REVIEW

Staphylococcal enterotoxins and possibilities to prevent their production in food

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Summary
Foodborne diseases represent a significant global public health problem. For this reason, food safety and quality are among the main priorities of many international and global organisations. An important risk factor for bacterial food poisoning is the consumption of food contaminated with toxinogenic bacteria. The most important causative agent of alimentary intoxications is *Staphylococcus aureus*. Staphylococcal poisoning occurs after ingestion of food containing staphylococcal enterotoxins produced by toxinogenic strains of staphylococci, especially *S. aureus*. Recent studies focus on the presence and toxinogenic character of *S. aureus* isolates from food; however, toxin production has also been detected in other coagulase-positive and even coagulase-negative staphylococci. If such toxinogenic staphylococci are also antibiotic resistant, the risk of poisoning, as well as the duration and cost of treatment, and the possibility of the spread and survival of these strains in the environment are increased. The main aim of this review is to summarise the current knowledge regarding the characteristics, expression, and control of staphylococcal enterotoxins in food matrices.

Keywords
Staphylococcus aureus; staphylococcal food poisoning; inhibition of enterotoxin production; food processing factors

It is well known that food has a direct impact on human health, and therefore, food safety is considered a global challenge. A supply of safe food free from harmful contaminants ensures the maintenance of human health, economic development, social stability, and international trade, thereby promoting sustainable development. For these reasons, the European Union seeks to ensure the safety of food that reaches the Community market. In 2002, the so-called general food law was adopted in the form of Regulation (EC) No 178/2002 of the European Parliament and of the Council [1], laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. In 2004, following the adoption of the general food law, the European Parliament and the Council adopted the so-called hygiene package, followed by the harmonisation of microbiological criteria in the form of Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs [2]. This regulation more specifically sets limits for microbiological contamination of different food categories and also specifies rules for sampling and sample preparation.

Staphylococcal food poisoning (SFP) accounts for an estimated 14 % of all foodborne illnesses and thus is considered one of the most common causes of foodborne outbreaks (FBO) worldwide [3, 4]. In 2022, there were 7 reported outbreaks of SFP in the USA, which resulted in 64 documented cases of illness [5]. In comparison, the EU recorded 137 SFP outbreaks, representing 2.4 % of all documented FBOs [6]. In Slovakia, no cases of unspecified bacterial enteric infection caused by *S. aureus* have been reported since 2020; the last outbreak with proven *S. aureus* as the causative agent was recorded in 2018 [7]. The real incidence of SFP is likely much higher, as most affected individuals recover from the infection without seeking medical attention [4, 8]. This underestimation of SFP real incidence also includes misdiagnosis, improper sampling, or unavailability of suspect foods needed for laboratory testing [4]. Although life-threatening SFP cases are rare, they pose a serious

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problem for the food industry as well as healthcare systems [9, 10]. For these reasons, the study of the food environment's effect on bacterial growth and toxin production is essential for the control and prediction of staphylococcal enterotoxin (SE) production in relation to the food environment. Understanding the mechanisms of SE production and then finding ways to regulate them through food composition treatment, preservative content, pH value, represents the greatest challenge to overcome in the fight against SFP [11].

The aim of this review was to highlight the complexity of bacterial food safety related to the potential presence of toxinogenic strains of *S. aureus* in the food production chain. We also address the issue of antibiotic-resistant staphylococci in food. The survival of unwanted bacteria under food processing conditions is of concern to many manufacturers, particularly in terms of the stability and quality of the final products. Therefore, we also aimed to summarise the currently available knowledge on food processing factors that can significantly influence *S. aureus* growth and survival as well as toxin production in food, thus enhancing food safety.

Bacteriological food safety

Many factors affect food safety, but foodborne pathogens (bacteria, viruses, fungi and parasites), allergens, pesticides and chemical residues are of greatest concern [12, 13]. It has been reported that almost 70 % of foodborne diseases are caused by bacteria possessing virulence factors that allow them to induce disease in consumers [12, 14]. This includes, in addition to structural factors, the production of toxins that bacteria release directly into the food or only in the digestive tract after colonisation [14].

Among the bacteria that cause food poisoning, some are particularly important in terms of the frequency and severity of illnesses caused by them [12]. Although 31 pathogens have been identified as causative agents of foodborne diseases, the most reported worldwide are *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perfringens*, and *Vibrio* spp. [12, 15, 16]. Gram-negative bacteria have been reported to be involved in almost 70 % of bacterial foodborne disease cases [12]. However, the distribution of pathogens varies from region to region due to cultural and economic factors that affect proper food handling and storage [14]. Thousands of foodborne outbreaks have been reported in the EU in recent years (5 763 in 2022, 4 005 in 2021), with thousands of human cases, thousands of hospitalisations, and approximately one hundred deaths [6, 17]. Campylobacteriosis, followed by salmonellosis, has been the leading cause of zoonoses reported for several years [6, 17]. The European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) summary report lists yersiniosis as the third most frequently reported zoonosis in 2022, followed by Shiga toxin-producing *E. coli* (STEC) and *L. monocytogenes* infections [6].

A significant risk factor for bacterial food poisoning is the consumption of food contaminated with pathogenic toxin-producing bacteria [18]. Bacteria cause the highest number of FBOs in the EU (25.1 % in 2022; 28.5 % in 2021), followed by bacterial toxins (19.8 % in 2022; 17.0 % in 2021) and viruses (6.1 % in 2022; 6.8 % in 2021). It should be noted that the causative agents of the majority (46.1 % in 2022 and 45.7 % in 2021) of FBOs remain unspecified. At the EU level, the most reported bacterial toxin producers causing FBOs are *Bacillus* spp. (*B. cereus*), *Staphylococcus* spp. (*S. aureus*) and *Clostridium* spp. (*C. perfringens* and *C. botulinum*). In 2022, FBOs caused by bacterial toxins were reported from 16 EU member states and 3 non-member states, while all seven deaths were associated with the outbreaks recorded in France [6, 17].

In terms of food safety, the occurrence of antibiotic-resistant zoonotic pathogens, which can contaminate foodstuffs at any stage of the food chain, so-called "from farm to fork", is also of increased concern [12]. Antimicrobial resistance (AMR) represents a complex problem, so it is unlikely that solutions to mitigate and reverse its impacts on human, animal, plant, and environmental health would be solved by the actions of any single group or organisation [19]. For this reason, a multidisciplinary and collaborative approach is necessary, and thus, the EU applies the One Health approach in the fight against AMR, which simultaneously addresses issues related to veterinary, human health, and the environment [20]. The aim of the One Health concept is to foster collaboration among different sectors and disciplines, thereby expanding knowledge about the emergence and transmission of AMR and enabling the implementation of evidence-based approaches to managing the risks associated with AMR.

Foodborne diseases caused by *Staphylococcus aureus*

SFP is attributed to the ingestion of one or more staphylococcal enterotoxins (SEs) already produced in foods contaminated with toxinogenic staphylococcal strains [3, 21]. While the genus *Sta-*

phylococcus spp. includes 53 species and 28 subspecies, SE production has been exclusively attributed to *S. aureus* for a long time [8, 9]. However, this paradigm has been shifting recently due to studies suggesting that other staphylococci are also capable of producing enterotoxins and thus may contribute to SFP outbreaks [9]. SE production has been detected not only in coagulase-positive staphylococci (CoPS) but also in coagulase-negative strains (CoNS), such as *S. epidermidis*, *S. haemolyticus, S. cohnii, S. xylosus* and *S. hyicus* [20, 22]. It is important to note that in cases of foodborne diseases caused by SEs, it is difficult to determine whether the SEs were produced by CoPS or CoNS, especially in heat-treated foods in which microorganisms have been devitalised, but SEs have retained stability. Routinely used tests can detect SEs even in the absence of viable cells in the incriminated food. Therefore, the potential involvement of CoNS in the onset of SFP cannot be excluded.

S. aureus is not only the main culprit of SFP but also the causative agent of a wide spectrum of diseases ranging in severity from mild skin infections to more severe diseases such as pneumonia, mastitis, meningitis, urinary tract infections, osteomyelitis, and endocarditis [12, 23]. Unlike many other bacterial pathogens that often rely on just one or a few toxins to cause disease, *S. aureus* possesses an overwhelming number of virulence factors. These include numerous toxins, immune evasion factors, as well as a wide range of proteinaceous and non-proteinaceous factors that enable host colonisation during infection [24]. The most significant secreted toxins include SEs, leukotoxins, hemolysins, exfoliative toxins, and toxic shock syndrome toxin (TSST-1) [25].

Furthermore, *S. aureus* has developed resistance to almost all commonly used antibiotics through horizontal transfer of antimicrobial resistance determinants and less frequently through gene mutation [26]. Methicillin-resistant *S. aureus* (MRSA) possesses *mecA* or *mecC* genes mediating resistance to methicillin and all other β-lactam antibiotics [27]. Currently, MRSA is globally recognised as a major cause of healthcare-associated infections, but apart from the hospital setting, these antibiotic-resistant pathogens have also been isolated from food [28].

Characteristics of staphylococcal enterotoxins

SEs are water-soluble, structurally stable polypeptides ranging in size from 22 kDa to 29 kDa, resistant to extreme conditions such as freezing and drying [4, 9]. They are also characterised by high resistance to heat, proteases (pepsin, trypsin, renin, papain), denaturing agents, and a wide range of pH, allowing them to maintain stability in the digestive tract [4, 29]. Thermal tolerance varies among SEs, with a decrease in the order SEC > SEB > SEA, and notably diminishes under acidic conditions [29]. The thermal resistance of SEs is influenced by several factors, including the type of food matrix in which the SEs are found, as well as pH and NaCl concentration. Common heat treatments used in food processing are ineffective in eradicating high levels of SEs implicated in SFP [29, 30].

The current literature lists 25 different SEs named from SEA to SElZ in chronological order of discovery, with the letter F dropped from the nomenclature because it originally denoted TSST-1 [25, 31]. It is anticipated that this list will expand in the future as automated characterisation techniques at the molecular and genetic levels become more widely utilised [22].

Several classification schemes have been proposed to categorise SEs into a limited number of coherent groups [22]. Based on emetic activity, SEs are divided into two groups referred to as “SE,” which includes toxins with demonstrated emetic potential in primates, and “SEl-,” encompassing staphylococcal enterotoxin-like proteins that lack proven emetic activity [22, 31, 32].

It has been reported that more than 90 % of SFP is caused by so-called classic SEs designated as A, B, C, D, and E [30, 33]. Among these types, SEA is responsible for 77 % of all SFP outbreaks, followed by SED (37 %) and SEB (10 %) [4, 34]. SEA is also the most common SE serotype causing SFP in the United States, Japan, France, and the United Kingdom [4]. SEA is predominantly produced by human strains; therefore, a link to food contamination is assumed during production. On the other hand, SEC is regarded as the primary cause of SFP linked to the consumption of dairy products [29].

Among the non-classical SEs, SEG, SEH, SEI, SEM, SEN, SEO, SER, SES, SEIY, and SET have been identified as potential toxins involved in SFP [10, 21, 33]. Since available commercial kits allow the detection of only the classical SEs, the contribution of other SEs and SEls to the induction of SFP remains unclear [22, 31]. The frequent co-occurrence of genes encoding SEs and SEls in staphylococcal strains isolated from patients with SFP suggests that novel SEs and SEls may also play a role in the onset of SFP. This assumption was confirmed in the SFP case in Japan in 2000 [22], where SEA was detected as the cause of the outbreak. However, five years later, Western blot analysis of samples from this outbreak revealed

the presence of SEH in almost the same quantities as SEA. The inadequacy of clinical laboratory analysis, when only the classical set of SEs is tested, is also indicated by the fact that in foods responsible for clinical symptoms typical of SFP, none of the classical SEs were detected.

Genes encoding SEs are carried on various mobile genetic elements (MGE) such as prophages, plasmids, transposons, *S. aureus* pathogenicity islands (SaPI), and highly variable genomic regions [31, 32]. MGEs can spread through horizontal gene transfer among species of the genus *Staphylococcus*, leading to the diffusion of SE not only among *S. aureus* strains but also among other staphylococci [21]. Genes encoding classical SEs are carried by a phage (*sea*), *seb* and *sec* are located on SaPIs, *sed* is found on a plasmid, and *see* is transported by a defective phage [9, 31]. Although most *se*/*sel* are generally found on MGEs, the *selx* and *selw* genes are located in the core chromosome [22].

S. aureus has a complex network of regulatory systems that act independently or in coordination and provide control of SE production. The most well-known of such regulatory systems is the accessory gene regulator (Agr), which uses quorum sensing in response to cell density. Although the *seb*, *sec*, and *sed* genes are carried on various MGEs, their expression is induced by the Agr system during the transition from the exponential to the stationary phase of growth at a cell density of 10^6–10^8 CFU·ml^{-1} or 10^6–10^8 CFU·g^{-1} [9, 22, 34]. The function of the Agr system is influenced by additional transcriptional regulators that indirectly affect the transcription levels of these SE genes. It is also important to note that these regulators respond to various environmental factors, which also affect SE synthesis [9, 34].

The expression of *sea* and *see* genes encoded by prophages is not controlled by the Agr system. The *sea* gene is carried by a polymorphic family of bacteriophages, and its transcription is associated with the prophage life cycle. Under conditions of environmental stress, the prophage can be induced to replicate the phage genome and release new bacteriophages. The polymorphic nature of prophages affects the amount of SEA produced by the bacterial strain carrying the prophage [9].

SEE is the most similar to enterotoxin A, sharing 90% homology in amino acid sequence [34]. The *see* gene is located on a defective prophage, and unlike *sea* expression, the expression of the *see* gene appears to be unaffected by bacterial growth [31, 34]. Regarding the regulation of non-classical enterotoxins, study results suggest that the expression of most newly described SE genes is not controlled by the Agr system [9, 31]. The obtained data show that only the transcript levels of *seh*, *ser*, and *sel* increase in the post-exponential phase, indicating possible regulation by Agr. Transcription levels of other SEs genes either remain unchanged during growth (*sej, sek, seq, sep*) or slightly decrease after exponential growth (*seg, sei, sem, sen, seo, seu*) [9].

Staphylococcal enterotoxins – mode of action

After contaminated food ingestion, SEs can pass through the gastrointestinal tract due to their proteolytic enzyme resistance [10]. SFP is an acute illness characterised by severe vomiting and nausea, often accompanied by abdominal cramps and watery diarrhoea [10, 31]. Symptoms can appear, with or without fever, within 1–8 h (on average 3 h) after consuming contaminated food [9, 10]. The illness usually resolves spontaneously within 24–48 h, but rare cases of fatal dehydration can occur, with a mortality rate ranging from 0.03 % in the general population to 4.4 % in children and the elderly [9, 31].

The mechanism of induction of vomiting and diarrhoea has not yet been fully investigated to provide direct evidence for SE mode of action [14, 22]. However, studies have suggested that SFP symptoms result from an inflammatory reaction in the gastrointestinal tract, manifesting as damage to the lower part of the small intestine [33]. The interaction of SEs with mast cells causes their degranulation and the release of the gastrointestinal neurotransmitter serotonin, which stimulates the vagus nerve, leading to the activation of the emetic centre [14, 34]. For the mechanism of diarrhoea induction, it is generally accepted that SEs act in a similar manner to cholera toxin. However, any comparison with this toxin remains speculative, and alternative mechanisms have been proposed, such as direct effects on mucosal transport, as well as changes in blood flow and gastrointestinal motility [22].

The severity of the illness depends on the amount and type of toxin in the ingested food and the overall health of the consumer [10]. Data regarding the intoxicating doses causing SFP vary depending on the source of information, the group of exposed consumers, and the type and number of SEs responsible for the intoxication [22]. According to the Food and Drug Administration (FDA) [35], the intoxicating dose of SEs is less than 1.0 μg, with the toxic dose generally reported to be in the range of 20–100 ng. An amount of approximately 1 ng of SEs per gram of contaminated food is sufficient to induce symptoms of SFP [8, 10, 31].

Staphylococcus aureus in the food chain

S. aureus can grow in a wide range of temperatures (7–48 °C, with an optimum of 30–37 °C) and pH levels (4.2–9.3, with an optimum of 7.0–7.5). A characteristic feature of this microorganism is its ability to tolerate sodium chloride concentrations up to 15 %, which is also linked to its resistance to high osmotic pressure and low water activity (a_w). It has been reported that the minimum a_w value for the growth of *S. aureus* ranges from 0.83 to 0.86. These properties enable *S. aureus* to survive in a wide variety of foods [12, 29, 31].

The occurrence of SFP is most associated with the consumption of protein-rich foods such as meat, egg, and dairy products, which are rich in amino acids and low molecular weight peptides. Such composition of food matrices promotes the survival and growth of *S. aureus* [31]. Salads, bakery products filled with cream, cakes, and sandwich fillings also contribute to many outbreaks of SFP. However, the foods associated with SFP outbreaks vary between countries, primarily due to differences in consumption patterns, dietary habits, culture, and geographic location [4, 34]. For example, in island countries like Japan and Malaysia, the foods most identified as being in SFP outbreaks are sushi, sashimi, and seafood. On the other hand, in Europe, the USA, and Russia, SE contamination is common in dairy products and beef products, while in China, this type of contamination is often detected in retail-ready-to-eat foods [34]. In the EU, SFP is most associated with the consumption of mixed foods, meat and meat products, cheese and dairy products, bakery products, fish and fish products [31].

The potential negative impact of the presence of *S. aureus* in food has led to the introduction of requirements for monitoring the presence of this microbial species, which are established in Commission Regulation (EC) No 1441/2007 on microbiological criteria for foodstuffs [36]. However, this regulation defines the criterion for SEs only in raw milk, dairy products, shellfish, and molluscs, even though different types of food matrices are involved in SFP epidemics.

In 2022, four EU member states (Croatia, Germany, Greece, Italy and Spain) provided data on the occurrence of *Staphylococcus* spp. in 7 494 food matrices, with 8.3 % of samples testing positive [6]. The highest positive detections were reported in food categories such as other processed food products and prepared dishes, meat products – ready-to-eat and cheeses soft and semi-soft. In the same year, four EU member states (Bulgaria, Italy, Slovakia and Spain) also provided data on SEs in the context of Regulation (EC) No 2073/2005. In total, 2 294 samples were analysed, with seven (0.3 %) testing positive, reported by Italy (five cases) and Spain (two cases). Samples positive for the presence of SEs included cheeses made from cows' milk unspecified and cheeses made from cows' milk – soft and semi-soft [6]

In Slovakia, 10 575 (11 073 in 2021) food samples were tested for CoPS in 2022, with the number of samples above the limit increasing slightly compared to previous years (1.73 % in 2022; 1.33 % in 2021). As of 2021, the highest proportions of positive samples were recorded for breast milk (7.14 % in 2022; 8.64 % in 2021) and for milk and milk products (7.88 % in 2022; 8.38 % in 2021). SEs were not detected in any sample. However, SE production was demonstrated in 33.3 % of the tested isolates from breast milk [7, 37].

Food contamination with toxinogenic strains of *S. aureus* often occurs as a direct result of the presence of these bacteria in food-producing animals, or is caused by inadequate hygiene during food production, storage, or sale of food products [14, 31]. From a food microbiological point of view, food handlers are considered the main source of food contamination [10]. *S. aureus* is a natural commensal of the human skin (especially hands, chest, and abdomen), gastrointestinal tract, and nasal cavities. About 30 % of people are considered intermittent carriers, while 15–35 % are permanent carriers. Asymptomatic workers can contaminate food through improper handling and respiratory secretions [10, 34].

Gelbíčová et al. [38] evaluated the occurrence and characteristics of *S. aureus* isolates from throat and hand swabs of employees from three cheese factories. *S. aureus* was detected in 58 % of hand swab samples and 47 % of throat swab samples. Strains carrying genes responsible for SEs production (58 %) and/or TSST-1 (25 %) were isolated from employees of all three facilities.

Cross-contamination was identified as one of the causes of SFP, leading to the transfer of microbes into foods from other foods and/or non-food items [10]. If food becomes contaminated at a central point from which it is further distributed, an SFP epidemic can have serious consequences affecting thousands of people. An example is the more than 13 000 cases of SFP in Japan in 2000 caused by milk contamination at a dairy food manufacturing plant [4].

Incorrect personal hygiene practices by food handlers can have serious consequences for food safety [10]. Evidence of this assertion is the SFP outbreak in Italy in 2015, which affected 24 out

of 42 customers at a local restaurant [39]. High levels of CoPS (3.4×10^8 CFU·g^{-1}) and SEA (2.12 μg·kg^{-1}) were detected in a cream dessert. Three *sea*-positive strains were isolated from the dessert, environment, and one of the chefs shared the same pulsed-field gel electrophoresis (PFGE) profile that belonged to the human biotype. This indicates that the contamination leading to the epidemic likely originated from a food handler. Additionally, improper storage of the dessert at room temperature facilitated microbial growth and SEA production.

Animals are also an important source of *S. aureus* contamination, with their fur and skin being common reservoirs. Farmers and the environment also pose a potential risk of spread, as staphylococci are commonly found in the air, dust, and wastewater. During the processing of slaughter animals and milk collection, enterotoxigenic bacteria can contaminate the carcass and raw milk. In addition, unhygienic operational habits can also increase the likelihood of contamination [34].

It is important to note that the risk to consumers increases if toxinogenic bacteria present in food are also antibiotic-resistant [10]. Data from several studies suggest that *S. aureus* isolated from food is resistant to most antibiotics, in particular penicillin, tetracycline, amoxicillin/clavulanic acid, erythromycin, gentamicin, chloramphenicol, clindamycin, cefoxitin, and oxacillin [34]. Oliveira et al. [40] tested the microbial susceptibility in isolates obtained from raw milk. Thirty-six percent showed resistance to at least one type of antibiotic, with the most common resistance being to penicillin (32 %), followed by resistance to tetracycline (24 %), ciprofloxacin (16 %), and chloramphenicol (16 %).

In the EU, monitoring of antibiotic resistance in *S. aureus* from food and animals is conducted on a voluntary basis. In 2022, Slovakia monitored antibiotic resistance in *S. aureus* in 1 065 samples (509 samples in 2021) from food, animals, water, and the environment, with resistance detected in 9.39 % (15.32 % in 2021) of samples [7, 37].

Currently, many studies focus on the detection of MRSA in foods. This pathogen represents a significant cause of morbidity and mortality worldwide. Transmission of zoonotic MRSA to humans can occur either through contact with animals or contaminated food. However, information on the potential transmission of MRSA to humans through the food chain remains limited [23]. In 2022, only four EU member states (Germany, the Netherlands, Slovakia and Spain) provided data on MRSA monitoring in food [41]. The prevalence of MRSA was investigated in broiler meat (Germany, the Netherlands, Spain), turkey meat (Germany, the Netherlands), duck meat (Germany) and pork meat (Slovakia, the Netherlands). In addition, the Netherlands reported data on MRSA in meat from bovine animals, wild game, deer, farmed game and fruit. The highest percentage of positive samples (43.6 %) was recorded in Germany in turkey meat samples. In Slovakia, almost 20 % of food samples tested positive for MRSA in 2022 (compared to 16 % in 2021) [7, 37]. Unlike in 2021, when the highest percentages of methicillin-resistant isolates were found in pork (32.84 %) and beef (4.68 %), in 2022, methicillin-resistant isolates were mainly obtained from turkey (30.69 %) and pork (19.29 %). Methicillin resistance was also monitored in 389 isolates from water and the environment, with 12.34 % of isolates being resistant, while isolates from swabs in food processing facilities showed no resistance [7, 37]. Information regarding the occurrence of MRSA in food in Slovakia is available in the annual Report on zoonoses, alimentary and waterborne infections in the Slovak Republic, issued by the Ministry of Agriculture and Rural Development of the Slovak Republic [7, 37], but it was not included in the report provided by EFSA [41].

In 2020, a study was conducted in Algeria [23], analysing 300 food samples, of which 17 % were contaminated with *S. aureus*. A total of 104 isolates were obtained, with 63 % carrying one or more genes encoding SEs. The highest level of resistance was observed to penicillin G (95 %), and five isolates showed methicillin resistance mediated by the *mecA* gene.

Most published studies focus on the detection of *S. aureus*, but other staphylococci, including CoNS, also contaminate food and are even more frequent in the food chain than CoPS. CoNS have been considered non-pathogenic; however, this view has been reconsidered, and they are now classified as significant nosocomial pathogens. Moreover, CoNS in food are important reservoirs of virulence factors, exotoxins, as well as antibiotic resistance genes that can potentially be transferred to CoPS through horizontal transfer [42]. Despite this knowledge, monitoring the presence of CoNS in foods is not part of the requirements of Commission Regulation (EC) No. 1441/2007 [36]. Given that CoNS are commonly found in food, it has been suggested that they may play an important role in the epidemiology of alimentary diseases.

Regecová et al. [43] focused on detecting the presence of *S. warneri* strains, their further characterisation in terms of resistance, and SE production in animal-origin foods. A total of 45 iso-

lates were obtained, with the presence of *sea* and *sed* genes confirmed in three and six isolates, respectively. The most detected resistance was to ciprofloxacin and tetracycline (73 %), with 22 % of isolates showing multidrug resistance. The isolates showing phenotypically confirmed β-lactam resistance were subsequently subjected to *mecA* gene detection, with its presence confirmed in four isolates. Although SE production was not confirmed, the results of the study highlighted the toxinogenic capacity of CoNS, which should not be ignored. Instead, the presence of this group of microorganisms should be monitored in food.

Occurrence of staphylococcal enterotoxins in food

The rate of toxin production is related to the growth rate of the producer. It is reported that the amount of SEs required to cause disease in food is reached when the population of *S. aureus* exceeds 10^5 CFU·g^{-1} [29]. Improper food storage conditions allow *S. aureus* strains to grow and reach the cell density necessary for SE production. The factors enabling SE production have a narrower range compared to the conditions necessary for the growth of the producing strain [30]. SEs are generally produced within a temperature range of 10–46.6 °C, with an optimal temperature of 34–40 °C. Their production decreases at 20–25 °C and is unlikely to occur at temperatures below 10 °C. The pH range for SE production varies among different types; for instance, SEA is synthesised over a broader pH range than SEB or SEC. Generally, SE production is possible within a pH range of 5.0–9.6, with optimal values between pH 7 and pH 8. However, SE production has also been reported at pH 4.0 in growth media, though in dairy products, a pH above 5.0 is generally required for SE production. Increased tolerance to pH changes has been observed under aerobic conditions compared to anaerobic growth conditions. Regarding aw requirements, the values fall within the same range as for growth, with the minimum value for SE production being reported as a_w 0.86 [3, 31, 44]. Nonetheless, Medveďová et al. [29] confirmed SED production at lower a_w values (0.842) at 37 °C. The a_w range also depends on the type of toxin produced; for example, SEB and SEC production is more sensitive to reduced water activity compared to SEA production [29, 31].

Most of the data available in the literature on the prevalence and characterisation of SE-producing *S. aureus* in livestock focus on dairy animals, with the occurrence of enterotoxigenic strains often associated with the diagnosis of mastitis [31].

Grispoldi et al. [45] analysed *S. aureus* isolates obtained from milk samples from 12 dairy farms in Italy. All genes encoding classical SE were detected in the isolates: *sea* 36 %, *seb* 6 %, *sec* 6 %, *sed* 29 % and *see* 47 %. The production of each SE was determined by enzyme immunoassay, with 41 % of the isolates producing SEA, 6 % SEB, 6 % SEC, 30 % SED and 35 % SEE.

Pasteurisation is considered an effective method for ensuring the quality and microbiological safety of dairy products. Although the high temperature used in this process can devitalise bacterial cells, SEs retain their biological activity [28]. Therefore, preventing contamination, growth, and potential SE production is crucial for minimising the risk of food poisoning [31].

A study conducted in China monitored the contamination of *S. aureus* in 258 samples of pasteurised milk [28]. The presence of *S. aureus* was detected in 3.9 % of the samples. Seventy-five percent of the isolates were resistant to three or more classes of antibiotics, and 7.7 % of the isolates possessed the *sec* gene.

There is less data available on SEs produced by *S. aureus* strains isolated from non-dairy samples. Zhang et al. [46] tested 130 *S. aureus* isolates from samples related to pork production for the presence of 18 SE-encoding genes. Ninety-five percent of the isolates from various stages of pork production harboured one or more SE genes, with *seb* being the most frequently detected gene (60 %). Almost 7 % of the isolates contained five SE genes.

Possible inhibition of enterotoxin production

Stakeholders involved in the food chain are making efforts to prevent, detect, and manage hazards arising from microbial food contamination. New challenges are constantly emerging in the fight against foodborne diseases. Among the causes are the changes in consumer eating habits and preferences. This assertion is particularly relevant to the increasing consumer demand for minimally processed foods, which provide a favourable environment for most pathogenic bacteria, including *S. aureus* [11].

In order to minimise the risk of SFP, it is particularly important to eliminate contamination, prevent the growth of *S. aureus* and toxin production, or avoid the accumulation of high levels of SEs (> 10–20 ng) in food [11, 31, 47]. In the food industry, food safety is ensured by preventive measures based on the potential risks associated with the production of a specific product. These are based on the principles of good hygiene practices (GHP) and on the Hazard Analysis and Critical Control Point (HACCP) system [48]. The most important preventive measures include improving

personal hygiene practices among food handlers, adequate cleaning and decontamination of equipment, surfaces and clothing, proper storage of food and preventing cross-contamination [4, 12].

Commission Regulation (EC) 1441/2007 [36] defines the maximum allowable limits of viable CoPS cells in food, which apply during or at the end of the production process, depending on the food category. If CoPS levels exceed 10^5 CFU·g^{-1}, food samples must be tested for the presence of SEs, which, as defined by the regulation, must not be present in 25 g of any sample. This requirement is partly based on earlier studies that demonstrated the critical population size required to determine detectable levels of SEs [47].

Although the use of these standards is well established, they have several limitations. One of these is that the number of *S. aureus* cells is not always a sufficient indicator of the presence of SEs in food. This assertion is based on the fact that not all strains of *S. aureus* are enterotoxigenic or capable of expressing genes encoding SEs [48].

On the other hand, the results of several studies suggest that SE can be detected even at cell densities lower than 10^5 CFU·g^{-1} or 10^5 CFU·ml^{-1}. Some studies have detected the presence of SEA and SEC at cell concentrations of 10^4 CFU·ml^{-1} [49, 50]. Medveďová et al. [29] determined the production of SED at a cell count of 3.6×10^3 CFU·ml^{-1}, at a_w 0.907 after 73 h and at a temperature of 18 °C and a_w 0.995 after 9 h, when the concentration of *S. aureus* cells reached 4.6×10^3 log CFU·ml^{-1}. From the results of these studies, it can be concluded that assessing the risk of SFP associated with a food product solely based on the number of colony-forming units of *S. aureus* present is not a completely reliable procedure.

It is also important to note that in heat-treated foods, where high temperatures ensure the elimination of viable cells, SEs retain their stability (biological activity of SEB is maintained after heating to 60 °C for 16 h and pH 7.3; heating of SEC for 30 min at 60 °C did not lead to any change in serological reactions; SEA loses serological activity after heating for 3 min at 80 °C or for 1 min at 100 °C) [29, 48].

Necidova et al. [51] evaluated the thermal stability of SEA, SEB and SEC in milk previously contaminated with enterotoxigenic *S. aureus* (10^4–10^5 CFU·ml^{-1}) incubated at 37 °C for 24 h. After heat treatment at 72 °C, 85 °C or 92 °C for 15 s, all samples were negative for *S. aureus*, but SEs were detected in 87.5 %, 52.5 % and 45.0 % of the samples, respectively.

Skim milk powder associated with staphylococcal intoxication in Japan in 2000 was processed at 130 °C for 2–4 s. Although *S. aureus* cells were eliminated, SEA, which was probably produced during the storage of raw milk, retained its biological and immunological properties [52].

The results of these studies suggest that SEs may exhibit biological activity and be the causative agent of SFP despite heat treatment. The thermal stability depends on the type of SE, the medium in which the toxin is present, salt concentration, pH, as well as other factors related to the level of protein denaturation [29].

The impact of environmental factors

In general, the growth of *S. aureus* is essential to produce SEs. Therefore, growth control is an essential step in preventing toxin production. Although bacteria adapt well to their environment, there are certain factors that promote their growth more than others. These include the type of food matrix, a_w, pH, temperature, time, oxygen availability and presence of competing microbiota [29, 31]. While the growth of the organism is suppressed by competing microbiota in many food matrices, *S. aureus* has shown a growth advantage in foods with low pH or high sugar or salt concentrations [47].

Modifying environmental factors to eliminate optimal conditions for pathogen growth and metabolite production may increase the stability of products with an increased incidence of *S. aureus*. Inhibition of *S. aureus* growth can be ensured by appropriate setting of production conditions, maximum permissible storage temperature or maximum salt addition [53].

In 2016, Medveďová et al. [29] published a comprehensive study dealing with the growth dynamics of *S. aureus* 14733, isolated from cheese, and SED production in relation to environmental factors. The results of the study showed that a decrease in a_w generally prolonged the duration of the lag phase and slowed the growth rate. At higher values of a_w (0.996 and 0.989), SED was detected already after 4 h of incubation. At 37 °C, SED was also detected at low values of a_w (0.857 and 0.842). SED production was not limited by incubation temperature and NaCl addition (up to 15 % at 18 °C and 21 °C and up to 20 % at 37 °C). The combination of reduced pH values (to values of 6.0 and 5.5) and a_w values (0.99 and 0.97) did not inhibit the growth of the organism. SED was not detected at any temperature when the pH was adjusted to 4.5 with lactic acid. Also, a longer time requirement for SED production was observed in the presence of lactic acid alone compared to the combination of lactic acid and NaCl at 21 °C and 37 °C. The study also aimed at the growth of

S. aureus 14733 in the presence of two different additions of Fresco starter culture at 15 °C, 18 °C and 21 °C in milk. When *S. aureus* 14733 was co-cultivated with a Fresco culture, inhibition of *S. aureus* growth was detected before a drop in pH that could significantly affect growth was observed.

An important factor to consider when estimating or monitoring SE production is the correlation between growth and toxin production in highly variable and complex food matrices under diverse environmental conditions, which may affect the expression of virulence factors in different and unpredictable ways [11, 47]. TSUTSUURA et al. [54] determined the production of SEA in brain heart infusion broth. The toxin was detected after 3 weeks of incubation at 10 °C, after 3–8 days at 15 °C, after 30–58 h at 20 °C and after 6–8 h at 37 °C. In contrast, GRISPOLDI et al. [45] reported slower SEA production at 37 °C and 20 °C in canned meat, while no SEA was detected at 10 °C.

MÁRTA et al. [55] pointed out specific levels of SE expression in different ham products in their study. The authors investigated the expression and production of SED in three types of ham products (cooked, smoked and dry ham) incubated at room temperature for 7 days. Continuous *sed* expression was observed in cooked and smoked ham during the incubation period. Nine times fewer SED per number of *S. aureus* CFU was detected in smoked ham than in cooked ham. In cooked ham, SED levels decreased unpredictably after three days of incubation. In dry ham, SED was detected after five days of incubation, although *S. aureus* growth was weak.

Currently, numerous publications focus on assessing the risk of *S. aureus* growth and toxin production in different types of food products under the inhibition of environmental factors [11]. However, most available studies are concentrated on determining the production of classical SEs, while knowledge about the expression of newer SE is limited [48]. This is understandable, given the proven potential of classical SEs to cause SFP. On the other hand, more recent studies have provided evidence of the contribution of newly described SEs to the occurrence of SFP [21, 22, 56].

GRISPOLDI et al. [45] analysed the growth and SEA production of three *S. aureus* strains in canned meat (with and without sodium nitrite treatment) before sterilisation at three different temperatures (10 °C, 20 °C and 37 °C). The presence of SEA was detected after 10 h of incubation at 37 °C and after 48 h of incubation at 20 °C. Toxin was produced during the transition from the exponential to the stationary growth phase. After incubation at 10 °C, SEA was not detected. Statistical analysis of the data revealed no significant difference between the meat samples treated and untreated with sodium nitrite.

WANG et al. [57] tested the inhibitory effects of varying pH, nisin content and a_w after adding sorbitol (7 %, 14 %, 21 %) on the growth of *S. aureus* and SEA production in whipped cream stored at 36 °C for 36 h. In this study, the reducing a_w after sorbitol addition proved to be the most effective method in inhibiting the growth of *S. aureus* and delaying SEA production. In contrast to cream with a pH of 5.5 or containing 0.5 g·kg^{-1} nisin (maximum values in the respective tested groups), SEA production was delayed in cream with minimal sorbitol levels (7 %). Cream containing 14 % and 21 % sorbitol significantly inhibited *S. aureus* growth, and SEA remained undetectable for 36 h. Considering costs and effects, the addition of 7–14 % sorbitol proved to be the most suitable choice.

Molecular aspects

Each step of food processing acts as a stress factor on the cell that can induce an increase in enterotoxicity. Stress conditions (changes in pH, temperature and osmotic pressure) lead to metabolic changes in bacterial cells through the expression of specific gene sets, including enterotoxins encoding genes [48].

The aim of the study published by ZEAKI et al. [58] was to evaluate the effect of NaCl and sorbic acid at concentrations relevant to food production on prophage induction and SEA production. In this study, the authors demonstrated that the use of NaCl as a food preservative could potentially increase the risk for SFP because it triggers phage induction and increases *sea* gene levels. Furthermore, pH was shown to be an important parameter with respect to SEA production, because at levels around pH 5, the effect of sorbic acid on phage induction was suppressed.

Understanding the genetic mechanisms that regulate the phenotypic responses of bacteria and integrating this knowledge into existing predictive models will significantly enhance hazard identification and pathogen control. Risk assessment related to SFP is further complicated by the need to evaluate SE production rather than solely the presence or absence of the microorganism itself. This entails assessing the *S. aureus* strain, the type and quantity of SE produced, and the potential correlation between growth and SE production [11, 47].

Another challenge to overcome in assessing the risk of *S. aureus* intoxication is the number of genes encoding SEs carried by a single strain.

As demonstrated by reported SFP outbreaks, it is quite common for more than one SE-encoding gene to be detected in *S. aureus* strains involved in cases of intoxication [11]. One example is the outbreak documented in the publication by JOHLER et al. [56], which broke out in 2014 at a Swiss boarding school and affected 14 people. The *S. aureus* strain identified as the source of this epidemic possessed the *sea* and *sed* genes. Both SEA and SED were detected in the food (soft cheese made from raw cow's milk) at levels > 6 ng·g^{-1} of cheese and > 200 ng·g^{-1} of cheese, respectively. As the production of SEA and SED is regulated by different mechanisms, more information is needed on how these mechanisms are triggered in different food environments [11].

Even though *S. aureus* can possess not only one but several enterotoxin genes, this does not mean that it can express all of them [22]. This phenomenon is likely caused by the different localisation of individual SE genes in the bacterial genome as well as the complexity of genetic expression consisting of a network of regulatory systems that can act independently as well as in coordination [11, 31]. According to this, it is important to study the expression of each SE gene individually.

Understanding the effect of different stressors on the expression of individual SEs is critical for improving risk assessment and adapting food production parameters to minimise the risk of intoxication to the consumer. For instance, SEB expression is highest during the transition from exponential to stationary growth phase, coinciding with the peak of Agr system activity [47]. SIHTO et al. [59] investigated the effect of NaCl (4.5 %), nitrite (150 mg·l^{-1}), glucose (30 %) and lactic acid (pH 6.0) on the activity of the *seb* promoter. The results of the study indicated that NaCl, nitrite and glucose resulted in a significant decrease in *seb* promoter. This indicates that the presence of NaCl, nitrite and glucose resulted in a significantly decreased *seb* promoter activity, while lactic acid stress led to increased promoter activity. Moderate stress conditions encountered during food production and preservation can induce significant changes in *seb* promoter activity.

Compared to other SEs, SEA and SED are produced in food over a broader range of pH, redox potential and a_w. This explains why SEA and SED are the primary toxins implicated in SFP outbreaks. SEA expression occurs from the mid-exponential phase of growth but is not regulated by the Agr system [29]. The effect of stress on *sea* prophage induction and SEA production can vary, depending on environmental factors (pH, temperature) as well as the prophage variant present in the *S. aureus* strain [31, 47]. Mild acetic acid stress (pH 5.5–7) can lead to prophage induction and subsequent increase of SEA expression in ham products, while no or very low levels of SEA were found at lower pH (4.5–5) [60]. Another study reported little or no effect of sorbic acid (0.15 %, pH 5) on phage induction [58].

The addition of NaCl (4.5 %) and glucose stress can reduce the expression of *sed*, whereas lactic acid stress had no significant effect on the expression of the gene [60, 61]. Interestingly, various studies assessing *sed* expression at both the transcriptional and translational levels have discovered that the relative levels of *sed* expression do not correlate with the detected levels of SED protein. This discrepancy might be due to regulation occurring at the translational level. Another possibility is that the accumulation of amino acids such as proline and glycine, which occurs under osmotic stress to maintain hydrostatic pressure, could impair exotoxin secretion [47].

There is limited data on the impact of food-related stressors on *sec* expression, especially data that consider different SEC variants. However, *sec* expression has been shown to be affected by glucose and NaCl stress [47].

It is also important to consider that SE production depends on the type of SE, strain, and environmental conditions. This means that, under identical conditions, different strains can generate varying levels of SE during different growth phases. This significant variability is also evident in the amounts and types of SE that *S. aureus* produces under optimal conditions [29]. The study by ZEAKI et al. [62] demonstrated that even though all three toxinogenic isolates used in the study exhibited very similar growth and viability, at 15 °C during a 14-day incubation on pork sausages in the presence or absence of lactic acid, the levels of SEA produced, as well as the rates of SE production, differed significantly. No apparent link was found between the absolute number of cells or growth rate and SE production.

The effect of competitive microbiota

The effect of competitive bacteria on SE production is an important subject of research. It has been found that the growth of the organism and the production of SEs depend not only on the strain of the tested pathogen but also on the type of competitive microbiota [29, 63]. It is not surprising that SE levels are not affected by proteolytic and enteric bacteria due to the weak ability of proteolytic enzymes to influence the biological activity of SEs [29].

Inhibition of *S. aureus* growth and SE production was detected during co-cultivation with CoNS. The addition of *S. carnosus* at a high concentration (10^7 CFU·ml^{-1}) was able to inhibit SE production in two strains of *S. aureus*, making it undetectable for up to 72 h of incubation. The presence of a non-enterotoxigenic strain of *S. aureus* in milk prevented SE production by an enterotoxigenic strain when added at 100 to 1000 times the initial concentration of one of the tested enterotoxigenic strains; however, such results were not reproduced with the second isolate [63].

Aljasir and D'Amico [44] published a study aimed at determining the potential of eight commercially available protective cultures to control the growth and attenuate the virulence of *S. aureus* in raw milk and laboratory media. The cultures of *Lactococcus lactis* and *Hafnia alvei* were found to be the most effective in inhibiting the growth of *S. aureus* in raw milk compared to the control when co-cultivated according to the time and temperature profile of cheese production. The cultures *H. alvei, Lactobacillus plantarum* and *Lc. lactis* in raw milk reduced SE levels by 25 %, 62 %, and 76 %, respectively. The observed reduction in SE was probably due to a slight decrease in *S. aureus* numbers compared to the control. A significant reduction in SE production (up to 10 %) without inhibiting pathogen growth was also achieved in laboratory media. Interestingly, *Lc. lactis* inhibited SE production in raw milk but not in brain heart infusion broth. Overall, these results highlight the potential of protective cultures to inhibit the growth of *S. aureus* and reduce SE production.

Another study examined the behaviour of *S. aureus* and SE production during storage of brine cheese prepared with or without a starter culture, in 10% or 15% NaCl solution at 10 °C and 25 °C for 28 days. SE production was detected only in unsalted UHT milk. However, the toxin was not determined in milk with 10% and 15% NaCl addition nor in any of the cheeses stored at 37 °C for 1, 3, or 7 days. *S. aureus* grew in cheese stored in both brines at 10 °C and 25 °C, regardless of the presence of a starter culture, which significantly reduced the growth of *S. aureus* in cheese and brine at 10 °C. At a storage temperature of 10 °C, *S. aureus* counts increased by 2.78 log CFU·g^{-1} and 2.96 log CFU·g^{-1} in the absence of the starter culture, and by 2.26 log CFU·g^{-1} and 0.47 log CFU·g^{-1} in cheese stored in 10% and 15% NaCl solutions, respectively, in the presence of the starter culture [64].

Impact of natural origin substances

In recent years, there has been an increasing interest in the use of compounds of natural origin due to their antimicrobial properties [45, 65]. Many natural products can inhibit SE production and/or its toxicity and have the potential to be used as a replacement for synthetic preservatives in food. Natural products inhibit SE through two mechanisms of action:

- by inhibiting SE production and secretion through inhibition of transcription, translation and the Agr regulatory system,
- by inhibiting superantigen activity or toxicity through neutralising or binding of SEs in the food matrix and human circulatory system [34, 45].

Terpenoids, flavonoids and other antimicrobial compounds found in grapes, citruses, garlic, honey, as well as in other foods, can influence the occurrence of SFPs and the spread of antimicrobial resistance. Combining various natural compounds could enhance antimicrobial or antitoxin effects, but further research is needed to assess their potential synergistic effects [42].

Phenolic compounds can be used to control haemolytic activity and the secretion of certain SEs [42]. In a 2010 study [66], it was shown that licochalcone A can reduce the expression and secretion of SEA and SEB in a dose-dependent manner. Olive oil contains many phenolic compounds with antimicrobial effects, making it an excellent natural product for food preservation. The antimicrobial activity of polyphenols in olive oil has been convincingly demonstrated. For example, 4-hydroxytyrosol, a phenolic derivative found in olives, exhibits bactericidal activity and can inactivate the biological activity of SEA [67]. Another polyphenol of plant origin is eugenol, found in clove oil, which suppresses the production of SEA, SEB, TSST-1 as well as the expression of α-haemolysin [68]. Menthol, a terpene alcohol from plants of the genus *Mentha*, also inhibits the expression of genes encoding α-haemolysin, SEA, SEB, and TSST-1 in *S. aureus* [69].

Shi et al. [65] evaluated the inhibitory effects of tea tree oil on the growth of *S. aureus* and the production of α-haemolysin, SEA, and SEB. Tea tree oil showed better bactericidal activity in milk than in tryptone-soy broth. The results of the study revealed that the transcription of genes encoding α-haemolysin, SEA and SEB was reduced after the exposition of *S. aureus* to tea tree oil at a concentration of 0.0625–0.5 mg·ml^{-1}.

Despite the considerable potential of certain natural compounds, their utilisation in food

remains restricted due to specific attributes, including unpleasant taste or aroma, instability, low bioavailability, poor water solubility, adverse effects on the sensory properties of food, and even low toxicity [34].

CONCLUSIONS

The purpose of this review is to summarise current knowledge about *S. aureus* and SEs from the perspective of food safety, with particular emphasis on the risks of staphylococcal food poisoning. We present the latest data on the occurrence of *S. aureus* and its toxins in food at the European Union level, with a special focus on the Slovakia. The text emphasises the necessity of adopting a broader legislative approach that would include not only the detection of the potential presence of coagulase-positive staphylococci in food. We have referred to several deficiencies in the current EU legislation, especially in relation to SE detection, the wider spectrum of staphylococci (including coagulase-negative strains), and the possibility of toxin production even at lower concentrations of viable cells than provided by current regulations. This review also aims to examine the impact of environmental factors during food processing and storage that can affect the growth of *S. aureus* and SE production. Research highlights the variability in SE production as a response to stress conditions in food matrices, indicating that prediction based only on the viable cell count may not be sufficient. Specific parameters of food matrices can modulate *S. aureus* virulence and SE production. Knowledge in this area is constantly advancing. However, further investigation of the mechanisms of gene expression regulation associated with SE production is still needed. Future research should focus not only on a better understanding of the mechanisms related to toxin production but also on the practical implementation of these findings in the food industry. Additionally, the development of new methodologies aimed at faster and more cost-effective detection of SE in food would allow testing a higher number of food samples, thereby significantly reducing the risk of SFP.

Acknowledgements

This work was supported by the call for doctoral students and young researchers of Slovak University of Technology in Bratislava to start a research career (Grant 23-04-02-A) by the Scientific Grant Agency VEGA under the contract 1/0464/21.

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Received 21 June 2024; 1st revised 13 December 2024; accepted 18 December 2024; published online 20 February 2025.