Staphylococcus aureus food-poisoning outbreak associated with the consumption of ice-cream

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1. Introduction

Staphylococcus aureus is an important food-borne pathogen due to the ability of enterotoxigenic strains to produce staphylococcal enterotoxins (SEs) preformed in food. Up to present, 22 SEs have been described, designated SEA to SEl, in the chronological order of their discovery (Hennekinne et al., 2010). Staphylococcal food poisoning is characterized by a sudden onset of symptoms, with vomiting, abdominal pain, and stomach cramps being the most common (Tranter, 1990). Occasionally it can be severe enough to warrant hospitalization, with S. aureus asymptomatically, who handle food can introduce the bacteria into the food chain (Argudin et al., 2010). Approximately 20–30% of humans persistently carry S. aureus as a commensal of the skin and mucosal membranes, respectively (Kluytmans and Wertheim, 2005). One-half of the isolates found among humans proved to be enterotoxigenic (Becker et al., 2003).

Food poisoning caused by staphylococcal enterotoxins is among the leading causes of food-borne outbreaks in the European Union (EFSA, 2013). The real incidence of SFP is probably underestimated for a number of reasons, which include unreported minor outbreaks, improper sample collection and laboratory examination (Argudin et al., 2010). Diagnosis of SFP is mainly based on at least one of the following: the recovery of > 10⁵ S. aureus/g from food remnants, the detection of SEs in food remnants and the isolation of identical S. aureus clones from both patients and food remnants (Bryan et al., 1997).

Foods that favour growth of bacteria, e.g., (raw) food of animal origin with high protein content such as milk, milk products, meet, meat products and salads, bakery products, particularly cream-filled pastries and cakes, and sandwich fillings have been frequently incriminated in SFP outbreaks (Hennekinne et al., 2012).

Here, we report on a food poisoning outbreak due to staphylococcal enterotoxins in ice-cream which occurred in April 2013, among...
participants of a christening party, that took place at a hotel in Freiburg, Baden-Wuerttemberg, Germany.

2. Methods

2.1. Outbreak investigation and sampling

Outbreak investigation was carried out immediately after notification via the emergency service by the staff members of the competent public health authority and the food and veterinary office Freiburg, namely, inspection, sampling and interviewing of participants of the christening party and staff members of the hotel.

The following samples were collected:

Four human samples (three stool samples, one specimen of vomit) were collected from affected and hospitalized children. In the course of the outbreak investigation all hotel employees handling or producing ice-cream were screened for S. aureus colonization, resulting in three additional nasal swab samples.

Left-overs from a variety of food items eaten by the participants of the christening party were also sampled: five different types of homemade ice-cream (yoghurt-lemon, vanilla, pistachio, chocolate, and strawberry) and seven different composed dishes/food components (foie gras terrine, mackerel, trout caviar, deep sea scallops, salad, mixed dried algae, and mixed fresh herbs), resulting in 12 foodstuff samples.

2.2. Laboratory methods

2.2.1. Isolation and phenotypic characterization of S. aureus from human samples

Human samples were processed and analysed by the State Health Office Baden-Wuerttemberg, Stuttgart. The stool samples and the specimen of vomit were cultured on mannitol salt phenol red agar (inhouse). Nasal swab samples were directly cultivated on Columbia blood agar (Thermo Fisher Scientific, Wesel, Germany) with optochin disc (Thermo Fisher Scientific, Wesel, Germany). Suspect isolates were further tested for the production of coagulase, catalase, and DNase/staphylococcal thermonuclease and by means of the VITEK2 compact system (bioMérieux, Nuertingen, Germany) according to the manufacturer’s instructions. In addition, all positive samples were tested for the occurrence of staphylococcal enterotoxins A, B, C and D by reverse passive latex agglutination (SET-RPLA-Toxin Detection kit, Thermo Fisher Scientific, Wesel, Germany) according to the manufacturer’s instructions with one exception (use of brain heart infusion broth (Becton-Dickinson Heidelberg, Germany) instead of tryptone soya broth).

For confirmation and further characterization all human isolates were forwarded to the German Reference Centre for staphylococci and enterococci (NRC for staphylococci) at the Robert-Koch-Institute (RKI), Wernigerode Branch.

2.2.2. Enumeration of coagulase-positive staphylococci, phenotypic characterization of S. aureus and detection of staphylococcal enterotoxins in food samples

Food samples were processed and analysed at the Chemisches und Veterinäruntersuchungsamt (CVUA) Stuttgart. For detection and enumeration of coagulase positive staphylococci (CPS) ISO-standard 6888−1 was applied with slight modifications (use of Brilliance Staph 24 agar plate (Thermo Fisher Scientific, Wesel, Germany), instead of Baird-Parker agar). Isolates were further differentiated and identified as S. aureus by detection of DNase/staphylococcal thermonuclease (according to DIN 10197) and MALDI−TOF mass spectrometry (Biotyper system, Version V3.3.1.0, BrukerDaltronics, Bremen, Germany).

All foodstuff isolates were further characterized at the National Reference Laboratory for Coagulase Positive Staphylococci Including S. aureus (NRL-Staph) at the Federal Institute for Risk Assessment (BfR), Berlin.

Detection of SEs was conducted by use of Vidas SET 2 kit (bioMérieux, Nuertingen, Germany) according to the manufacturer’s instructions. Food samples from the different types of ice-cream were measured in duplicate.

2.2.3. FT-IR: Fourier transform infrared spectroscopy (FT-IR)

Analysing the total composition of components of the cell by using infrared spectroscopy (Naumann et al., 1990; Wenning and Scherer, 2013), FT-IR was used for rapid species identification and for comparison of S. aureus isolates (Johler et al., 2013). For this purpose, all of the isolates were cultivated on sheep blood agar plates (Thermo Fisher Scientific, Wesel, Germany) at 37 °C for 24 h. Cells of each isolate were harvested with a platinum loop and suspended in 80 µl of deionized water. An aliquot of 35 µl was placed in the sample zone of a zinc selenide optical plate (BrukerOptics GmbH, Ettlingen, Germany) and dried under reduced pressure for 30 min to a homogeneous solid film, which was used directly for FT-IR spectroscopy (Kuhm et al., 2009). FT-IR spectroscopy was performed using a Tensor27 spectrometer with an HTS-XT module (BrukerOptics, Ettlingen, Germany) in the wave number range from 500 to 4000 cm−1 (Stamm et al., 2013). Acquisition and analysis of data were carried out using OPUS Software (vers. 4.2, BrukerOptics) and an artificial neural network built by the NeuroDeveloper software (Synthon, Heidelberg, Germany) (Udelhoven et al., 2003). IR double spectra of isolates were compared by cluster analysis (cf. Johler et al., 2013; Stamm et al., 2013). For cluster analysis the vector normalized spectra of the wave number range from 500 to 1500 cm−1 in second derivation were used for calculation with Ward’s algorithm (OPUS 4.2) (Ward, 1963). Dendrograms obtained show the arrangement of the isolate-spectra according to their spectral differences.

2.2.4. Antibiotic susceptibility testing

All isolates were subjected to susceptibility testing by the broth microdilution method, according to DIN 58940. The 18 antimicrobials tested were ciprofloxacin, clindamycin, dapтомycin, erythromycin, fosfomycin, fusidic acid, gentamicin, linezolid, moxifloxacin, mupirocin, oxacillin, penicillin, rifampicin, trimethoprim/sulfamethoxazol, tetracycline, tigecycline, teicoplanin and vancomycin. For interpretation of results, epidemiological cut-off values according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were applied (http://www.eucast.org). S. aureus strain ATCC 25923 was used as a control.

2.2.5. Genotypic characterization of isolates

As a prerequisite, DNA was extracted using the DNAeasy Blood and Tissue kit (Qiagen, Hilden, Germany) using lysostaphin to obtain bacterial lysis. Hence, isolates were genotypically confirmed as S. aureus by means of multiplex-PCR and simultaneous detection of 23S rDNA (Straub et al., 1999) and nuc (Poulsen et al., 2003).

Strains were further characterized by spa-typing according to Harmsen et al. (2003), and in some cases multi-locus-sequence-typing (MLST) (Enright et al., 2000). In addition, a commercially available microarray kit (Identibac S. aureus Genotyping, Alere Technologies GmbH, Jena, Germany) was applied. This array covers 333 target sequences corresponding to approximately 185 distinct genes and their allelic variants. These include among others species-specific controls, genes encoding for relevant antibiotic resistance determinants and virulence factors (SE encoding genes) as well as agr group and capsule typing markers. The array was performed according to the manufacturer’s instructions and analysis of the array profiles based on the presence or absence of the enquired genes was done with Bionumerics Software (version 6.6.4; Applied Maths, Sint-Martens-Latem, Belgium).
### 3. Results

#### 3.1. Epidemiological information

On 13 April 2013, 31 people participated at a christening party that took place at a hotel in Freiburg, Baden-Württemberg. Among them, 13 developed typical SFP associated symptoms 3–4 h after lunch. Seven people, including four young children, two adults and one teenager, were either hospitalized or obtained an ambulatory treatment. All of them recovered fully the next day. None of the personnel of the hotel presented symptoms of SFP. Participants of the party did only meet for the purpose of the christening and did not meet before. Interviewing of cases and participants without symptoms was limited as most of them departed immediately after the party as the majority of them either live in other regions of Germany or in a neighbouring country (Switzerland, France).

#### 3.2. Laboratory investigation

##### 3.2.1. Human S. aureus isolates

From all of the four samples taken from cases enterotoxigenic S. aureus of spa-type t127 (clonal complex CC1), displaying phenotypic fusidic acid (FUS) resistance and harbouring the sea gene together with further enterotoxin genes (seh, sek, seq) could be isolated. All four isolates produced enterotoxin A in the culture supernatant (SEA+).

In addition, from one out of three employees of the hotel restaurant an enterotoxigenic, seh and sep (sea N315) gene positive S. aureus of spa-type t160 (CC12) was identified. The isolate produced enterotoxin B in the culture supernatant (SEB+).

##### 3.2.2. Enumeration of CPS, detection of S. aureus and SE from food

CPS were detected in varying numbers from all of the five different types of ice-cream samples: vanilla and chocolate (>3.0 × 10^6 CFU/g), pistachio (5.6 × 10^6 CFU/g), strawberry (2.4 × 10^6 CFU/g) and yoghurt-lemon (1.7 × 10^3 CFU/g). The amount of CPS in all other food left-overs was below the detection limit (<10^2 CFU/g).

The S. aureus isolates detected in four of five ice-cream samples (vanilla, chocolate, pistachio and strawberry) were of spa-type t127 (CC1). All of these isolates were enterotoxigenic, harboured the sea gene together with further enterotoxin genes (seh, sek, seq) and displayed phenotypic FUS resistance. Whereas, a non-enterotoxigenic S. aureus of spa-type t084 (MLST-type ST15) was detected in the ice-cream sample of type yoghurt-lemon.

Beside the detection of CPS, SE could be detected in three of the five different types of ice-cream: vanilla and pistachio displaying the highest amount of SE with a two times positive result and values of up to 2.27 and 2.78, respectively (cut-off: 0.13). SE was also detectable in chocolate ice-cream once, though at a lower level (value of 0.76) than the previous two. Neither in the two types of ice-cream strawberry and yoghurt-lemon, nor in one of the other food left-overs could SE be detected.

##### 3.2.3. Comparison of strains from human and food origin

Overall, ten different isolates, five from humans and five from food left-overs, i.e. ice-cream of different types (Table 1), were compared by means of FT-IR and DNA microarray analyses.

In Fig. 1 a dendrogram obtained by FT-IR shows the arrangement of isolates in groups according to their spectral differences.

In Fig. 2 a minimum spanning tree displaying the results of the microarray cluster analysis is shown.

Considering all phenotypic and genotypic typing results, the human S. aureus isolates from cases (1H–4H) and those obtained from four of the five different types of ice-cream (1F–4F) were almost indistinguishable indicating a common origin. The one human isolate from the employee of the hotel (5H) and the one isolate derived from yoghurt-lemon ice-cream (5F) were clearly distinct from the others.

#### 4. Discussion

Here, we describe a food poisoning outbreak due to staphylococcal enterotoxins in ice-cream. The findings allow for the conclusion of a strong microbiological and epidemiological evidence outbreak according to the European Food Safety Authority (EFSA, 2011).

Clearly, ice-cream was the incriminated food vehicle of the outbreak as neither CPS nor SEs were detectable in any of the other food remnants. Ice-cream has regularly been involved in SFP outbreaks in the past (Enhuber, 1971; Seidel et al., 1962), and not just in Germany (Williams and Swift, 1946). By far the most common cause of staphylococcal food poisoning worldwide is SEA (Argudin et al., 2010). This was also the case in the present outbreak with findings of enterotoxin A on pheno- and genotypic level among S. aureus strains from human cases and food left-overs.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>SFP isolates included in this study and their main characteristics.</th>
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<tbody>
<tr>
<td>ID</td>
<td>Origin</td>
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<tr>
<td>1-H</td>
<td>Human</td>
</tr>
<tr>
<td>2-H</td>
<td>Human</td>
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<tr>
<td>3-H</td>
<td>Human</td>
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<tr>
<td>4-H</td>
<td>Human</td>
</tr>
<tr>
<td>5-H</td>
<td>Human</td>
</tr>
<tr>
<td>1-F</td>
<td>Food</td>
</tr>
<tr>
<td>2-F</td>
<td>Food</td>
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<td>3-F</td>
<td>Food</td>
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<tr>
<td>4-F</td>
<td>Food</td>
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<tr>
<td>5-F</td>
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Clonal complex (CC); multi-locus-sequence type (MLST); Fusidic Acid Resistance (FUS-R); Penicillin Resistance (PEN-R); Staphylococcal Enterotoxin Type A/B positive (SEA/B+); staphylococcal enterotoxin a/b/h/q gene (sea/seb/she/sek/seq); staphylococcal enterotoxin p gene (sea (N315)).
Whether the enterotoxigenic *S. aureus* strain was introduced by humans or a particular common ingredient, or whether contaminated equipment was used during processing and/or handling of the ice-cream, remains unknown. In contrast, a SFP outbreak that occurred in 2008 at a wedding celebration in Baden-Wuerttemberg, Germany, could clearly be linked to the source, as identical enterotoxigenic strains were found in a patient, food leftovers and the nasal cavity of a food handler (Johler et al., 2013). Likewise, a food-handler was the most likely common source of a SFP gastroenteritis occurring in 2011 in Turin, Italy, following a catered dinner party at a private home (Gallina et al., 2013).

Among the three employees of the hotel restaurant handling or producing ice-cream, only one harboured an enterotoxigenic *S. aureus*. However, this strain differed substantially from the outbreak strain in terms of pheno- and genotype. Both, the outbreak causing *S. aureus* of spa-type t127 (CC1) and the one of spa-type t160 (CC12) found in the nasal cavity of the employee, are Methicillin-sensitive *S. aureus* (MSSA) clones, having the potential for colonization and causing endogenous disease (Strommenger et al., 2008; NRC for staphylococci, unpublished results). Most recently, MSSA of spa-type t127 was found in the nasal cavity of food handlers in China (Ho et al., 2014). Though, the two clones must not be of human origin, only. E.g. MSSA of spa-

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*Fig. 1.* Similarity tree of the FT-IR cluster analysis.
type t160 were obtained from chicken carcasses in Switzerland (Ebner et al., 2013), whereas Methicillin-resistant *S. aureus* of spa-type t127 were found in holdings of breeding pigs in Italy (Franco et al., 2011). Approximately 20–30% of humans persistently carry *S. aureus* as a commensal of the skin and mucosal membranes, respectively (Kluytmans and Wertheim, 2005). Persistent carriage of *S. aureus* increases the risk of developing an endogenous disease (Wertheim et al., 2005). One-half of the isolates found among humans proved to be enterotoxigenic (Becker et al., 2003). This is in line with the present findings. However, in some humans *S. aureus* are not persistently present in the nasal cavity (Van Belkum et al., 2009). This might explain why none of the employees of the hotel restaurant carried the outbreak strain at the time of sampling which took place at a substantially later time than the time of onset of symptoms among patients. Findings of enterotoxigenic *S. aureus* in humans handling food, as in the case of the *sec* positive t160 of the employee in the present outbreak, always have to be seen as a potential contamination source, posing a risk of food intoxication.

The manufacturing process of ice-cream harbours several critical points in terms of biological hazards, including growth of CPS and subsequent SE production (Timm, 1965). For example, one is the insufficient heating of the pre-mix, allowing for the survival of pathogenic bacteria naturally or artificially present in one of the main components of ice-cream. Another is the addition of ingredients after any heat-treatment or application of another risk eliminating processing step. In the present outbreak, the ice-cream was produced at the restaurant kitchen of the hotel itself, where the outbreak occurred. Unfortunately, no detailed records on the manufacturing process were available to allow for the retrospective identification of such a critical processing step.

Interestingly, SE was detectable in three of the five different types of ice-cream, only: vanilla, pistachio and chocolate, with all three harbouring high amounts of CPS, too. In contrast, SE was detectable neither in strawberry nor in yoghurt-lemon ice-cream. This allows for the speculation, that a common ingredient was used, possibly being contaminated with either SE or CPS. In case of the latter, the manufacturing process must allow for growth of CPS and subsequent SE production. The different types of ice-cream were produced on several days prior the christening party all by use of the same ice-cream-maker device. The two fruit-containing ice-cream types were produced first, while the three types of milk ice-cream (chocolate, vanilla, and pistachio) were produced from pasteurized milk as ground mass. But also ice-cream with fruit components contains milk or milk products in general. Therefore, it cannot be excluded that the pathogen was introduced via contaminated milk. As neither recipes for the different types of ice-cream, nor details about the origin of its components were available at the time of investigation a common ingredient could not be identified. Despite the possible introduction via an ingredient, it might also be assumed that the ice-cream was contaminated through the used equipment. *S. aureus* is capable of developing biofilms on food-processing surfaces, a pathway leading to cross contamination of food (Vázquez-Sánchez et al., 2013). However, as the equipment used for the manufacturing and handling of ice-
cream, as well as the kitchen environment was properly cleaned prior to the inspection by the food and veterinary office Freiburg took place, no sampling was done in the course of the outbreak investigation.

In Germany, only very few numbers of foodborne outbreaks (FBOs) caused by SE are reported per year compared to the EU (EFSA, 2013). Reasons being manifold: on the one hand, there is no mandatory commercial Enzyme-Linked-Immunosorbent-Assay (ELISA)-based analytical methods for the detection of SE types A–E available. Method of choice in terms of sensitivity is the screening method of the European Reference Laboratory for CPS, ANSES, Maires-Alfort, France, which includes dialysis concentration as an essential step (Hennekinne et al., 2010). However, preliminary results of a proficiency trial organized by the NRL-Staph show that in Germany the majority of competent laboratories in charge of official checks of food apply SE detection methods without dialysis concentration (Fetsch et al., unpublished results). This might also explain the low number of reports on FBOs caused by SE.

In conclusion, to our knowledge this is the first description of a food poisoning outbreak due to staphylococcal enterotoxins in ice-cream in recent time. An enterotoxin type A producing S. aureus of spa-type t127 was found in human cases and different types of ice-cream, respectively, and displayed almost indistinguishable pheno- and genotypes with regard to the methods applied in this study. Suitable hygienic measurements, including personal hygiene when handling food, are needed at all processing steps to reduce the possibility of introduction of a severe hazard into the food chain. In case of a future food-borne outbreak, investigations should include the analysis of nasal samples taken from all people handling the food, as well as samples from all food components. Subsequent in-depth analyses of strains are needed as the results allow for the identification of epidemiological correlations of strains. Hence, an interdisciplinary team taking together experts from both, the human and veterinary health side, epidemiologists, microbiologists and food hygiene experts, all of them working together hand in hand is a crucial prerequisite for successful outbreak investigation. Particularly, if hazards such as SE, with high estimated numbers of unreported cases are the causing agent.

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Appendix A. Supplementary data
Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijfoodmicro.2014.06.017.

References