Staphylococcus: Food Poisoning

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Introduction

Staphylococci, and their most prominent food poisoning representative *Staphylococcus aureus*, are probably among the most established foodborne pathogens. It was in 1871 when Recklinghausen, a German scientist, observed cocci in a diseased kidney and called them 'micrococci,' incriminating staphylococci as human pathogens. Later, after several changes in their nomenclature, Sir Alexander Ogston coined the name *Staphylococcus*. The foodborne disease involving staphylococci was for the first time investigated in Michigan (the United States) in 1884 by Vaughan and Sternberg, as a response to the food intoxication involving cheese probably contaminated by staphylococci. Proof of the involvement of staphylococci in food poisoning was first brought in 1914 by Barber who discovered that a toxin substance produced by staphylococci was responsible for staphylococcal food poisoning. The capacity of some strains of *S. aureus* to produce food poisoning was conclusively demonstrated in 1930 by Dack et al. who showed that the symptoms could be produced by feeding culture filtrates of *S. aureus*. Today, it is well established that food poisoning caused by staphylococci is a foodborne intoxication, with staphylococcal enterotoxins (SEs) as etiologic agents. SEs are produced in food during exponential and stationary growth phase of enterotoxigenic strains causing foodborne intoxication after the consumption of contaminated food. *S. aureus* is the species almost invariably involved, although enterotoxin production by several other species has been reported.

Species of Concern in Food

Genus *Staphylococcus* contains 47 species and 24 subspecies as reported in the List of Prokaryotic Names with Standing in Nomenclature as of the full update in 6 August 2013 (http://www.bacterio.net/s/staphylococcus.html). The genus *Staphylococcus* comprises Gram-positive, catalase-positive cocci. Metabolism of carbohydrates may be either oxidative or fermentative. Cells are small (~1 mm diameter) and often form characteristic clumps resembling bunches of grapes. Their classification distinguishes between coagulase-producing strains, designated as coagulase-positive staphylococci (CPSs), and noncoagulase-producing strains, called coagulase-negative staphylococci (CNSs). Staphylococci are predominantly of animal origin, although some species were isolated from the environmental sources. They may be present as part of the normal microflora of humans and animals. *S. aureus* is carried on skin and nasal cavities of about 30% of the healthy human population. Among those, enterotoxigenic species are of potential interest in food safety, although not all of them have been documented in foods. Table 1 shows an overview of reported enterotoxigenic *Staphylococcus* spp. The most important member of genus *Staphylococcus* in relation to food safety is *S. aureus*, followed by *S. hyicus* and *S. intermedius*. Next to the well-established role of CPS in food poisoning, several CNSs have also been potentially involved in foodborne outbreaks. Among CNS isolates reported to produce detectable enterotoxins are those belonging to *S. saprophyticus*, *S. epidermidis*, *S. cohnii*, and *S. warneri*. Recently, following consumers' complaint, a coagulase-negative *S. saprophyticus* was found in an oyster sauce (Rapid Alert System for Food and Feed, RASFF, alert 2012.0605). It is also important to note that CNS isolates play an important role in mastitis etiology and that milk from subclinically infected cows can be hazardous to human health not only because of the risk of pathogen transmission but also because of contamination with enterotoxins.

Regarding the percentage of strains that are enterotoxigenic, widely different percentages have been found depending on the source of isolates. The numbers vary from 10% (of 236 raw milk isolates) to ~60% (200 food isolates). The relative incidence of specific enterotoxins among strains recovered from various sources varies widely, too. The existing data should be critically interpreted as the methods used to determine enterotoxigenic potential differ among studies, including types of SE that were screened for. It is however important to note that one strain can produce multiple enterotoxins.

S. aureus in Foodborne Intoxications

Symptoms

The incubation period and severity of symptoms depend on the amount of enterotoxins ingested and the susceptibility of each individual. However, it is generally understood that all people are susceptible to staphylococcal food intoxication. *Symptoms* are of rapid onset and are commonly described by nausea and violent vomiting. Early symptoms comprise nausea followed by incoercible characteristic vomiting (in spurts) and appear within 30 min to 8 h (about 3 h on average) after ingestion of contaminated food. Other commonly described symptoms include abdominal pain, diarrhea, dizziness, shivering, and general weakness sometimes associated with a moderate fever. In the most severe cases, headache, prostration, and low blood pressure have been reported. In the majority of cases, recovery occurs within 24–48 h without specific treatment, while diarrhea and general weakness can last 24 h or longer. Death is rare, occurring primarily in those susceptible to dehydration (infants and elderly people) and in those affected by an underlying illness. The vomiting symptoms of staphylococcal intoxication are identical to that of *Bacillus cereus* emetic intoxication, caused by cereulide. It is interesting to mention that the symptoms of *B. cereus* diarrheal toxicoinfection are identical to that of *Clostridium perfringens* toxicoinfection. An overview of the symptoms of these toxigenic species is provided in Table 2.

Table 1 Overview of reported enterotoxigenic *Staphylococcus* spp.

Staphylococcus *spp.*	*Coagulase*	*Enterotoxins produced (some of the SE types produced/or* se *encoding genes detected)*
S. aureus	+	+ (All known SE types)[a]
S. caprae	−	+
S. carnosus	−	+ (SEA, SEE, SHE)
S. cohnii		+ (SEA, SEB)
S. chromogenes	−	+
S. delphini	+	+ (SEA, SEE)
S. epidermidis	−	+ (SEA, SEC, SED)
S. equorum	−	+ (SED, SEH)
S. haemolyticus	−	+
S. hyicus	(+)	+ (SEA, SEC, SED)[b]
S. intermedius	+	+ (SEA, SEB, SEC, SED, SEE)[c]
S. lentus	−	+
S. piscifermentans		+ (SEH)
S. saprophyticus	−	+ (SEA)
S. schleiferi subsp. *schleiferi*	−	+
S. sciuri	−	+
S. warneri	−	+
S. xylosus		+ (SEH)

[a]Staphylococcal enterotoxins are members of a family of more than 20 different staphylococcal and streptococcal exotoxins that are functionally related and share sequence homology. Those with established emetic activity, involved in food intoxications, are SEA, SEB, SEC, SED, SEE, SEF, SEG, SHE, and SEI.
[b]Until recently, it has been thought that *S. hyicus* produced SE other than classical SEA–SEE.
[c]Even though *S. intermedius* is known to produce SE (next to *S. aureus* the most often cited as enterotoxigenic staphylococci), only one outbreak has been associated with *S. intermedius* since the identification of this species.

Reservoirs

The staphylococci are ubiquitous in nature, with humans and animals as the primary reservoirs. They are present in the nasal passages and throat, in the hair, and on the skin of many healthy individuals. About 40–45% of healthy individuals are nasal carriers of staphylococci, and 8–22% have them on their skin. In fact, estimates range from 20% to 30% for persistent and 60% for intermittent colonization. Staphylococci can be isolated from animals, with the bovine being the most important because of the involvement of staphylococci in mastitis. The prevalence of subclinical and clinical bovine mastitis caused by *S. aureus* ranges from 5% to 50% in different countries.

Much uncertainty remains about the origin and public health implications of livestock-associated methicillin-resistant *S. aureus* (LA-MRSA), but it is known that it is capable of causing zoonotic infections. Recent reports of MRSA in livestock, particularly pigs, and in humans with contact to livestock provided the first evidence of the existence of a true LA-MRSA reservoir throughout Europe. Since then, LA-MRSA has been identified in Canada, the United States, China, Malaysia, Korea, Thailand, etc.

Although animals and humans are the major source, staphylococci also can be found in the air, dust, water, and human and animal wastes. From these sources, they can be transferred to different types of foods.

Implicated Foods and Outbreaks

Foods that have been frequently incriminated in staphylococcal intoxication include meat and meat products; poultry and egg products; milk and dairy products; salads; bakery products, particularly cream-filled pastries and cakes; and sandwich fillings. In the European Union, the largest proportion of 345 outbreaks caused by staphylococcal toxins in 2011 were attributed to mixed food (40.0%). The second most frequently single food category reported was dairy products (14.3%), followed by bakery products (11.4%). Broiler meat and products thereof, cereal products, eggs and egg products, meat and product thereof, vegetables and juices and other products thereof, and other foods were also implicated. In the United States, pork – particularly baked ham – is the food most frequently involved in outbreaks; poultry, salads (meat, potato, etc.), and cream-filled bakery goods are responsible for many of the outbreaks. Table 3 shows an overview of foods involved in staphylococcal outbreaks in the United States in the period 1998–2008. Salted food products, such as ham, have also been implicated, resulting from the capacity of *S. aureus* to grow at relatively low water activity ($a_w = 0.83$). For a number of dried meat products, *S. aureus* remains one of the main microbial safety issues. In RASFF database, about 83 different notifications were issued on the presence of staphylococci or their enterotoxin in many different foods of different geographic origin. Perhaps, surprising to the common knowledge is that more than 20 of these alerts were related to fish and fishery products and crustaceans and products thereof.

Outbreaks of staphylococcal food poisoning are numerous. *S. aureus*, *B. cereus*, *and C. perfringens* create the group of three most important causative agents of bacterial exotoxin-related foodborne diseases. It is estimated that of 9.4 million foodborne illnesses caused by a known pathogen annually in the United States, 1.3 million are caused by *B. cereus*, *C. perfringens*, or *S. aureus*. Meat or poultry dishes were commonly implicated in *C. perfringens* (63%) and *S. aureus* (55%) outbreaks, and rice dishes were commonly implicated in *B. cereus* outbreaks (50%). Errors in food processing and preparation were commonly

Table 2 Comparison of syndromes caused by toxins of *B. cereus*, *S. aureus*, and *C. perfringens*

		B. cereus		S. aureus	C. perfringens
Bacteria/syndrome/toxin		*Diarrheal syndrome (hemolysin BL (HBL), nonhemolytic enterotoxin (NHE),* B. cereus *enterotoxin T (bceT), and cytK)*	*Emetic syndrome (cereulide)*	*Enterotoxins*	*Enterotoxin (CPE)*
Symptoms		Abdominal pain, cramps, watery diarrhea (secretory type), and occasionally nausea	Nausea, vomiting, malaise, and ultimately a fatal liver failure	Nausea, vomiting, sometimes diarrhea	Intense abdominal cramps, diarrhea, and flatulence
Usual incubation time (h)		8–24 (or longer depending on the dose and host susceptibility)	0.5–5	1–8	6–24
Usual resolution time (h)		12–24 (up to several days in severe cases)	6–24	24–48	Within 24
Intoxication/infection dose		Ingestion of more than 10^5 CFU of diarrheal toxin-producing *B. cereus* strains	$\sim$10 μg kg^{-1} bw, 0.01 μg g^{-1} of food (*B. cereus* concentration more than 10^5 CFU g^{-1} food, depending on the strain, food, and conditions)	100 ng of ingested toxin, 0.05 ng ml^{-1} of food (produced when *S. aureus* counts reach $\sim10^5$ CFU ml^{-1} (g^{-1}))	10^6–10^7 CFU g^{-1} of food (ingested vegetative cells produce CPE during intestinal sporulation possibly under impact of bile and stomach acids)
Toxin produced		In the small intestine of the host	Preformed in the food	Preformed in the food	In the small intestine of the host
Food involved		Milk- and meat-containing products, soups, vegetables, puddings	Rice, pasta, potato puree, noodles, sauces, paella, meat, polenta	Meat- and milk-containing products, creams, chickens salads, canned mushrooms	Meats, meat products, gravy, cooked beans, stews, soups
Toxin stability	Heat	5 min at 56 °C	90 min at 121 °C	SEA 3 min at 80 °C, 1 min at 100 °C; SEB 87 min at 99 °C	5 min at 60 °C
	pH	4–8	2–11	Wide range, resistant to gastric pH	5–10
	Proteinases	Nonresistant	Resistant	Resistant	Increased activity

reported (93%), regardless of etiology; contamination by a food handler was only common in *S. aureus* outbreaks (55%). The incidents involving *S. aureus* are related to different establishments but are very frequent in catering services where food is premade in larger volumes so that cooling is not as fast as required. Moreover, the food is maintained at convenient temperatures for fast serving, and those are temperatures that can support growth and enterotoxin formation. Other studies confirm that the food handler is the principal source of food contamination. In a recent outbreak in Germany, an *S. aureus* isolate from the stool sample was matched to the food isolate and isolate coming from the food handler. Reporting of outbreaks is mandatory and the confirmation of *S. aureus* outbreak, based on the guideline of Centers for Disease Control and Prevention, consists of the isolation of organism of same phage type from stool or vomitus of 2 or more ill persons, or the detection of enterotoxin in epidemiologically implicated food, or the isolation of 10^5 organisms per gram from epidemiologically implicated food. There is an internationally agreed basic set of phages used for typing strains of *S. aureus* of human origin, which is updated regularly to reflect changes in the typability of human clinical isolates. There are problems of nontypability, since 15–20% of isolates may be untypable. Today, more robust, reliable, and convenient molecular typing methods exist, such as pulsed field gel electrophoresis, which is the method of choice in most of the EU member states and is also used worldwide. Also, CDC had published recently a unified pulsed-field gel electrophoresis (PFGE) protocol for Gram-positive bacteria. However, the typing of *S. aureus* is not the common practice in the world. In its report, the European Food Safety Agency states that 19 member states (MSs) and 3 non-MSs currently perform molecular typing for *S. aureus* isolates from animals, food, and feed. Six MSs and one non-MS reported that they neither perform or purchase molecular typing of *S. aureus* isolates.

Control and prevention

As mentioned earlier, humans and animals are usual carrier of *S. aureus*. Consequently, the contamination of foods often happens via the factory or kitchen staff by the hand contact or their respiratory system. Therefore, the good personal hygiene is essential as a preventive measure for *S. aureus*

Table 3 Foods implicated in outbreaks caused by *Staphylococcus aureus*, in the United States, 1998–2008 (for details, see Bennett et al., 2013)

	Staphylococcus aureus *outbreaks*		
Food type	*Confirmed*	*Suspected*	*All Outbreaks*
Outbreak food ethnicity	$n=150$	$n=242$	$n=1074$
Any ethnic	18 (12)	34 (14)	241 (22)
Mexican style	7 (5)	13 (5)	132 (12)
Asian	9 (6)	10 (4)	81 (8)
Single food groupings	$n=106$	$n=199$	$n=820$
Meat or poultry	58 (55)	53 (27)	336 (41)
Beef	6 (10)	8 (15)	116 (35)
Poultry	8 (14)	26 (49)	115 (34)
Pork	39 (67)	14 (26)	92 (27)
Unspecified origin	4 (7)	4 (8)	11 (3)
Other	1 (2)	1 (2)	2 (1)
Rice dishes	5 (5)	5 (3)	85 (10)
Sandwiches	8 (8)	45 (23)	68 (8)
Pasta dishes	9 (8)	10 (5)	43 (5)
Burritos and tacos	4 (4)	5 (3)	39 (5)
Soups and stews	3 (3)	3 (2)	36 (4)
Bean dishes	0 (0)	0 (0)	32 (4)
Sauces	2 (2)	4 (2)	32 (4)
Salads	4 (4)	14 (7)	31 (4)
Pizza	1 (1)	25 (13)	30 (4)
Seafood	3 (3)	11 (6)	21 (3)
Other produce	1 (1)	6 (3)	19 (2)
Pocket foods	2 (2)	1 (1)	16 (2)
Dairy	1 (1)	5 (3)	9 (1)
Baked goods	2 (2)	3 (2)	8 (1)
Other foods	3 (3)	9 (5)	15 (2)

Data represent number of outbreaks and (%).

contamination. Nevertheless, *S. aureus* is also able to nest itself on the surfaces of process equipment due to inadequate and insufficient cleaning and disinfection. CPSs, which serve as analytic indicator of the presence of toxin-producing *S. aureus*, not only are determined because of their pathogenic nature but also are often detected as an indication of the level of personal hygiene and good manufacturing practice (GMP) performance. Low numbers (100–1000 CFU g^{-1}) can be tolerated, depending on the inherent properties of the food and envisaged storage conditions and length of the shelf life.

It is generally understood and accepted that *S. aureus* as a food intoxicant presents a risk only when the growth to high numbers occurs ($>10^5$ CFU g^{-1}). Since *S. aureus* cannot grow at temperatures below 7 °C and that multiplication of *S. aureus* in raw products is inhibited or slowed down by competing accompanying microflora, these high numbers are normally not reached under the conditions of maintained cold chain. However, the mere presence (and not the growth) of *S. aureus* is associated with the number of cold stored foods and even with ice creams. Nevertheless, problems with *S. aureus* are most often an indication of temperature abuse or postcontamination. With respect to growth temperature, SEB production in ham has been recorded already at 10 °C, as well as lower amounts of SEA, SEB, SEC, and SED in cooked ground beef, ham, and bologna-type sausage.

A simplified scenario in *S. aureus* food poisoning encompasses foods in which production involves a number of manipulations through which the intrinsic factors (especially a_w – given the high salt tolerance of *S. aureus*) have been amended so that the competitive flora becomes inhibited and *S. aureus* is free to multiply at insufficient cooling and produces one or more of its enterotoxins (Figure 1). Similar scenario is when postpasteurization contamination occurs. If in the analyzed food *S. aureus* is found in numbers of 10^5 CFU g^{-1} and higher, then also the presence of SE should to be suspected. However, the SE production is largely strain- and food-dependent, and evidence of SE production at lower numbers and the lack of production at counts even higher than 10^5 CFU g^{-1} exists. Due to the heat stability of enterotoxins, it is possible that *S. aureus* is eliminated in the food, which was heated for the consumption, but that its toxin(s) is still present and active (Table 2). *S. aureus* cells are considerably more sensitive to heat than the SEs, with *D* values ranging from ~2 to 15 at 60 °C. The actual heat stability of each of SE is largely dependent on the environment in which heat treatment occurs.

Knowing that the main source of food contamination with *S. aureus* comes from food handlers, food contact materials, or contaminated raw materials, the main preventive measure is strict application of good hygienic practices and good manufacturing practices. The factors that contribute to staphylococcal food poisoning are inadequate refrigeration, inadequate cooking or heating processing, and poor personal hygiene of infected handlers that results in cross contamination.

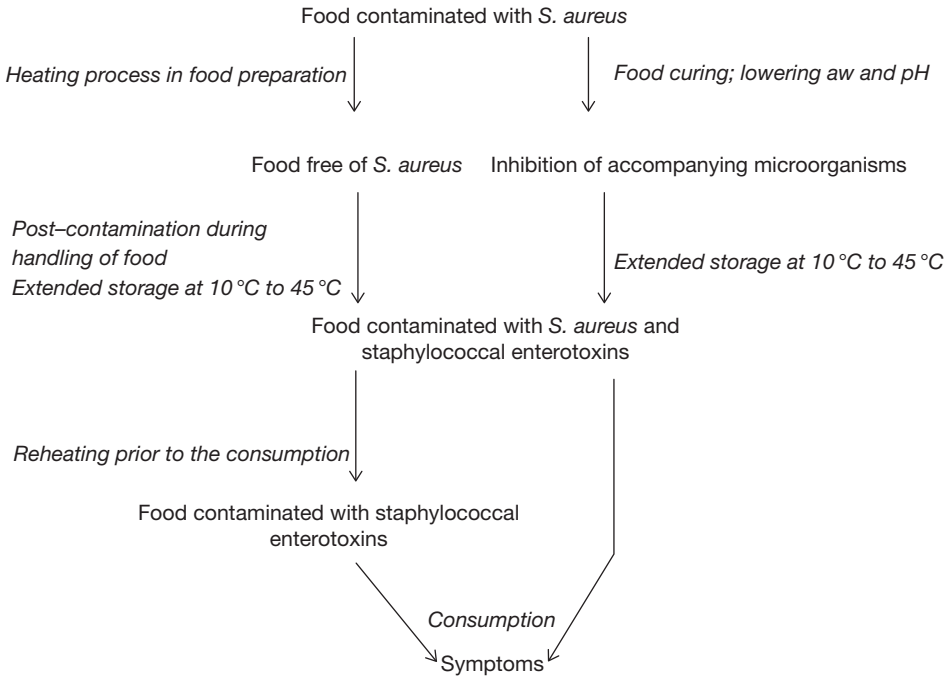

Figure 1 Simplified scholastic representation of usual scenarios leading to staphylococcal foodborne intoxication.

Keeping food below 5 °C and above 50 °C will not allow SE production, nor *S. aureus* multiplication.

Enterotoxins

SEs are the most notable virulence factors associated with *S. aureus*. SEs belong to a great family of staphylococcal and streptococcal pyrogenic exotoxins (PT), characterized with common phylogenetic relationships, structure, function, and sequence homology. SEs function not only as potent gastrointestinal toxins causing emesis but also as superantigens that stimulate nonspecific T-cell proliferation. Although these are two separate functions localized on separate domains of the proteins, certain evidence exists that these two activities can exist independent from each other.

To date, 21 SEs and enterotoxin-like (SEl) types have been described: enterotoxins A (SEA), B (SEB), C1 (SEC1), C2 (SEC2), C3 (SEC3), D (SED), E (SEE), G (SEG), H (SEH), I (SEI), J (SElJ), K (SElK), L (SElL), M (SElM), N (SElN), O (SElO), P (SElP), Q (SElQ), R (SER)[13], S (SES), T (SET) [14], U (SElU), and U2 and V, as reviewed in detail by Hennekinne et al. in 2010 in 'Toxins' (see 'Further Reading' for more information). The incidence of enterotoxin producers among human isolates is reported to be much higher than among nonhuman isolates.

Next to the SEs with demonstrated emetic activity (SEA to SEE, SEG to SEI, and SER to SET), there are also staphylococcal-like (SEl) proteins, which are not emetic in a primate model (SElL and SElQ) or have yet to be tested (SElJ, SElK, SElM to SElP, SElU, SElU2, and SElV). SEs and SEls have been traditionally subdivided into classical (SEA to SEE) and new (SEG to SElU2) types. Several studies attempted to characterize the emetic potential of SEl. In one of them, an emetic potential of SElK, SElL, SElM, SElN, SElO, SElP, and SElQ was assessed using a monkey-feeding assay. All the SEls that were tested induced emetic reactions in monkeys at a dose of 100 μg kg^{-1}. Although a number of affected monkeys were significantly smaller than a number that were affected after consuming SEA (six out of seven) or SEB (four out of four) at the same concentration and the latency periods of the classical SEs seems to be shorter than those of the SEls, these results indicate that SEl may nevertheless play certain role in staphylococcal food poisoning. However, the role of SEls in human food poisoning currently remains unclear, but is it well known that not all of the SEs have equal emetic potential (Table 4). The enterotoxin gene that is most frequently detected in clinical isolates of foodborne staphylococcal intoxications is *sea*. Of note, strains of human origin are highly prevalent in the strains isolated from SFP outbreaks, and *sea* is predominantly carried by human strains. This information does not necessarily correspond to incidence of specific SE genes in food isolates and cow mastitis isolates.

The currently known SEs form a group of serologically distinct, extracellular proteins that share important properties. Among these are the abilities to cause emesis and gastroenteritis in primates, exhibit superantigenicity through an unspecific activation of T lymphocytes followed by cytokine release and systemic shock, and resist heat treatments and digestion by pepsin and structural similarities. SEs are resistant to proteolytic enzymes, hence retaining their activity in the digestive tract after ingestion. Their known heat stability is not the same for all SEs and also depends on the food matrix and toxin concentration. So, for example, *SEA* is more *heat*-sensitive than *enterotoxin* SEB, and SEB is more sensitive than SEC. The biological activity of SEB was retained after heating for 16 h at 60 °C and pH 7.3. Heating of one preparation of SEC for 30 min at 60 °C resulted in no change in serological reactions. The heating of SEA at 80 °C for 3 min or at 100 °C for 1 min

Table 4 Overview of reported SE doses required for intoxication in different hosts

Organism	*Toxin*	*Minimal dose to induce toxic effect or (ED_{50} per kg body weight)/host*
S. aureus	SEA	(25 μg)/rhesus monkey
		(10 μg)/rhesus monkey
		(∼10 mg)/rhesus monkey
		(144 ± 50 ng)/humans
		0.60 ng ml^{-1} of chocolate milk
	SEB	(0.4 μg)/humans
		(0.9 μg)/rhesus monkey
	SEC1	(1.0 μg)/pigtail monkey
	SEG	∼80 μg/rhesus monkey
	SEI	>150 μg/rhesus monkey
	SET	0.05–1.0 ng ml^{-1} milk/humans
		0.1–1.0 μg/humans

resulted in the loss of serological reaction. When evaluating heat inactivation of SE, one should be aware that SE may lose serological activity in detection immunologic assay, but remain biologically active. Crude toxin preparations have been found to be more resistant than purified toxins.

Enterotoxin and enterotoxin-like proteins are globular, single polypeptides with molecular weights ranging from 22 to 29 kDa. They can be encoded in prophages, plasmids, or chromosomal pathogenicity islands. The *seb* gene is carried on the *S. aureus* pathogenicity island, SaPI3, 59 while enterotoxin C (SEC) exists in multiple variants, C1, C3, and Cbov, which are situated on SaPI4, SaPIn1/m1, and SaPIbov, respectively. The *sed* gene is situated on a pIB485 plasmid, likewise *sej*. The expression of *seb*, *sec*, and *sed* genes is induced during the transition from the exponential to the stationary phase, an expression pattern characteristic of proteins encoded by genes regulated by the Agr regulatory system. *Seb* and *sec* undergo a much stronger induction than the plasmid-encoded *sed*. The *sea* gene is carried by a polymorphic family of temperate bacteriophages, and the transcription of sea is linked to some extent to the life cycle of the SEA-encoding prophage. The polymorphic nature of the prophages has been found to affect the amount of SEA produced by the bacterial strain carrying the prophage. Sequence analysis of the *sea* gene and its neighboring genomic regions has further indicated that SEA-producing strains can be grouped into two major groups, SEA_1 and SEA_2. SEA is produced throughout the growth phases. The amount of SEA produced has been related to the group and to the expression level of *sea*. The *see* gene is situated on a (defective) prophage and its expression appears to be unaffected by bacterial growth. It seems that virulence expression of *se* genes is upregulated in iron-depleted environment and in the conditions of the host environment. In other words, *in vivo* expression seems to be higher than *in vitro* expression.

The regulation of production of SE is SE-dependent, as well as strain- and environment-dependent. Under the same conditions, different strains may produce different amounts of SE. Different SEs are synthesized by *S. aureus* in different growth phases. This reflects also in the amounts of different SEs produced by *S. aureus* growing under optimal conditions in laboratory media. For SEB and SEC, the amounts may exceed 100 μg ml^{-1}, compared with 1–10 μg ml^{-1} for SEA and SED. Some indications exist that low amounts of SEB are produced already in early exponential growth phase. Reports exist that show that SEB appears in cultures as early as 4–6 h. However, SEA and SED are produced in foods under a wider range of pH, Eh, and aw than are the other SEs, which explain why SEA and SED are principal toxins involved in staphylococcal food poisoning.

Based on large-scale outbreak data, the intoxication dose of SEs (SEA) can be as low as 94–184 ng, corresponding to about 0.3 ng SEA per ml of chocolate milk.

There are different ways to detect SE in foods, each with its own characteristics. Described assays include those based on animal testing; immunologic, molecular, and biological assays; biosensors; and lately also chromatography-based methods. There are several commercial kits/systems available for SE detection, which are in routine use. ELISA-based kits for enterotoxin detection are commercially available from various manufacturers like 3 M™ Tecra™ (Staph Enterotoxins Visual Immunoassay; screening of the presence of SEA–SEE using AOAC International official method 3 M™ Tecra™ SET VIA and/or specific identification of present SEA–SEE using 3 M™ Tecra™ SET ID VIA; both methods have limit of detection at about 1 ng ml^{-1} of sample extract according to the manufacturer), bioMérieux (VIDAS SET2, which is a reference method for the European screening method of the Committee Reference Laboratory for milk and milk products and is also recommended as the primary screening method for suspect food samples and culture isolates in FDA BAM Chapter 13A and an official method on a variety of foods by AOAC International), Diffchamb AB (Transia Plate SE official methods recommended by Ministere de l'Agriculture, France, Transia Tube SE), R-Biopharm (RIDASCREEN A, B, C, D, and E with sensitivity of below 0.031 ng ml^{-1} in buffer for SEA, SEB, SEC, and SEE and 0.062 for SED and performance equal or better than VIDAS SET2 in cheese), and Toxin Technology (SET-EIA). In the latest years, some very prominent research groups in the field are working on proteomics-based methods, mainly using different formats of LC–MS, to provide new sensitive, robust, and instrumental detection and quantification of SE. Since as little as 100–200 ng of the toxin can cause symptoms of staphylococcal intoxication, the methods are required to have very high sensitivity.

European Legislation

In its latest Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs, the European Commission provided two kinds of microbiological criteria, the process hygiene criteria and food safety criteria. The rationale behind is that the safety of foodstuffs is mainly ensured by a preventive approach, such as implementation of good hygiene practice and application of procedures based on hazard analysis and critical control point (HACCP) principles. Process hygiene criteria that can be used in validation and verification of HACCP procedures and other hygiene control measures were set to define the acceptability of the processes. The food safety microbiological criteria set a limit above which a foodstuff should be considered unacceptably contaminated with the microorganisms for which the criteria are set. The food safety criteria were not set for *S. aureus*, but for SEs. This

was done only for cheeses, milk powder, and whey powder, with safety criteria defined as follows: $n=5$, $c=0$, and $m=$absence in 25 g. The criteria are valid for these products placed on market during their shelf life. As for process hygiene criteria, CPSs were defined for cheeses made from raw milk ($n=5$, $c=2$, $m=10^4$ cfu g^{-1}, and $M=10^5$ cfu g^{-1}), cheeses made from milk that has undergone a lower heat treatment than pasteurization and ripened cheeses made from milk or whey that has undergone pasteurization or a stronger heat treatment ($n=5$, $c=2$, $m=100$ cfu g^{-1}, and $M=1000$ cfu g^{-1}), unripened soft cheeses (fresh cheeses) made from milk or whey that has undergone pasteurization or a stronger heat treatment ($n=5$, $c=2$, $m=100$ cfu g^{-1}, and $M=1000$ cfu g^{-1}), milk powder and whey powder ($n=5$, $c=2$, $m=10$ cfu g^{-1}, and $M=100$ cfu g^{-1}), and shelled and shucked products of cooked crustaceans and molluscan shellfish ($n=5$, $c=2$, $m=100$ cfu g^{-1}, and $M=1000$ cfu g^{-1}).

See also: *Bacillus cereus* and Other *Bacillus* sp. Causing Foodborne Poisonings, Detection of; *Clostridium*: Food Poisoning by *Clostridium perfringens*; *Clostridium*: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin; Cured Foods: Health Effects; Diarrheal Diseases; *Staphylococcus*: Detection; *Staphylococcus*: Occurrence and Properties; Toxins in Food: Naturally Occurring.

Further Reading

Argudin MA, Mendoza MC, and Rodicio MR (2010) Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins* 2: 1751–1773.

Asao T, Kumeda Y, Kawai T, et al. (2003) An extensive outbreak of staphylococcal food fpoisoning due to low-fat milk in Japan: estimation of enterotoxin a in the incriminated milk and powdered skim milk. *Epidemiology and Infection* 130: 33–40.

Bennett SD, Walsh KA, and Gould LH (2013) Foodborne disease outbreaks caused by *Bacillus cereus*, *Clostridium perfringens*, and *Staphylococcus aureus* – United States, 1998–2008. *Clinical Infectious Diseases* 57: 425–433.

Bergdoll MS and Wong ACL (2006) Staphylococcal intoxications. In: Riemann HP and Cliver DO (eds.) *Foodborne infections and intoxications*, pp. 522–552. Amsterdam: Elsevier.

Dinges MM, Orwin PM, and Schlievert PM (2000) Exotoxins of *Staphylococcus aureus*. *Clinical Microbiology Reviews* 13: 16–34.

EFSA (2009) Scientific report of EFSA. Report on the availability of molecular typing methods for *Salmonella*, *Campylobacter*, verotoxigenic *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* isolates from food, animals and feedingstuffs in European Union Member States (and in some other reporting countries). *EFSA Journal* 272: 1–52.

Gill DM (1982) Bacterial toxins – a table of lethal amounts. *Microbiological Reviews* 46: 86–94.

Hennekinne JA, De Buyser ML, and Dragacci S (2012) *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. *FEMS Microbiology Reviews* 36: 815–836.

Honeyman A, Friedman H, and Bendinelli M (2001) Staphylococcus aureus *infection and disease*. New York: Kluwer Academic.

ICMSF (1996) Staphylococcus aureus. In: Roberts TA, Baird-Parker AC, and Tompkin RB (eds.) *Microorganisms in foods 5: characteristics of microbial pathogens*, pp. 299–333. London: Blackie Academic & Professional.

Jay JM (2000) Staphylococcal gastroenteritis. In: Jay JM (ed.) *Modern food microbiology*, pp. 441–459. Maryland: Aspen Publishers Inc, 6th ed.

Larkin EA, Carman RJ, Krakauer T, and Stiles BG (2009) *Staphylococcus aureus*: the toxic presence of a pathogen extraordinaire. *Current Medicinal Chemistry* 16: 4003–4019.

Larsen J, Imanishi M, Hinjoy S, et al. (2012) Methicillin-resistant *Staphylococcus aureus* ST9 in pigs in Thailand. *PloS One* 7(2).

Lawley R, Curtis L, and Davis J (2008) Staphylococcus aureus. In: *The food safety hazard guidebook*, pp. 70–74. Cambridge: Royal Society of Chemistry.

Schelin J, Wallin-Carlquist N, Cohn MT, Lindqvist R, Barker GC, and Radstrom P (2011) The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. *Virulence* 2(6): 580–592.

Relevant Websites

http://www.cdc.gov/foodsafety/outbreaks/investigating-outbreaks/confirming_diagnosis.html – Centers for Disease Control and Prevention (CDC) USA. Guide to Confirming a Diagnosis in Foodborne Disease.

http://ec.europa.eu/food/food/rapidalert/rasff_portal_database_en.htm – RASFF porta.

www.efsa.europa.eu/ – European Food Safety Agency, EFSA.

http://www.nlm.nih.gov/medlineplus/staphylococcalinfections.html – U.S. National Library of Medicine.

http://textbookofbacteriology.net/staph.html – Todar's Online Textbook of Bacteriology. "The Good, the Bad, and the Deadly".

Staphylococcal food poisoning is due to the absorption of staphylococcal enterotoxins preformed in the food. Here, we briefly review the latest data on staphy-lococcal 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. Staphylococcal food poisoning is a gastrointestinal illness. It is caused by eating foods contaminated with toxins produced by Staphylococcus aureus. The most common way for food to be contaminated with Staphylococcus is through contact with food workers who carry the bacteria or through contaminated milk and cheeses. Staphylococcus is salt tolerant and can grow in salty foods like ham. As the germ multiplies in food, it produces toxins that can cause illness. 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.