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FOODBORNE PATHOGENS (S JOHLER, SECTION EDITOR)

Staphylococcus aureus as a Foodborne Pathogen

Alexandra Fetsch[1] · **Sophia Johler**[2]

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Abstract

Purpose of Review We present recent insights on *S. aureus* as a foodborne pathogen, thus providing readers with an update of current findings impacting prevention and control measures.

Recent Findings Advances in disease burden assessment show the burden of *S. aureus* foodborne disease around the globe. In recent years, recent research has provided valuable new data improving the understanding of the pathobiology of *S. aureus* foodborne disease as well as proteomics and genomics of this foodborne pathogen. In particular, recent findings shed new light on the role of newly described enterotoxins and methicillin-resistant *S. aureus*. These new findings guide the way towards improved prevention and control strategies.

Summary *S. aureus* is the leading cause of foodborne intoxications worldwide. Control strategies are focused on hygiene measures to avoid food contamination and limit *S. aureus* growth. Outbreak investigations remain challenging and would strongly benefit from additional data on enterotoxin formation under stress conditions and novel tools allowing for detection of newly described enterotoxins.

Keywords Staphylococcal Food Poisoning · Staphylococcal enterotoxins · Detection methods · Foodborne outbreak · MRSA

Introduction

The genus *Staphylococcus* currently comprises more than 50 species all known as common colonizers of the skin and mucous membranes of many animal species including humans. One of these different species is *S. aureus*, so-named because of the color of the pigmented colonies ("aureus" means golden in Latin). *S. aureus* is one of the most important pathogens of humans and animals and a leading cause of foodborne disease around the globe. In addition, the issue of antimicrobial resistance of *S. aureus*, in particular of Methicillin-resistant *S. aureus* (MRSA), is receiving widespread attention with important initiatives to improve reporting and develop new strategies for prevention and control [1].

S. aureus can be considered a "heirloom disease," that is, one that has been passed on for millennia from person-to-person [2]. The health of humans and animals is closely inter-dependent and many human diseases are shared with animals and vice versa. Molecular epidemiology suggests that *S. aureus* has jumped from humans to livestock several times in the past and has more rarely switched host species from livestock back to people [3].

The largest ecological reservoir of *S. aureus* strains causing disease in humans is the human nose. However, the skin, hair, and mucous membranes may also be colonized. Although nasal carriage is strongly associated with staphylococcal infections, only a tiny minority of carriers will ever fall ill [4]. In contrast, the high rate of human carriers contributes to the frequent occurrence of Staphylococcal Food Poisoning, which has largely been attributed to faulty food handling. Therefore, control of *S. aureus* foodborne disease is based on hygiene measures to avoid contamination of food. The widespread application of approaches such as Risk Assessment and Hazard Analysis and Critical Control Points (HACCP) and Good Hygienic Practice (GHP) can help prevent contamination [5].

In this article, we review the current situation regarding *S. aureus* as a foodborne pathogen. After describing Staphylococcal Food Poisoning in general, we first provide an update on all staphylococcal enterotoxin types yet described as

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✉ Sophia Johler
sophia.johler@uzh.ch

[1] National Reference Laboratory for coagulase-positive staphylococci incl. Staphylococcus aureus, German Federal Institute for Risk Assessment (BfR), Berlin, Germany

[2] Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

well as recent developments in detection strategies for staphylococcal enterotoxins. Moreover, we give an overview of outbreak investigation approaches as well as food safety and food process criteria put in place to control *S. aureus* along the farm-to-fork food chain. We then question the role of methicillin-resistant *S. aureus* (MRSA) as foodborne pathogen. Finally, we discuss these recent findings and developments and put these into the consumer health perspective.

Staphylococcal Food Poisoning

Staphylococcal Food Poisoning (SFP) is the most prevalent foodborne intoxication worldwide. In the USA, the Centers for Disease Control estimate that 240,000 cases occur per year, resulting in 1000 hospitalizations and six deaths [6]. In Europe, the number of SFP outbreaks reported by the European Food Safety Authority (EFSA) is rising, with 434 SFP outbreaks in 2015, which equals 10% of all outbreaks reported [7]. The true number of SFP outbreaks is likely much higher, as indicated by the fact that currently > 90% of SFP outbreaks are reported by France [7].

SFP is caused by ingestion of a sufficient amount of one or several staphylococcal enterotoxins preformed in food during growth of the organism (Fig. 1). Food handlers who contaminate food with *S. aureus* are the most common source of SFP outbreaks [8, 9]. However, outbreaks were also linked to consumption of raw milk or raw milk cheese originating from dairy animals suffering from *S. aureus* mastitis [10].

SFP symptoms appear 0.5–8 h (on average 3 h) after consumption of contaminated food [11]. There are indications that the incubation period may depend on the age of the patient, with earlier onset of symptoms in children and teenagers compared to adults [10]. Key symptoms are nausea and violent vomiting, often accompanied by watery diarrhea, abdominal pain, moderate fever, and shivering. To date, the underlying mechanisms of enterotoxigenicity and SE-induced vomiting are still poorly understood [12••, 13]. The disease is usually self-limiting within 24 h. However, rare cases of fatal dehydration and electrolyte imbalances occur, with fatality rates ranging from 0.03% in the general population to 4.4% in children and the elderly [14].

Staphylococcal Enterotoxins

Staphylococcal enterotoxins (SEs) are water-soluble, structurally stable, secreted polypeptides of 22–29 kDa and belong to the family of pyrogenic toxin superantigens able to unspecifically activate T-cells [15]. They display extreme tenacity in the face of stress conditions, which reliably inactivate *S. aureus*. This is of particular relevance, as loss of serological recognition, e.g., caused by heat treatment, does not guarantee loss of emetic activity [16]. The high stability of SEs and their resistance to most proteolytic enzymes such as pepsin and trypsin assure that these toxins remain emetically active in the gastrointestinal tract [16].

SE nomenclature follows an alphabetical system. Guidelines for the description of enterotoxins were proposed by the International Nomenclature Committee for Staphylococcal Superantigens and include verification of gene expression and characterization of the protein. In addition, toxins either not tested for or lacking emetic activity in a monkey feeding assay should not be designated SE, but "staphylococcal enterotoxin-like superantigens (se*l*)" [17, 18]. SEF is missing in the current alphabetical list of toxins, as the denomination "SEF" for the toxic shock syndrome toxin-1 was omitted due to lack of emetic activity [14, 19].

A comprehensive list of SEs providing a current overview of emetic potential and the location of the SE genes is presented in Table 1. SEs have been categorized into the classical SEs (SEA, SEB, SEC, SED, SEE), and the newly described SEs. The vast majority of SFP outbreaks has been attributed to the classical SEs, although newly described SEs also elicit an emetic response in the monkey feeding assay [39, 42] and in the house musk shrew [40, 48, 50]. Raised awareness of their emetic potential led to an increasing number of SFP outbreaks, in which newly described SEs were implicated as the causative agents [56–62]. SE coding regions exhibit high sequence variability [63, 64], with toxin genes located on a wide variety of different mobile genetic elements, including a multitude of pathogenicity islands, prophages, and plasmids (Table 1). While *sea* and *see* are carried by lysogenic phages, *seb* and *sec* are located on pathogenicity islands [65], and *sed* is located on pIB485, a 27.6 kb plasmid [66]. In contrast, many newly described staphylococcal enterotoxins are encoded by the enterotoxin gene cluster (*egc*) operon [33, 34], which acts as an enterotoxin nursery generating new SE genes through genomic rearrangements [33, 53, 67].

While over the last decades, the number of known species of the genus *Staphylococcus* kept steadily growing, SE production was for a long time exclusively attributed to *S. aureus*, with enterotoxigenic coagulase-negative *staphylococci* being considered mutants or variants of *S. aureus* [68]. Recently, this paradigm is shifting due to collected evidence of studies suggesting that *staphylococci* other than *S. aureus* are able to form enterotoxins and may contribute to SFP [69–71].

Detection of Staphylococcal Enterotoxins

While SEs can be detected in amounts of 200 ng or more by animal feeding assays using monkeys or the house musk shrew, substantially lower amounts of SEs were reported to elicit an emetic response in humans [11]. Therefore, more

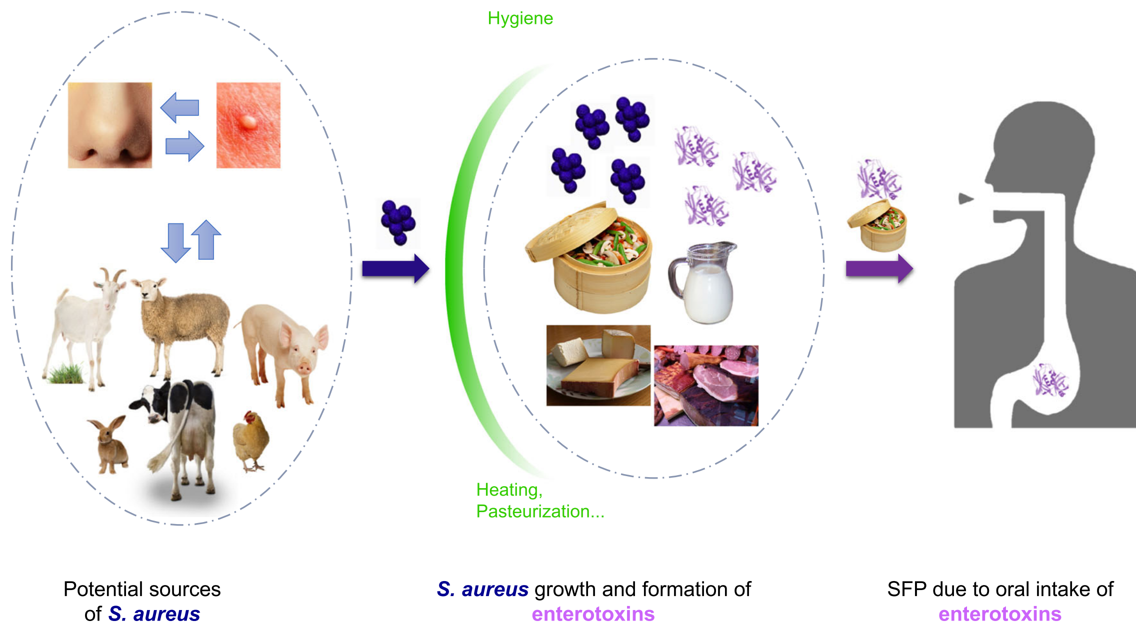

Fig. 1 Overview of Staphylococcal Food Poisoning

Table 1 Overview of currently known staphylococcal enterotoxins (SEs) and staphylococcal enterotoxin-like superantigens (SEls)

SE/SEl	Emetic activity	Location of respective SE gene	References
SEA	+	Prophage	[20–23]
SEB	+	SaPIs (SaPI1, SaPI2, SaPI3, SaPI4, SaPImw2, SaPIrki4)	[24–26]
SEC	+	SaPIs (SaPIbov1, SaPIn1, SaPIm1, SaPImw2, SePI1)	[27, 28]
SED	+	Plasmid (pIB485)	[29, 30]
SEE	+	Prophage	[31, 32]
SEG	+	egc, prophage	[33–35]
SEH	+	Transposon	[35–38]
SEI	+	egc	[33, 34, 39]
SEJ	+	Plasmid (pIB485)	[40, 41]
SEK	+	SaPIs (SaPI1, SaPI3), prophage	[35, 42, 43, 44••]
SEL	+	SaPIs (SaPIbov1, SaPI3, SePI1)	[42, 44••, 45, 46]
SEM	+	egc	[33, 34, 42, 44••]
SEN	+	egc	[33, 34, 42, 44••]
SEO	+	egc	[33, 34, 42, 44••]
SEP	+	Prophage	[42, 47]
SEQ	+	SaPI1, SaPI5	[42, 48, 49]
SER	+	Plasmid (pIB485)	[50, 51]
SES	+	Plasmid (pIB485)	[50]
SET	+	Plasmid (pIB485)	[50]
SElU	Questionable	egc	[52]
SElV	Questionable	egc	[53]
SElW	Questionable	egc	[53]
SElX	Questionable	Chromosome	[54]
SElY	Questionable	Chromosome	[55]

SaPI S. aureus pathogenicity island, *SePI S. epidermidis* pathogenicity island, *egc* enterotoxin gene cluster, *SElW* former SelU-2

sensitive assays are needed to be able to detect low but clinically relevant amounts of SEs in outbreak investigations.

Commercially available reverse passive latex-agglutination (RPLA) and enzyme-linked immunosorbent (ELISA) or enzyme-linked fluorescent assay (ELFA) kits are commonly used to screen for SEA-SEE (Table 2). These user-friendly and fast immunological assays require only limited pretreatment of the sample and allow for easy read-out either by eye or using a photometer. However, detection in food matrices and in particular in cheese suffers from low specificity and sensitivity, as well as false-positive results due to matrix components such as phosphatase and peroxidase or unspecific binding of IgG by protein A. Affinity chromatography and dialysis can be used to improve results through purification and concentration of the toxin in a given sample [72]. Recently, an ISO method (ISO 19020:2017) for SE screening in foodstuffs has been published. It comprises an extraction step, followed by concentration by dialysis and immunoenzymatic detection of SEA-SEE [73••].

While detection of SE genes does not provide any information on the expression of SEs in the food matrix, it is often used to complement immunological testing. Commercially available immunological systems currently only enable screening for SEA-SEE and cannot detect the newly described SEs.

Detection of SE genes by PCR, whole-genome sequencing, or DNA microarray analysis can be highly useful tools. As demonstrated in a massive SFP outbreak in Japan caused by reconstituted milk, PCR using DNA extracted directly from food can still be successful, even if the organism itself was inactivated through heat treatment [56]. However, results need to be treated with caution, as the presence of an SE gene does not guarantee SE formation in the food matrix. In addition, SE genes exhibit a high degree of sequence variation, complicating the search for primers able to bind to all allelic variants of the target gene [63].

Outbreak Investigations

In general, well-established approaches such as the ten steps of an outbreak investigation recommended by the European Centre for Disease Prevention and Control [74] are applicable to SFP outbreaks: (i) confirm outbreak and diagnosis, (ii) define a case, (iii) identify cases and obtain information, (iv) describe data collected, (v) develop hypothesis, (vi) test hypothesis (analytical studies), (vii) conduct microbiological investigation and additional studies, (viii) implement control measure, (ix) communicate results including outbreak report, and (x) evaluate and update procedures. However, SFP outbreaks often present with particular challenges that merit consideration.

Ideally, competent authorities suspecting a SFP outbreak due to characteristic clinical symptoms will obtain samples of food, food handlers (nasal swabs), and patients (feces), as well as comprehensive questionnaires providing data on diseased and non-diseased persons. Bacterial isolation and identification will be followed by fast and comprehensive strain typing and characterization, e.g., using whole-genome sequencing [75], DNA microarray profiling [76], or Fourier-transform infrared spectroscopy [77]. In the simplest of scenarios, one enterotoxigenic *S. aureus* strain present in food at numbers $\geq 10^5$ CfU/g can be matched to an isolate from a patient sample and will be further confirmed as the cause of the outbreak by detection of high levels of SEs in the respective food item, as well as fitting questionnaire results. However, real-life SFP outbreak investigations often become far more challenging.

Questionnaire data may be incomplete, unreliable (e.g., recall bias), or unavailable. Isolation of staphylococci from patient samples may have been unsuccessful and little or no food leftovers may be available for sampling. In the case of outbreaks associated with heated foods or long-ripened cheese, a large amount of highly stable SEs could have been produced in the food prior to subsequent food treatment steps that inactivated the organism itself [59•, 78, 79]. High levels of emetically active SEs could therefore still be present in foods that do not allow for isolation of the causative strain.

However, as *S. aureus* frequently carries enterotoxin genes, isolation of multiple different enterotoxigenic *S. aureus* strains from food samples and nasal swabs is possible. In addition, enterotoxigenic *staphylococci* other than *S. aureus* could be detected. Even with the help of cutting edge equipment, it is often highly difficult or impossible to determine, which one or which combination of these strains had contributed to the production of sufficient levels of one or more SEs leading to SFP. If however, resources are scarce, *spa* typing of coagulase-positive isolates can be used to discriminate strains [80, 81]. The enterotoxin gene profile can subsequently be determined by PCR and will, if possible, be matched to SE detection results in food.

In many outbreaks, strains harboring both classical and newly described enterotoxin genes can be detected. However, SE detection is only feasible for classical SEs, as there are currently no commercially available immunological detection methods for the newly described SEs. Therefore, if even small quantities of classical SEs are detected in food/feces, an outbreak will likely be attributed to these enterotoxins, even if genes encoding newly described SEs are present. However, if no classical SEs are detected, the outbreak will likely not be reported, as many investigators question the relevance of newly described SEs for SFP outbreaks.

Table 2 SE detection kits. The table provides an overview for commonly used commercially available kits for SE detection

Approach	Assay ID	SEs detected[a]	Time[b] (h)	Sensitivity[c] (ng/mL)	Provider
RPLA	SET RPLA	SEA, SEB, SEC, SED	24	0.5	Oxoid
ELISA	RIDASCREEN® SET	SEA, SEB, SEC, SED, SEE	3	0.25	R-Biopharm
	3 M® TECRA® Staph Enterotoxins	SEA-SEE	4	0.5	3 M®
	Transia® plate SET	SEA-SEE	1.5	0.2	Raisio diagnostics
ELFA	VIDAS® SET2	SEA-SEE	1.5	0.25	bioMérieux

[a] While some kits provide only a yes/no answer for the presence of classical enterotoxins (SEA-SEE), others are able to identify which enterotoxin is present in the sample

[b] Time expenditure for analysis of an already extracted sample

[c] Sensitivity varies based on matrix tested and extraction technique used

Increased awareness of these challenges could lead to improved outbreak reporting and could significantly extend the limited current knowledge of SFP outbreaks.

Food Standards to Combat *S. aureus* in the Food Chain

SFP has been associated with poor food hygiene, inadequate equipment cleaning, cross-contamination by raw ingredients or after a heating process, and time/temperature abuse during food processing [82–84]. Mitigation strategies that help control the risks must take into account factors such as variability in primary production, food processing, and cooking practices [85] as well as variability of SE production in various food products [86]. One effective approach to control *S. aureus* in the food chain is based on the establishment of specific food standards. The Codex Alimentarius Commission, a body that was established in early November 1961 by the Food and Agriculture Organization of the United Nations (FAO), and later joined by the World Health Organization (WHO), now acts as the international body to develop and coordinate standards that safeguard the food supply and food trade. Codex has prepared a document entitled "Principles and Guidelines for the Application of Microbial Risk Assessment" (http://www.fao.org/docrep/004/y1579e/y1579e05.htm), which became the reference standard for international trade. Moreover, the EU and individual countries in Europe such as the Netherlands and Germany, as well as the USA, have initiated various microbial risk analyses targeting different questions based on this document. At the core of all these microbial risk analyses, the presence of the pathogen as well as the level of the pathogen must be determined to be able to assess the potential for an undesirable outcome [86–88]. To this end, each nation has developed policies and standards regarding acceptable levels of pathogens in a variety of food products, e.g., specific food safety standards for the enumeration of coagulase-positive *staphylococci* (CPS) and/or *S. aureus*, as well as for the presence/absence of SE in food. For instance, in the EU, various food safety and process hygiene criteria on CPS and SEs have been established according to Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs (http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02005R2073-20140601&from=EN). A food safety criterion defines the acceptability of a product or a batch of foodstuff placed on the market and was established in the EU for a variety of cheeses, i.e., (a) cheese made from raw milk, (b) cheese made from raw milk that has undergone a lower heat treatment than pasteurization and ripened cheese made from milk that has undergone pasteurization or a stronger heat treatment, (c) unripened soft cheese made from milk that has undergone pasteurization or a stronger heat treatment, as well as for milk powder and whey powder. When testing a 25 g sample for SEs, these foods must yield a negative test result. On the other hand, the EU has established different process hygiene criteria not applicable to products placed on the market but measuring the functioning of the production process and setting an indicative contamination value above which corrective actions are required to maintain the hygiene of the process in compliance with food law. Food categories for which process hygiene criteria were set in the EU include milk and dairy products (various cheeses—for details see a–c above; milk and whey powder) and fishery products (i.e., shelled and shucked products of cooked crustaceans and molluscan shellfish). Within those categories of foods, the hygiene parameter is CPS (Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02005R2073-20140601&from=EN).

MRSA in Food

MRSA has been a major threat for public health worldwide. During the last decade, an expansive spread of MRSA with livestock origin along the farm-to-fork food chain has been shown (reviewed in [89]). Livestock-associated (LA)-MRSA evolved independently from common Hospital- or Community-Associated MRSA usually found in humans [90] and mainly belong to *S. aureus* clonal complex CC398 and associated *spa* types t011 and t034. However, also other CCs such as CC1, CC5, CC97, and CC130 are found in livestock around the globe [91]. Soon after its discovery, LA-MRSA emerged among humans indicating a zoonotic transmission from animals to humans [92–94]. Therefore, it is important to monitor MRSA from farm-to-fork and to compare isolates from livestock and food with those from humans. Also, the EFSA has suggested to monitor the occurrence and diversity of MRSA in primary production, including at slaughter, while monitoring in food may also help with the assessment of consumers' exposure via this route [95]. In addition, EFSA stated that antimicrobial susceptibility data on MRSA isolates are useful in directly informing on the emergence of strains of potential public health significance but can also provide important epidemiological information on the spread of particular strains between the animal and human populations, particularly when investigated in conjunction with molecular typing data [95]. For instance, in Germany, a monitoring system for zoonotic bacteria in the food chain was established in 2009 to fulfill the requirements of directive 2003/99/EC [96]. The general aim of the monitoring system is to investigate the prevalence of zoonotic bacteria such as MRSA along the different food chains and to collect isolates of the different bacterial classes for further characterization, for example, typing and antimicrobial resistance testing. Sampling plans cover various steps along the food value chains, starting from primary production to retail level and targeting different zoonotic bacteria. According to this monitoring over the years 2009–2015, MRSA is highly prevalent along the farm-to-fork food chain in Germany including raw meat at retail with mean prevalences of 38% (turkey meat), 24% (broiler meat), 13% (pork), and 11% (veal), respectively (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, 2010–2016: https://www.bvl.bund.de/EN/01_Food/_01_tasks/02_OfficialFoodControl/06_ZoonosesMonitoring/ZoonosesMonitoring_node.html). Other countries report similar findings [97–101]. However, the presence of MRSA in/on food intended for human consumption may not necessary render MRSA a foodborne pathogen [102]. Firstly, as those clonal lineages present in the farm-to-fork chain do not or only at a very minor percentage carry SE encoding genes [83, 103]. Secondly, the number of MRSA present in the food may be very low, too [104]. On the other hand, some cases of LA-MRSA carriage in humans cannot be explained by livestock contact [105]. Thus, one could speculate that humans might have acquired such MRSA colonization via contaminated food. Very recently, poultry meat, mainly from turkey meat, has been considered as a probable source of infections in humans with a novel hybrid LA-MRSA CC9/CC398 genotype [106•, 107]. Moreover, it was suggested that LA-MRSA subpopulations may have become adapted to humans [106•]. The high plasticity, the acquisition of different genetic elements related to host adaption, antimicrobial resistance, and virulence as well as its complex epidemiology need to be considered in any future research on MRSA along the farm-to-fork food chain.

Conclusion

S. aureus is a serious threat to human health and one of the main challenges to the food industry. The prevalence of Staphylococcal Food Poisoning remains high around the globe, and advances in disease burden assessment are showing the enormous burden of *S. aureus* foodborne disease. Several advances in detection, prevention, and control of *S. aureus* were seen in recent decades: The increase in whole-genome sequence data for *S. aureus* is transforming our understanding of population diversity, disease spread, and emergence. The exclusive use of the amount of colony forming units present in a food sample in order to determine the risk associated with a food item has shown to be unreliable, stressing the crucial need for methods enabling SE detection directly in food. Molecular and immunological methods are increasingly used in diagnosis of *S. aureus* and SE detection and very recently, the first international methodology standard for SE detection in food has been published targeting SEA-SEE. As novel evidence has led to an increased understanding of the relevance of newly described SEs in SFP outbreaks, suitable detection systems and standards targeting SEs other than SEA-SEE are urgently needed.

S. aureus is also a very dynamic bacterial organism that is in continuous evolution, as seen for instance by the emergence of zoonotic MRSA of livestock origin. The high capacity of *S. aureus* to acquire mobile genetic elements, which encode key proteins for host adaption, in addition to antimicrobial resistance or virulence characteristics, need also be considered from a consumer health perspective.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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