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

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ABSTRACT

In April 2013, a food poisoning outbreak caused by staphylococcal enterotoxins (SEs) in ice-cream occurred in Freiburg, Germany, among the 31 participants of a christening party. Of the 13 cases, seven were hospitalized or obtained ambulatory treatment. Different types of ice-cream, which was freshly produced at the hotel where the party took place, were found to contain SE and high amounts of coagulase positive staphylococci. Enterotoxigenic *Staphylococcus aureus* strains isolated from ice-cream and human cases were of the same *spa*-type (t127), harboured the *sea* gene and displayed identical phenotypic resistance-, Fourier transform infrared spectroscopy- (FT-IR) and microarray-profiles. Despite the strong microbiological and epidemiological evidence of ice-cream being the incriminated food vehicle of the outbreak, a common source of *S. aureus* from the ice-cream could not be deduced. As none of the employees carried the outbreak strain, either the equipment used for the production of the ice-cream or a contaminated ingredient is the most likely introduction source.

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 SE*l*V, 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, particularly among the group of YOPIs (young, old, pregnant, immunosuppressed persons) (Murray, 2005). Individual susceptibility to SE and the amount of SE ingested influence the onset and severity of the symptoms (Hennekinne et al., 2012; Tranter, 1990). As less as 100–200 ng of enterotoxin A (SEA) can lead to a disease (Evenson et al., 1988).

Typically, a staphylococcal food poisoning (SFP) occurs after ingestion of foods that are contaminated with *S. aureus* by improper handling and subsequent storage at elevated temperatures. People colonized 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 $>10^5$ *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, meat, 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

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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 home-made 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 Veterinaeruntersuchungsamt (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, daptomycin, 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 DNeasy 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).