developed a biotype schema, including six different biotypes (human, non-β-hemolytic human, avian, bovine, ovine, and nonspecific), based on biochemical characteristics.

Staphylococcus aureus is an important pathogen due to a combination of toxin-mediated virulence, invasiveness, and antibiotic resistance. This bacterium is a significant cause of nosocomial infections, as well as community-acquired diseases. The spectrum of staphylococcal infections ranges from pimples and furuncles to toxic shock syndrome and sepsis, most of which depend on numerous virulence factors. On the other hand, some infections, such as staphylococcal food poisoning, rely on one single type of virulence factor: the SEs. The symptoms of staphylococcal food poisoning are abdominal cramps, nausea, vomiting, sometimes followed by diarrhea (never diarrhea alone). The onset of symptoms is rapid (from 30 min to 8 h) and usually spontaneous remission is observed after 24 h.

ENTEROTOXIGENIC POTENTIAL OF OTHER STAPHYLOCOCCI

Several staphylococcal species other than *S. aureus* reportedly produce SEs (Jay, 1992). For example, among the coagulase negative species, *S. cohnii*, *S. epidermis*, *S. xylosus* and *S. haemolyticus* have been isolated from ewe's milk and were found to produce one or several SEs (Bautista et al., 1988). The coagulase positive *S. intermedius* is the predominant non-*S. aureus* species isolated from food; some strains have been shown to produce SEs (Becker et al., 2001). *Staphylococcus intermedius* is the only non-*S. aureus* species that has been clearly involved in staphylococcal food poisoning outbreaks (Khambaty et al., 1994).

THE STAPHYLOCOCCAL ENTEROTOXINS

Studies on SEs started from the analysis of *S. aureus* strains involved in staphylococcal food poisoning. In the first SEs identified, the peptide sequence was available before the nucleo-

tide sequence. This was the case for SEA (Huang et al., 1987), SEB (Huang and Bergdoll, 1970) and SEC (Schmidt and Spero, 1983). The abundance of literature on SEs varies considerably among the types, according to the chronology of their identification and their importance in staphylococcal food poisoning. To date, 14 different SE types have been identified, which share structure and sequence similarities (Table 2). The SEs are short proteins secreted in

Table 2. Percentage of amino acid identity in different staphylococcal enterotoxins (SE).

Toxin	SEA	SEB	SEC1	SED	SEE	SEG	SEH	SEI	SEJ	SEM	SEN	SEO
SEA	100	33	30	50	83	27	37	39	64	35	39	37
SEB		100	68	35	32	43	33	31	33	29	32	36
SEC1			100	31	29	41	27	26	30	26	29	33
SED				100	52	27	35	33	51	41	38	39
SEE					100	27	35	35	63	37	39	37
SEG						100	34	28	29	28	31	30
SEH							100	33	35	38	34	31
SEI								100	34	31	31	57
SEJ									100	38	42	33
SEM										100	28	31
SEN											100	42
SEO												100

After Jarraud et al., 2001. Names were corrected by the authors after the correction note published in *J. Immunol.* 166: 4260. Amino acid sequences of the precursors were compared using Blast2 sequence method (open gap of 11 and extension gap penalties of 1).

the medium and soluble in water and saline solutions. Some of their biochemical characteristics are summarized in Table 3. They are rich in lysine, aspartic acid, glutamic acid, and tyrosine residues. Most of them possess a cystine loop required for proper conformation and which is probably involved in the emetic activity (discussed below). They are highly stable, resist most proteolytic enzymes, such as pepsin or trypsin, and thus keep their activity in the digestive tract after ingestion. They also resist chymotrypsin, rennin and papain. Nevertheless, SEB and SEC1 have been cut in the cystine loop by mild trypsin digestion. Staphylococcal enterotoxin B can be destroyed by pepsin digestion at pH 2 but it is pepsin resistant at higher pHs, which are normal conditions in the stomach after food ingestion (Bergdoll, 1983). Staphylococcal enterotoxins are highly heat resistant as well; they are thought to be more heat resistant in foodstuffs than in a laboratory culture medium (Bergdoll, 1983), but can be inactivated by heat treatments used in the sterilization of canned foods when they are present at low concentrations (Bergdoll, 1983). Great efforts have been applied to the development of detection methods for SEs, based on immunological and activity assays. Although immunological assays are quicker and cheaper, the relevance of the immunological approach is still under discussion. Heat treatment in an acidic medium usually leads to a loss of immunological activity and a concomitant loss of biological activity. Nevertheless, SEA and SED were found to be undetectable (loss of serological recognition) but still active (in kittens in an *in vivo* assay) after heat treatment (Bennet, 1992). Heat inactivation of SEA, SEB, and SEC has been shown to vary according to the food matrix and to the pH (Schwabe et al., 1990). It is thus quite difficult to foresee the impact of heat treatment on SE activity, since it depends on SE type, SE concentration and the food matrix. Furthermore, in some cases, heat inactivation is spontaneously reversible by an alkaline pH (Schwabe et al., 1990) or by urea treatment (Bennet, 1992). Taken together, these data show that SEs resist conditions (heat treatment, low pH) that easily destroy the bacteria that produced them.

Table 3. Major characteristics of the staphylococcal enterotoxins (SE).

SE type	ORF length (bp)	Precursor length (aa)	Mature SE length (aa)	Molecular mass (kDa)	pI	Reference
A	774	257	233	27,100	7.3	Betley and Mekalanos, 1985, 1988
B	801	266	239	28,336	8.6	Johns and Khan, 1988
C1	801	266	239	27,531	8.6	Bohach and Sclievert, 1987
C2	801	266	239	27,531	7.8	Bohach and Sclievert, 1989
C3	801	266	239	27,563	8.1	Hovde et al., 1990
C (bovine)				27,618	7.6	Marr et al., 1993
C (sheep)				27,517	7.6	Marr et al., 1993
C (goat)				27,600	7.0	Marr et al., 1993
D	777	258	228	26,360	7.4	Chang and Bergdoll, 1979
						Bayles and Iandolo, 1989
E	774	257	230	26,425	7.0	Couch et al., 1988
G	777	258	233	27,043	5.7	Munson et al., 1998
H	726	241	218	25,210	Nd	Su and Wong, 1995
I	729	242	218	24,928	Nd	Munson et al., 1998
J	806	268	245 ²	28,565 ²	8.65 ²	Zhang et al., 1998
K	729	242	219	25,539	6.5	Orwin et al., 2001
L	723 ¹	240 ¹	215 ¹	24,593 ²	8.66 ²	Fitzgerald et al., 2001
M	722 ¹	239 ¹	217 ¹	24,842 ²	6.24 ²	Jarraud et al., 2001
N*	720 ¹	258 ¹	227 ¹	26,067 ²	6.97 ²	Jarraud et al., 2001
O*	783 ¹	260 ¹	232 ¹	26,777 ²	6.55 ²	Jarraud et al., 2001

After J.P. Rosec PhD thesis, 1999. Université de Montpellier II. Les staphylocoques entérotoxiques: étude épidémiologique de souches d'origine alimentaire et détection par PCR multiple.

*Named SEK and SEL in Jarraud et al., 2001, renamed SEM and SEO, respectively, in a correction note published in *J. Immunol.* 166: 4260 (2001).

¹Length of the mature moiety determined by the authors after Henrik Nielsen, Jacob Engelbrecht, Søren Brunak and Gunnar von Heijne: Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Protein Eng.* 10: 1-6 (1997).

²Molecular weight and isoelectric point of the mature moiety determined by the authors using the software MWALC, Infobiogen. http://www.infobiogen.fr/services/analyseq/cgi-bin/mwcalc_in.pl

Nd, not determined.

Genes encoding SEs have different genetic supports, most of which are mobile genetic elements (Table 4). For example, *sea* is carried by a family of temperate phages (Betley and Mekalanos, 1985; Coleman et al., 1989). *Seb* is chromosomally located in some clinical isolates (Shafer and Iandolo, 1978), whereas it has been found in a 750-kb plasmid in other *S. aureus* strains (Shalita et al., 1977). *SEC_{bovine}* is encoded by a gene located on a pathogenicity island (Fitzgerald et al., 2001) and *see* is carried by a defective phage (Couch et al., 1988). The main regulatory system controlling the gene expression of virulence factors in *S. aureus* is the accessory gene regulator (*agr*; Kornblum et al., 1990) that acts in combination with the staphylococcal accessory regulator (*sar*; Cheung et al., 1992; for a review, see Novick, 2000). Some but not all of the SE genes are controlled by the *agr* system. The *seb*, *sec* and *sed* genes have been demonstrated to be *agr* dependent, whereas *sea* and *sej* are *agr* independent (Tremaine et al., 1993; Zhang et al., 1998). Recent research by Vojtov et al. (2002) demonstrated that SEB, like toxic shock syndrome toxins (TSST), is a negative global regulator of exoprotein gene transcription, and that it acts via the *agr* system. As *agr* expression is tightly linked to quorum sensing (Novick, 2000), the production of *agr*-dependent SEs in foodstuffs is dependent on the ability of *S. aureus* to increase to a high cell density (estimated 10⁶ cfu/g) in the foodstuffs, and environmental factors play an important role in SE gene expression (see below).

Table 4. Genetic support of some staphylococcal enterotoxin (SE) genes.

Gene	Genetic location	Reference
sea	prophage	Betley and Mekalanos, 1985; Borst and Betley, 1994
seb	chromosome, plasmid, transposon	Shafer and Iandolo, 1978 Shalita et al., 1977; Altboum et al., 1985
sec1	Plasmid	Altboum et al., 1985
sec _{bov}	pathogenicity island	Fitzgerald et al., 2001
sed	plasmid (pIB485)	Bayles and Iandolo, 1989
see	defective phage	Couch et al., 1988
seg	<i>Enterotoxin gene cluster (egc)</i> , chromosome	Jarraud et al., 2001
sei	<i>egc</i> , 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	<i>egc</i> , chromosome	Jarraud et al., 2001
sen*	<i>egc</i> , chromosome	Jarraud et al., 2001
seo*	<i>egc</i> , chromosome	Jarraud et al., 2001

*Renamed after the correction note published in *J. Immunol.* 166: 4260 (2001).

STAPHYLOCOCCAL ENTEROTOXIN ACTIVITIES

The SEs belong to a family of the so-called pyrogenic toxins originating from staphylococcus and streptococcus species. Pyrogenic toxins include SEs, TSST, exfoliatins A and B and streptococcus pyrogenic toxins. These toxins share some structure, function and sequence similarities. They have phylogenetic relationships as well (for a review, see Balaban and Rasooly, 2000). Until recently, SEs were discovered in studies of *S. aureus* strains implicated in FBD outbreaks, and they were classified in distinct serological types. Thus, SEA to E and SEH have been clearly demonstrated as being capable of more or less potent emetic activity. More recently, increasing data resulting from partial or complete genome sequence analyses have allowed the identification of several new SE types. These new SEs were first identified on the basis of sequence and structural similarities with existing SEs. There is experimental evidence for their superantigenic *in vitro* and/or *in vivo* activities, but rarely their emetic activity. Although pyrogenic toxins are involved in distinct pathologies, they have common biological activities: they are pyrogenic, and they cause immunosuppression and nonspecific T-cell proliferation. These activities are referred to as superantigen activity. Besides these common features, some toxins are able to cause other symptoms. Among superantigens, only SEs have emetic activity. Superantigen and emetic activity of the SEs are two separate functions localized on separate domains of the protein (Hovde et al., 1994; Dinges et al., 2000). Nevertheless, a high correlation exists between these activities since, in most cases, genetic mutations resulting in a loss of superantigen activity result also in a loss of emetic activity (Harris et al., 1993). Superantigen activity results from direct interaction of SEs with T-cell

antigen receptors (TCR) and the major histocompatibility complex (MHC) of antigen-presenting cells (APC). A normal antigen is presented to TCR in the form of peptides bound to MHC class I or II, after processing in APC (Figure 1A). MHC are protein complexes displayed on the surface of the APC. T-cell antigen receptors are glycosylated heterodimers composed either of α and β , or of δ and γ chains. This recognition of the antigen is a primary step in the cellular immune response and is a key to the high specificity of the immune response. Only a few T-cells can recognize a specific antigen presented in the MHC of an APC (McCormick et al., 2001). Superantigen toxins interact with many T-cells by the recognition of specific V β chains of the TCR. They are able to cross-link the TCR and the MHC class II of APC, thus causing activation (Figure 1B). It seems that an interaction between SE and MHC is first required for binding to the V β chain of the APC. This cross-link results in the nonspecific activation and proliferation of T-cells and a massive secretion of interleukins that may be involved in the mechanism of SE toxicity. Through this interaction, SEs activate T-cells at orders of magnitude above antigen-specific activation. This dramatic activation causes toxic shock syndrome (McCormick et al., 2001). The domains of SE involved in these interactions are well characterized through genetic and crystallographic studies. SEB has been particularly well studied due to its potent superantigen activity. SEB-producing strains are considered as potential microbiological weapons of warfare and terrorism (Greenfield et al., 2002).

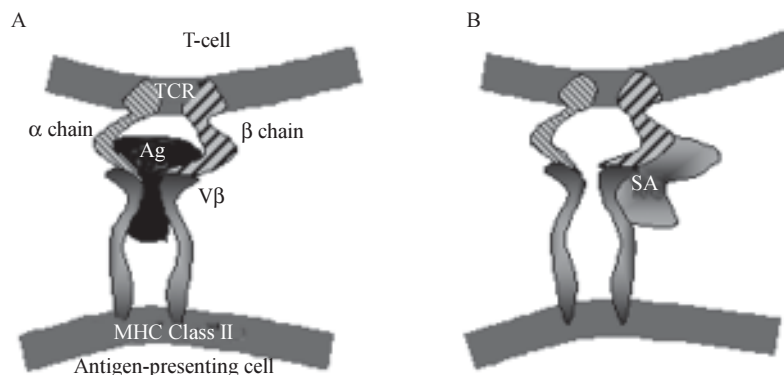


Figure 1. Specific and nonspecific activation of a T-cell. **A**, T-cell activation by conventional antigens. After processing by the antigen-presenting cell, the antigen peptide (Ag) is displayed in the major histocompatibility complex (MHC) class II. This complex attracts T-cells bearing T-cell receptors (TCR) with variable (V β) chains specific to the Ag presented. **B**, Nonspecific T-cell activation by superantigen. The superantigen binds directly the outside of the MHC class II and cross-links it to the V β chain. This event initiates nonspecific T-cell activation. SA stands for superantigen. After Balaban and Rasooly, 2000.

The emetic activity of SEs is not as well characterized as superantigen activity. The enterotoxin activity is uniquely characterized by the SE ability to cause emetic responses when administered orally to monkeys, whereas other superantigens are not emetic (Dinges et al., 2000). Little is known about how SEs cause symptoms of food poisoning; they may have a direct effect on intestinal epithelium and on the vagus nerve, causing stimulation of the emetic center and of gut transit (Bergdoll, 1983; Arbuthnott et al., 1990). The infective dose required to induce staphylococcal food poisoning in humans is estimated to be around 0.1 μ g and it may vary with patient sensitivity (Evenson et al., 1988). Although there is considerable data on the structure-function relationships of SE superantigen activity, emetic activity has not been precisely localized. One common feature of SEs is a cystine loop, thought to be important for emetic activity based on mutant analyses (Hovde et al., 1994; Dinges et al., 2000). However,

SEI lacks the cystine loop structure and is both superantigenic and emetic; this emetic activity is nevertheless significantly lower than that of other SEs (Munson et al., 1998). Sequence analysis of two other recently identified SEs, SEK and SEL, also indicated absence of the cystine loop (Orwin et al., 2001; Fitzgerald et al., 2001). These latter SEs have not been tested yet for their emetic activity. SEs possess two distinct activities. How these activities are linked remains unclear. A working hypothesis is that enterotoxin activity may facilitate transcytosis, thus enabling the toxin to enter the bloodstream and interact with T-cells, leading to superantigen activity (Hamad et al., 1997; Balaban and Rasooly, 2000).

ENVIRONMENTAL FACTORS THAT AFFECT STAPHYLOCOCCAL ENTEROTOXIN PRODUCTION

Many studies have investigated the conditions in which *S. aureus* is able to produce SEs (for reviews, see Bergdoll, 1989; Genigeorgis, 1989). SE production has been studied in strains grown in laboratory media and in diverse foodstuffs. The abundance of literature is much greater for SEs with *agr*-dependent expression. Conditions of expression of *agr* are so well documented because it regulates most of the virulence factors in *S. aureus* (Novick, 2000). *Staphylococcus aureus* is considered an exigent bacterium in terms of nutritional requirements. Valine is necessary for growth and arginine and cystine are necessary for both growth and SE production in five strains of *S. aureus* that produce SEA, SEB or SEC. The necessities for other amino acids vary with the strains (Onoue and Mori, 1997). Glucose has been shown to have an inhibitory effect on SE production, especially for SEB and SEC (Bergdoll, 1989). This inhibitory effect has been attributed to a drop in pH, as a consequence of glucose metabolism. These observations could also be correlated with *agr*-dependent synthesis of these SEs. Glucose and low pH indeed have an inhibitory effect on *agr* expression (Regassa et al., 1992; Novick, 2000). SE production is optimal in neutral pH and decreases in acidic pH. Usually, SE production is inhibited in pH below 5. At a given pH, substances used to acidify the medium may have more or less effects. For example, acetic acid has a greater inhibitory effect than lactic acid on SE production. High concentrations of sodium chloride increase the inhibitory effect of acidic pH, with no SE production at salt concentrations above 12%, independent of the pH (Notermans and Heuvelman, 1983). On the other hand, alkaline pH also decreases the production of SEB, SEC, and SED via decreased expression of *agr* (Regassa and Betley, 1992). *Staphylococcus aureus* is quite sensitive to microbial competition. This feature has been particularly well studied in fermented food products. Genigeorgis (1989) demonstrated that the higher the concentration of competing microorganisms in milk, the lower the rate of *S. aureus* growth and SE production. Competition with lactic acid bacteria has been reported in other research on cheese (Otero et al., 1988; Vernozy-Rozand et al., 1998) and fermented sausage production (Sameshima et al., 1998). The effect of lactic acid starter is mainly due to lactic acid production, lowered pH, oxygen peroxide production, competition for nutrients and is sometimes due to the synthesis of antimicrobial substances, such as bacteriocins.

FOODS INVOLVED IN STAPHYLOCOCCAL POISONING

In all cases of staphylococcal food poisoning, the foodstuff or one of the ingredients, was contaminated with an SE-producing *S. aureus* strain and was exposed, at least for a while, to temperatures that allow *S. aureus* growth. Most of the time the foodstuff reaches this temper-

ature because of a failure in the refrigeration process, or because a growth-permissive temperature is required during processing (e.g., cheese making). Many different foods can be a good growth medium for *S. aureus*, and have been implicated in staphylococcal food poisoning, including milk and cream, cream-filled pastries, butter, ham, cheeses, sausages, canned meat, salads, cooked meals and sandwich fillings. Various examples of staphylococcal food poisoning are described in the literature (Bergdoll, 1989). In one case, cheese was involved in an outbreak because it had been made from milk contaminated after pasteurization and before inoculation with lactic starter culture. In this particular case, the starter culture did not grow properly, resulting in a fermentation accident that allowed the *S. aureus* strain to develop and produce SE (Bergdoll, 1989). In 1985, chocolate milk was the origin of a staphylococcal food poisoning in the Kentucky, USA. This chocolate milk was contaminated and stored at too high a temperature for 4 to 5 h, before pasteurization. Pasteurization killed the staphylococci but had no effect on the SEs. These, and many other examples, illustrate the importance of the elimination of any contamination sources during the processing and refrigeration of food and food ingredients whenever possible. The products can be refrigerated before, as well as after, heat treatment (pasteurization). In the case of canned foods that have been correctly processed, bacteria and SEs are usually destroyed. Nevertheless, some cases of staphylococcal food poisoning involving canned mushrooms that were correctly processed were reported in the USA (Bennet, 1992). These kinds of examples raise questions about the heat stability of SEs (see above).

The foods that are most often involved in staphylococcal food poisoning differ widely from one country to another. In the United Kingdom, for example, 53% of the staphylococcal food poisonings reported between 1969 and 1990 were due to meat products, meat-based dishes, and especially ham; 22% of the cases were due to poultry, and poultry-based meals, 8% were due to milk products, 7% to fish and shellfish and 3.5% to eggs (Wieneke et al., 1993). In France, things are different. Among the staphylococcal food poisonings reported in a two-year period (1999-2000), among the cases in which the food involved had been identified, milk products and especially cheeses were responsible for 32% of the cases, meats for 22%, sausages and pies for 15%, fish and seafood for 11%, eggs and egg products for 11% and poultry for 9.5% (after Haeghebaert et al., 2002). In the United States, among the staphylococcal food poisoning cases reported between 1975 and 1982, 36% were due to red meat, 12.3% to salads, 11.3% to poultry, 5.1% to pastries and only 1.4% to milk products and seafoods. In 17.1% of the cases, the food involved was unknown (Genigeorgis, 1989). Thus, the origins of staphylococcal food poisoning differ widely among countries; this may be due to differences in the consumption and food habits in each of the countries. In France, for example, the consumption of raw milk cheeses is much higher than in Anglo-Saxon countries. This may explain the relative importance of milk products involved in staphylococcal food poisoning in France.

In any case, the main sources of contamination are humans (handlers contaminate food via manual contact or via the respiratory tract by coughing and sneezing), and contamination occurs after heat treatment of the food. Nevertheless, in foods such as raw meat, sausages, raw milk, and raw milk cheese, contaminations from animal origins are more frequent and due to animal carriage or to infections (e.g., mastitis).

FREQUENCY OF ENTEROTOXIGENIC STRAINS

Foods and raw ingredients are subjected to regular microbiological controls. Among the *S. aureus* strains isolated from food samples, the percentage of enterotoxigenic strains is

estimated to be around 25% (Bergdoll, 1989). Nevertheless, estimations vary considerably from one food to another and from one report to another. Here are some examples of variations concerning strains isolated from cows with mastitis and milk products. In France, among 61 strains isolated from raw milk cheeses, 15.9% were enterotoxigenic (Rosec et al., 1997). In Denmark, another study performed on strains isolated from cows with mastitis found that only 1 of 414 *S. aureus* isolates carried an SE gene (Larsen et al., 2000). A similar study was performed in Minas Gerais, Brazil, where 54 (43%) of 127 *S. aureus* isolates from bovine mastitis were found to be SE producers (Cardoso et al., 1999). In these latter studies, the authors investigated the strains for SEA to E (SEA to D for Cardoso et al.), but not for newly described SEs. More recently in Germany, similar work on strains isolated from the milk of cows with mastitis showed that up to 72.8% of the strains were enterotoxigenic when SEA to SEJ were considered (Akineden et al., 2001). There is thus a great increase in the percentage of enterotoxigenic strains when the newly described SEs are taken into account. Along with the SEA, SEC, and SED often found in these studies, SEG, SEI, and SEJ seem to be the predominant SEs in strains isolated from cattle with bovine mastitis (Akineden et al., 2001). This situation also applies to other kinds of foodstuffs. A study on SE genes of “classical” (i.e., SEA to E) and new types detected by PCR of 332 *S. aureus* strains isolated from a variety of foods in France revealed a very high frequency of strains harboring the SEG, H, I, and J genes (57%), greater than that of strains harboring classical genes, which had been previously established as being predominant (Rosec and Gigaud, 2002). Nevertheless, data based on PCR-based detection of SE genes (including the newly described genes) has to be examined with precaution. Sandwich enzyme-linked immunosorbent assays (ELISA) have been developed for SEA to SEE (Thompson et al., 1986), and are commercially available. Most of the studies on SEA to SEE demonstrate a good correlation between the occurrence of the *sea* to *see* genes and the production of the corresponding SEs. This is not so clear for other SEs. Recently, ELISA for SEH (Su and Wong, 1996), and for SEG and SEI have been described (Omoe et al., 2002). One strain producing SEH has already been involved in some staphylococcal food poisoning (Su and Wong, 1996), and most *seh*-harboring strains produce significant amounts of SEH. However, *S. aureus* isolates harboring *seg* and most of the isolates harboring *sei* do not produce detectable levels of SEG or SEI, though the corresponding mRNA is detected (Omoe et al., 2002). This demonstrates the importance of quantitative assessment of SE production in foods in order to have a clear idea of the relationship between the recently described SEs and food poisoning. Thus, recent PCR-based studies demonstrate that newly described SE genes are widely distributed among *S. aureus* strains, but their true incidence in staphylococcal food poisoning has still to be clarified.

CONCLUDING REMARKS

Staphylococcal food poisoning is of major concern in public health programs worldwide. Predictive models for *S. aureus* growth and SE production would be powerful tools for microbial risk assessment in food industries. However, many factors affect *S. aureus* growth and SE production in foodstuffs and further studies are still necessary in order to develop such predictive tools. To date, in most countries, microbial risk assessment for *S. aureus* relies on the identification and the quantification of coagulase positive isolates in end products. As *S. aureus* is widely spread in raw ingredients and since low contamination levels do not induce FBD, the microbial norms in most countries tolerate *S. aureus* contamination. For example, in France, the norms for coagulase positive staphylococci range from 0 cfu/g in semi-canned foods, (e.g.,

rollmops or anchovies) up to 10^3 cfu/g in some raw milk cheeses. Nevertheless, SE production rather than *S. aureus* itself should be taken into account in risk assessment. *Staphylococcus aureus* can indeed be easily eliminated from foodstuffs by heat treatment (in pasteurized foods) or by competition with other flora (in fermented foods), whereas SEs resist most of the treatments used during food processing. Moreover, most of *S. aureus* food isolates are not SE producers. Staphylococcal species other than *S. aureus* are also capable of SE production but are not looked for in routine tests. Thus considerable research effort is still required for better understanding of the interactions between *S. aureus* and the food matrix, and of the mechanisms of SE production in foodstuffs. Research is also needed for the identification of new SEs and of new enterotoxigenic staphylococci. Much effort is being applied towards the development of new, and more sensitive methods for SE detection in foodstuffs. Taken together, these studies should lead to better control and a subsequent reduction of staphylococcal food poisoning outbreaks.

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