



Year: 2013

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DOI: <https://doi.org/10.1089/fpd.2013.1503>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-81833>

Journal Article

Published Version

Originally published at:

Johler, Sophia; Tichaczek-Dischinger, P; Rau, J; Sihto, H M; Lehner, Angelika; Adam, M; Stephan, Roger (2013). Outbreak of Staphylococcal food poisoning due to SEA-producing *Staphylococcus aureus*. *Foodborne Pathogens and Disease*, 10(9):777-781.

DOI: <https://doi.org/10.1089/fpd.2013.1503>

Outbreak of Staphylococcal Food Poisoning Due to SEA-Producing *Staphylococcus aureus*

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Abstract

In 2008, 150 people gathered for a wedding celebration in Baden-Württemberg, Germany. Three hours after ingestion of a variety of foods including pancakes filled with minced chicken, several guests exhibited symptoms of acute gastroenteritis such as vomiting, diarrhea, fever, and ague. Twelve guests were reported to have fallen ill, with nine of these seeking medical care in hospitals. At least four patients were admitted to the hospital and received inpatient treatment, among them a 2-year-old child and a woman in the 4th month of pregnancy. Within 24 h of the event, an investigative team collected a variety of samples including refrigerated leftovers, food in the storage unit of the caterer, nasal swabs of the caterer, as well as 21 environmental swabs. Five stool samples from patients were provided by the hospitals. *Staphylococcus aureus* isolates were gathered from eight samples, among them nasal swabs of the caterer, food samples, and one stool sample. Fourier transform-infrared spectroscopy was used for species identification and for primary clustering of the isolates in a similarity tree. The isolates were further characterized by *spa* typing and pulsed-field gel electrophoresis, and a DNA microarray was used to determine the presence/absence of genes involved in virulence and antimicrobial resistance. We were able to match an enterotoxigenic strain from the stool sample of a patient to isolates of the same strain obtained from food and the nasal cavity of a food handler. The strain produced the enterotoxin SEA and the toxic shock syndrome toxin-1, and was also found to exhibit the genes encoding enterotoxins SEG and SEI, as well as the enterotoxin gene cluster *egc*. This is one of only a few studies that were able to link a staphylococcal food poisoning outbreak to its source.

Introduction

WHILE *STAPHYLOCOCCUS (S.) AUREUS* represents an ubiquitous commensal that persistently colonizes the anterior nares of 20–30% of the global population (van Belkum *et al.*, 2009), it can also cause severe infections, toxinoses, and life-threatening illnesses in humans and animals. Staphylococcal food poisoning is one of the most prevalent causes of foodborne intoxication worldwide. It is typically self-limiting, presenting with violent vomiting following a short incubation period. Within 2–6 h after ingestion of food containing staphylococcal enterotoxins (SEs), symptoms of acute gastroenteritis can be observed (Tranter *et al.*, 1990).

A variety of SEs and enterotoxin-like superantigens produced by *S. aureus* has been described, but only some were demonstrated to elicit an emetic response in a monkey-feeding assay. SEs that were shown to exhibit emetic activity include the classical enterotoxins SEA/SEB/SEC/SED/SEE, as well as

the newly described enterotoxins SEG/SEH/SEI/SEJ (Thomas *et al.*, 2007). Although the toxic shock syndrome toxin-1 (TSST-1) shares many biological properties of SEs, it displays no emetic activity. However, TSST-1 can induce massive proliferation of T-cells and production of cytokines, leading to multisystem failure and lethal shock (Thomas *et al.*, 2007).

As clinical symptoms of staphylococcal food poisoning are often self-limiting, only 10% of patients with staphylococcal food poisoning are estimated to visit a hospital (Holmberg *et al.*, 1984). In a few of these patients, *S. aureus* can be isolated from a stool sample, and in an even far lower number of cases, the isolated strain can be matched to isolates collected from foodstuff, environmental samples, or food handlers. In many outbreak reports, stool samples are missing or negative, thus forcing the authors to try to evaluate the relevance of numerous enterotoxigenic staphylococcal isolates collected from different sources (Do Carmo *et al.*, 2004; Colombari *et al.*, 2007; Schmid *et al.*, 2009).

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In this study, we aim to (1) provide a comprehensive overview of an outbreak of staphylococcal food poisoning, and (2) identify and characterize the *S. aureus* strain responsible for the outbreak.

Outbreak Report

On June 21, 2008, 150 people gathered for a wedding celebration in Baden-Württemberg, Southwest Germany. Food was served at 4 p.m., with a caterer providing a buffet of pancakes filled with minced chicken, as well as a variety of meat (chicken, beef, pork), spaetzle, salad, soup, and cake. The wedding party contributed a watermelon.

Three hours later, the first guests complained about nausea and abdominal pain that progressed quickly to emesis with or without additional symptoms such as diarrhea, fever (39–40°C), cardiovascular problems, ague, and aching limbs. A total of 12 guests (8%) were reported to have fallen ill the night of June 21, with nine of these (6%) seeking medical care in local hospitals. While the youngest of them was only 2 years of age, the oldest patient was 53 years old. At least four patients (3% of the guests) were admitted to the hospital and received inpatient treatment over a period of 1–2 days, among them the 2-year-old child and a woman in the 4th month of pregnancy. All patients were diagnosed with gastroenteritis due to a presumptive food intoxication and were treated symptomatically by administration of intravenous fluids. As the general condition of one patient deteriorated on the second day, the patient was additionally treated with metazolol and metoclopramide. All patients recovered within 72 h, and no sequelae were reported. Eight of the nine patients who visited a hospital provided detailed anamnestic information to the investigative team by filling out a questionnaire.

Materials and Methods

Bacterial isolation and identification

Samples were taken on the day of the celebration and the day after, including refrigerated leftovers, food samples obtained from the storage unit of the caterer, nasal swabs of both nasal cavities of the caterer, as well as 21 environmental swabs. The hospitals provided stool samples of five patients for further investigation.

S. aureus were isolated from food samples using methods in accordance with §64 of the German Food and Feed Code (Amtliche Sammlung, L06.00-16 and L00.00-55). This corresponds to enumeration of coagulase-positive staphylococci by direct plating of decimal dilutions as described in EN ISO 6888-1: "Horizontal method for enumeration of coagulase-positive staphylococci." Coagulase-positive *Staphylococci* were identified by inoculating KRANEP agar (Oxoid, Wesel, Germany). Environmental swabs were streaked directly onto the selective agar plate that was subsequently incubated under aerobic conditions for 48 h at 37°C. RapiDEC staph® (bioMérieux, Nürtingen, Germany) was used to detect aurease production, and *S. aureus* species identification was confirmed using the latex agglutination test Staphytect Plus® (Oxoid). Fecal samples were fractionated on mannitol salt agar (MSA) and incubated at 37°C for 24 h. Subcultures of yellow colonies were used to inoculate Columbia blood agar (bioMérieux) that was incubated for 24 h at 37°C. Presumptive

S. aureus isolates were confirmed by VITEK GP ID card (bioMérieux).

Fourier transform infrared spectroscopy (FT-IR)

FT-IR allowed for rapid species identification of *S. aureus* isolates (Spohr *et al.*, 2011). For this purpose, bacterial isolates were cultivated on sheep blood agar plates (Oxoid) at 37°C for 24 h. Sample preparation, FT-IR spectroscopy (using IFS 28/B spectrometer, BrukerOptics, Ettlingen, Germany) and data acquisition were performed as described previously (Kuhm *et al.*, 2009). Analysis of data was carried out using OPUS Software (vers. 4.2, BrukerOptics) and an artificial neural network built by the NeuroDeveloper software (Synthon, Heidelberg, Germany). The differentiation of *S. aureus* was performed with a method described previously (Spohr *et al.*, 2011).

IR spectra of isolates were compared by cluster analysis (Amiali *et al.*, 2007; Stamm *et al.*, 2013). For cluster analysis, the vector-normalized spectra of the wave number range 500–1600 cm⁻¹ in second derivation were used for calculation with Ward's algorithm (OPUS 4.2; Ward *et al.*, 1963). The dendrogram obtained shows the arrangement of isolates in groups according to their spectral differences (Fig. 1).

Spa typing

The sequence of the polymorphic X region of the *spa* gene of the eight *S. aureus* isolates was determined as previously described (Aires de Sousa *et al.*, 2006), with minor modifications. Total DNA was isolated using the Qiagen DNeasy kit (Hilden, Germany), following the manufacturer's instructions. The *spa* gene was amplified as previously described (Wattinger *et al.*, 2012). Amplicons were purified and the concentration of nucleic acids was determined using a Nanodrop ND-UV/Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE). Sequencing was outsourced (Microsynth, Balgach, Switzerland). The sequences were assigned to *spa* types using the *spa*-server (<http://www.spaserver.ridom.de/>).

Microarray-based genotyping

The StaphyType ArrayStrip platform was used for DNA microarray profiling according to the manufacturer's instructions (Clondiag chip technologies, Jena, Germany). Similar to Coombs *et al.* (Coombs *et al.*, 2010), microarray profiles were compared using SplitsTree4 (<http://www.splittree.org>).

PFGE

Preparation of chromosomal DNA and PFGE analysis of *Sma*I digested fragments was performed as previously described (Bannerman *et al.*, 1995). Electrophoresis was carried out in a CHEF-DR III electrophoresis cell (Bio-Rad). *Salmonella* serotype Braenderup strain H9812 digested with *Xba*I (50 U, 12 h, 37°C) was used as a molecular size standard.

RPLA toxin detection

S. aureus isolates collected from food samples were examined for production of superantigenic toxins including enterotoxins SEA/SEB/SEC/SED, as well as TSST-1 (toxic

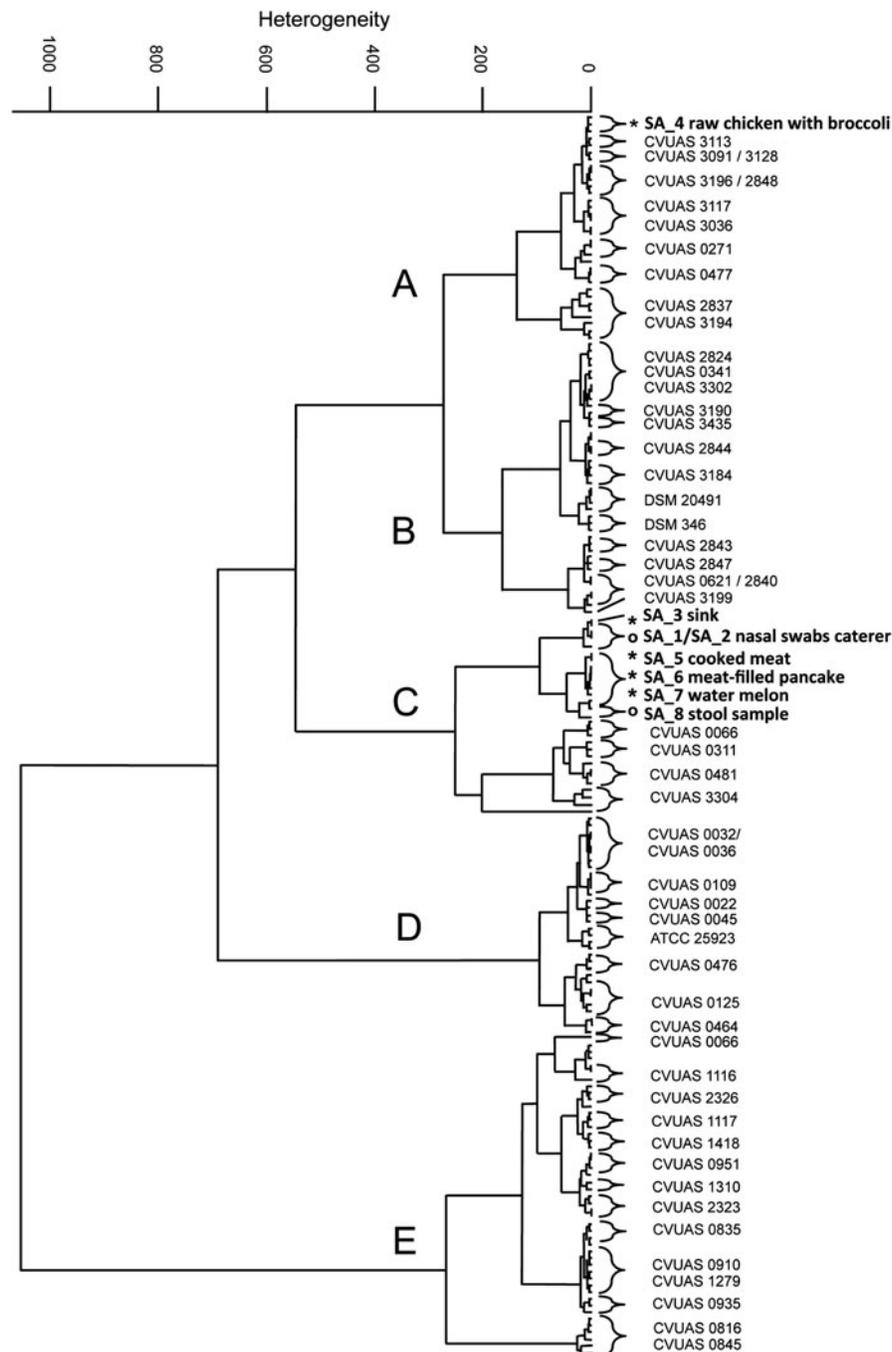


FIG. 1. Similarity tree depicting the results of the Fourier transform infrared spectroscopy analysis. Seven isolates (SA_1-3 and SA_5-8) formed a cluster of closely related isolates, while spectra of SA_4 were found on a different branch.

shock syndrome toxin-1) using RPLA toxin detection kits (Oxoid).

Results

S. aureus was isolated from eight different samples (Table 1), including nasal swabs of the caterer's nose, different foods, a swab from a kitchen sink at the site of the celebration, as well as the stool sample of one patient.

FT-IR was used for rapid species identification and for a preliminary determination of the degree of relatedness of the

isolates. We compared the results of the FT-IR in a dendrogram (Fig. 1) that also included a number of *S. aureus* strains for reference that were not involved in the outbreak investigation (strains designated CVUAS, DSM, and ATCC). Seven of the eight obtained *S. aureus* isolates clustered together, including the isolate obtained from the stool sample of a patient, while SA_4 obtained from raw chicken represented the only isolate located on another branch of the similarity tree. This was confirmed by *spa* typing that assigned SA_4 to t002, while assigning the seven other *S. aureus* isolates to t018.

TABLE 1. CHARACTERISTICS OF ALL *STAPHYLOCOCCUS AUREUS* ISOLATED BY THE INVESTIGATIVE TEAM

ID	Source	RPLA toxin detection					Spa type	CFU/g food
		SEA	SEB	SEC	SED	TSST-1		
SA_1	Nasal swab of caterer's nose #1	+	—	—	—	+	t018	n.a.
SA_2	Nasal swab of caterer's nose #2	+	—	—	—	+	t018	n.a.
SA_3	Sink at the site of the celebration	+	—	—	—	+	t018	n.a.
SA_4	Raw chicken filled with broccoli	—	—	—	—	—	t002	10 ²
SA_5	Cooked meat	+	—	—	—	+	t018	10 ⁶
SA_6	Meat-filled pancake	+	—	—	—	+	t018	10 ⁶
SA_7	Watermelon	+	—	—	—	+	t018	10 ⁴
SA_8	Stool sample of patient	+	—	—	—	+	t018	n.a.

n.a., not applicable.

DNA microarray was used to further characterize the seven isolates assigned to *spa* type t018. All tested isolates exhibited either identical profiles or differed only in the result determined for one of over 250 probes. DNA microarray confirmed that all tested isolates represent *S. aureus* and assigned the isolates to clonal complex 30. Among others, genes encoding enterotoxins SEA/SEG/SEI (*entA*, *entA-320E*, *entG*, *entI*), the enterotoxin-like superantigens SEM/SEN/SEO/SEU (*entM*, *entN*, *entO*, *entU*), as well as TSST-1 (*tst-1*) were detected. For a full list of hybridization results of all isolates, see Supplementary Table S1 (Supplementary Data are available online at www.liebertpub.com/fpd).

PFGE patterns are depicted in Figure 2. The seven isolates that were assigned to t018 and exhibited highly similar FT-IR and DNA microarray profiles also display identical PFGE patterns (SA_1, SA_2, SA_3, SA_5, SA_6, SA_7, SA_8).

The questionnaires revealed that the 2-year-old child had exclusively eaten pancakes filled with minced chicken and that this dish had also been ingested by all other patients. The average incubation time before onset of symptoms equalled 4.2 h. The clinical symptom common in all patients was emesis, while only some patients also reported diarrhea, abdominal pain/cramps, nausea, fever (39–40°C), cardiovascu-

lar problems, ague, and aching limbs. The 2-year-old child suffered from severe dehydration.

Discussion

FT-IR analysis as well as *spa* typing suggested that the seven *S. aureus* isolates SA_1-3 and SA_5-8 are very closely related. *Spa* typing assigned these strains to t018, a *spa* type previously found in common methicillin-resistant *S. aureus* clones in the United Kingdom and Denmark (Bartels *et al.*, 2009; Khandavilli *et al.*, 2009), as well as *S. aureus* strains associated with nasal colonization, infections, and food poisoning (Wattinger *et al.*, 2012). These seven highly similar isolates originated from food samples, nasal swabs of the caterer, as well as a stool sample from a patient.

In contrast, isolate SA_4 exhibited a different *spa* type and FT-IR spectrum and could not be linked to the outbreak. Therefore, only the seven isolates assigned to t018 were further characterized by DNA microarray and PFGE. The identical PFGE patterns and highly similar DNA microarray profiles of these isolates suggest that they represent the same *S. aureus* strain.

DNA microarray showed that the enterotoxin genes *sea*, *seg*, *sei* encoding SEA, SEG, and SEI were present, consistent with the identification of this strain as the source of the food-poisoning outbreak. Expression of the *sea* gene was confirmed by RPLA, detecting the SEA protein. SEA represents the most common enterotoxin recovered from food-poisoning outbreaks in the United States (77.8%) and was shown to exhibit exceptionally high emetic activity, with a total dose of only 200 ng being sufficient to cause gastroenteritis (Balaban *et al.*, 2000).

We detected isolates of the same SEA producing *S. aureus* strain in samples of the nose of the caterer, different foodstuffs served at the celebration including meat-filled pancakes, and the stool sample of one patient. It is therefore highly likely that the caterer inadvertently contaminated the food and that the organism was able to multiply and produce enterotoxins due to insufficient refrigeration before and/or during the buffet.

Conclusions

This is one of only a few studies (Argudín *et al.*, 2010; Johler *et al.*, 2010) that was able to link a staphylococcal food poisoning outbreak to its source. We were able to match an enterotoxigenic strain in the stool sample of a patient to isolates of the same strain obtained from food and the nasal cavity of a food handler. The strain produced SEA and TSST-1, and was

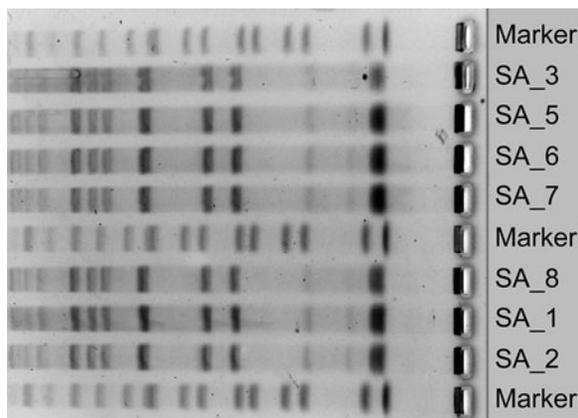


FIG. 2. The seven isolates that were assigned to t018 and exhibited highly similar Fourier transform infrared spectroscopy and DNA microarray profiles also display identical pulsed-field gel electrophoresis patterns (SA_1, SA_2, SA_3, SA_5, SA_6, SA_7, SA_8). Molecular size standards were used in lanes 1, 6, and 10. Lanes 2–5, as well as lanes 7–9, each contain a different staphylococcal isolate.

also found to exhibit the genes encoding enterotoxins SEG and SEI, as well as the enterotoxin gene cluster *egc*.

Acknowledgments

This study was partially funded by a fellowship provided by the Swiss National Science Foundation (PBZHP3-138695).

Disclosure Statement

No competing financial interests exist.

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