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Comparison of *Staphylococcus aureus* isolates associated with food intoxication with isolates from human nasal carriers and human infections

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Abstract *Staphylococcus aureus* represents an organism of striking versatility. While asymptomatic nasal colonization is widespread, it can also cause serious infections, toxinoses and life-threatening illnesses in humans and animals. Staphylococcal food poisoning (SFP), one of the most prevalent causes of foodborne intoxication worldwide, results from oral intake of staphylococcal enterotoxins leading to violent vomiting, diarrhea and cramps shortly upon ingestion. The aim of the present study was to compare isolates associated with SFP to isolates collected from cases of human nasal colonization and clinical infections in order to investigate the role of *S. aureus* colonizing and infecting humans as a possible source of SFP. *Spa* typing and DNA microarray profiling were used to characterize a total of 120 isolates, comprising 50 isolates collected from the anterior nares of healthy donors, 50 isolates obtained from cases of clinical infections in humans and 20 isolates related to outbreaks of staphylococcal food poisoning. Several common *spa* types were found among isolates of all three sources (t015, t018, t056, t084). DNA microarray results showed highly similar virulence gene profiles for isolates from all tested sources. These results suggest contamination of foodstuff with *S. aureus* colonizing and infecting food handlers to represent a source of SFP.

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Introduction

Staphylococcus aureus is not only a commensal colonizer, but can also cause serious infections, toxinoses and life-threatening diseases, such as skin and soft tissue infections, toxic shock syndrome and septicemia. *S. aureus* colonizes skin and mucosa of humans and animals, with nasal carriage rates between 30% and 50% among the adult human population [1–4]. While colonization of the anterior nares is usually asymptomatic, it serves as a reservoir for the spread of the organism [1, 5]. Carriers are at increased risk to develop nosocomial bacteremia which in 80% of cases is caused by the strain colonizing their nares [6, 7]. The rapid emergence of antibiotic resistance among *S. aureus* is also known to play a crucial role in the epidemiology of staphylococcal infections. Recently, infections with methicillin resistant *S. aureus* (MRSA) have been estimated to constitute the leading cause of death due to one single infectious agent in the United States [8].

S. aureus also represents the cause of staphylococcal food poisoning (SFP), one of the most prevalent foodborne intoxications worldwide. SFP results from ingestion of staphylococcal enterotoxins preformed in food, typically presenting with violent emesis, nausea, diarrhea and prostration. While in most cases symptoms subside spontaneously after 24 h, fatality rates range from 0.03% in the general population to 4.4% in children and the elderly [9]. As staphylococcal colonization and infection is widely spread, contamination of foodstuff by food handlers may represent a major source of SFP. As SFP isolates are difficult to obtain, to date, there is only very limited information on the original source of enterotoxigenic *S. aureus* strains that lead to cases of food poisoning.

Different techniques are established for typing *S. aureus*. The most widely used method for epidemiological inves-

tigations is *spa* typing, based on the determination of the polymorphic X region of the gene encoding staphylococcal protein A (*spa*). DNA microarray is used for rapid detection of a multitude of virulence genes (genes encoding enterotoxins, hemolysins, leukocidins, etc.), resistance determinants, and typing markers. The resulting hybridization pattern can be used to assign isolates to clonal complexes [10].

In this study, *spa* typing and DNA microarray analysis were performed with a total of 120 *S. aureus* isolates, comprising *S. aureus* isolates obtained from nasal colonization in healthy donors, isolates gained from clinical cases of infection and isolates associated with outbreaks of staphylococcal food poisoning. The objective was to compare SFP isolates to isolates obtained from *S. aureus* nasal colonization (SANC) and clinical cases of infection (SAI) in order to determine the role of *S. aureus* colonizing and infecting humans as a possible source of SFP.

Materials and methods

Bacterial isolates

A total of 120 *S. aureus* isolates were examined, constituting 50 SANC isolates, 50 SAI isolates and 20 isolates associated to outbreaks of SFP in humans. Nasal swabs of the anterior nares were collected from randomly chosen

volunteers in Switzerland between November and December 2010. Samples from both nostrils were taken using sterile cotton swabs moistened with saline. Fifty SAI isolates were obtained from the Institute of Medical Microbiology of the University of Zurich, Switzerland, between November and December 2010. The 20 SFP isolates were provided by the Bavarian Authorities for Health and Food Safety (LGL, Munich, Germany), the German National Reference Center for Staphylococci (Robert Koch Institute, Wernigerode, Germany), the Cantonal Laboratory Fribourg (Fribourg, Switzerland) and the Medical Department of the German Federal Armed Forces (Kronshagen, Germany) (Table 1). Ethical clearance was granted by the locally cognizant ethics commission (cantonal ethics commission, Zurich).

DNA extraction and species identification

Swabs were streaked directly onto rabbit plasma fibrinogen (RPF) plates (Oxoid, Basel, Switzerland), incubated at 37°C and examined for coagulase activity after 48 h. Two *S. aureus* typical colonies (colonies surrounded by an opaque halo) each were subcultured on RPF plates (48 h at 37°C). One colony of each plate was transferred to blood agar and incubated overnight at 37°C. DNA isolation kits were obtained from QIAGEN (Hilden, Germany) and handled according to the manufacturer's instructions. The PCR consumables were supplied by Promega (Madison, Wisconsin,

Table 1 Staphylococcal food poisoning (SFP) isolates included in this study

ID	Sample	Institution providing the isolate
SFP1	Food	Medical Department of the German Federal Armed Forces
SFP2	Food	Medical Department of the German Federal Armed Forces
SFP3	Food	Cantonal Laboratory of Fribourg
SFP4	Feces	Bavarian Authorities for Health and Food Safety
SFP5	Feces	Bavarian Authorities for Health and Food Safety
SFP6	Feces	Bavarian Authorities for Health and Food Safety
SFP7	Feces	Bavarian Authorities for Health and Food Safety
SFP8	Feces	Bavarian Authorities for Health and Food Safety
SFP9	Feces	Bavarian Authorities for Health and Food Safety
SFP10	Feces	Bavarian Authorities for Health and Food Safety
SFP11	Feces	Bavarian Authorities for Health and Food Safety
SFP12	Feces	Bavarian Authorities for Health and Food Safety
SFP13	Feces	Bavarian Authorities for Health and Food Safety
SFP14	Feces	Bavarian Authorities for Health and Food Safety
SFP15	Feces+food	Robert Koch Institute
SFP16	Food	Robert Koch Institute
SFP17	Feces+food	Robert Koch Institute
SFP18	Feces	Robert Koch Institute
SFP19	Feces	Robert Koch Institute
SFP20	Feces	Robert Koch Institute

USA). The DNA concentration was measured by using a Nanodrop ND-1000 UV/Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Spa typing

The sequence of the polymorphic X region of the *spa* gene of each *S. aureus* isolate was determined as described by Aires-de-Sousa et al. [11], with minor modifications. Briefly, the *spa* gene was amplified with spa-1113f and spa-1514r primers (Table 2) using the GoTaq PCR system (Promega AG, Dübendorf, Switzerland) at the following reaction conditions: (i) 5 min at 94°C; (ii) 35x [45 s at 94°C; 45 s at 60°C; 90 s at 72°C]; (iii) 10 min at 72°C. PCR purification and sequencing was outsourced (GATC Biotech, Constance, Germany and Microsynth, Balgach, Switzerland). The sequences were assigned to *spa* types using the *spa*-server (http://www.spaserver.ridom.de/) [12]. Clonal complexes were determined using Ridom StaphType 2.0.3 software and the Based Upon Repeat Pattern (BURP) algorithm.

Microarray based genotyping

For DNA microarray profiling the StaphyType ArrayStrip platform was used according to the manufacturer's instructions (Clondiag chip technologies, Jena, Germany). Similar to Coombs et al., microarray profiles were compared using SplitsTree4, a software designed to compute unrooted phylogenetic networks from molecular sequence data [13, 14]. DNA microarray gene profiles were converted to "sequence-like" strings of information, defining present genes as "A" (positive), absent genes as "T" (negative) and spots with ambiguous signal intensities as missing.

Statistical analysis

The distribution of genes among SANC, SAI, and SFP isolates was compared based on the hybridization results of the DNA microarray. SPSS Statistics 19 was used to run Pearson's chi-squared test, identifying significant associations between the source the isolates were collected from and the presence of the examined genes. *P*-values<0.05 were considered statistically significant.

Results

Screening of nasal swabs for the presence of *S. aureus* showed a nasal carriage rate of 37.6% among the 133 healthy test persons.

The 120 staphylococcal isolates tested, including 50 SANC, 50 SAI, as well as 20 SFP isolates, could be assigned to 20 clonal complexes comprising a total of 79 different *spa* types (see Table 3). Among SANC, SAI, and SFP isolates, clonal complexes CC8, CC15, CC30, CC45, CC78, and CC101 could be found. Isolates from all three sources were frequently assigned to CC45 (SANC: 16%, SAI: 20%, SFP: 30%). While high prevalence of CC30 was found among SANC (24%) and SFP isolates (15%), SAI isolates were more often assigned to CC59 (14%). The 50 isolates from nasal swabs were grouped into 39 *spa* types with *spa* type t015 and t012 being found most frequently (8% each). The 50 SAI isolates grouped into 38 different *spa* types, with t216 representing the most common *spa* type (12%). The 20 isolates associated with SFP were grouped into 15 *spa* types. Some common *spa* types were found among isolates of all three sources (t015, t018, t056, and t084). Isolates obtained from nasal colonization and cases of clinical infections were overlapping in *spa* types t002, t127, t148, and t216. Spa type t008 was found in both clinical and food poisoning isolates of *S. aureus*.

DNA microarray was used to determine gene profiles of all 120 strains. Hybridization results for *agr* and capsule types are depicted in Table 4. While *agrI* was found to represent the most frequent *agr* type among all three sources, SANC and SAI isolates differed significantly in the number of isolates assigned to *agrI* (36% SANC, 70% SAI; *p*=0.001) and *agrIII* (28% SANC, 8% SAI, *p*=0.009).

Most isolates possessed one or several genes involved in resistance to antimicrobial agents (see Table 5). The *blaZ* gene conferring resistance to beta lactams was found most frequently among isolates from all three sources (SANC: 74%, SAI: 76%, SFP: 85%). Antibiotic resistance profiles were highly similar for SANC, SAI, and SFP isolates. Only *fosB*, which is involved in resistance to fosfomycin and bleomycin, was present in significantly higher numbers in SANC than in SAI isolates (SANC: 68%, SAI: 38%; *p*=0.002). While genes involved in vancomycin resistance (*vanA/B/Z*) were not found, few isolates exhibited genes

Table 2 Primers used in this study	Name	Nucleotide sequence (5′ → 3′)	Product size	Reference
	spa-1113f	5′ TAA AGA CGA TCC TTC GGT GAG C 3′	Variable	[11]
	spa-1514r	5′ CAG CAG TAG TGC CGT TTG CTT 3′		
	Staur 4	5′ ACG GAG TTA CAA AGG ACG AC 3′	1250 bp	[31]
	Staur 6	AGC TCA GCC TTA ACT AGC AG 3′		

Table 3 *Spa* types and predicted clonal complexes of *S. aureus* nasal colonization (SANC), clinical cases of infection (SAI) and staphylococcal food poisoning (SFP) isolates investigated in this study

Clonal complex	Spa type	Numerical code assigned to repeats	SANC(n=50)	SAI(n=50)	SFP(n=20)
CC1	t127	07-23-21-16-34-33-13	1	1	0
	t189	07-23-12-21-17-34	0	1	0
	t8021	07-23-12-21-23-12-21-17-34	0	1	0
CC5	t002	26-23-17-34-17-20-17-12-17-16	2	3	0
	t010	26-17-34-17-20-17-12-17-16	1	0	0
	t105	26-23-17-34-17-20-17-17-16	1	0	0
	t857	26-23-17-34-17-13-17-16	1	0	0
	t954[a]	26-23-17-34-17-17-16	0	1	0
	t1062	26-23-17-34-17-02-17-12-17-16	1	0	0
	t8017	35-17-34-17-20-17-17-12-17-16	0	1	0
	t8020	07-22-17-20-17-12-17-17-16-16	1	0	0
CC6	t701	11-10-21-17-34-24-34-22-25-25	0	1	0
CC7	t091	07-23-21-17-34-12-23-02-12-23	0	2	0
CC8	t008	11-19-12-21-17-34-24-34-22-25	0	1	2
	t024	11-12-21-17-34-24-34-22-25	0	0	1
	t148	07-23-12-21-12-17-20-17-12-12-17	1	1	0
	t334	11-12-21-17-34-22-25	1	0	0
	t648	11-21-17-34-24-34-22-25	0	0	1
	t8016	07-23-13-21-22-34-34-34-34-33-34	1	0	0
CC9	t209	07-16-12-23-34	1	0	0
	t733	26-23-02-12-23-02-34-34-34	0	0	1
CC12	t156	07-06-17-21-34-34-22-34	0	1	0
	t5444	14-12-33-22-17	1	0	0
CC15	t084	07-23-12-34-34-12-12-23-02-12-23	3	3	2
	t085	07-23-12-34-34-12-23-02-12-23	0	1	0
	t279	07-23-12-34-34-34-12-12-23-02-12-23	0	0	1
	t328	07-23-12-34-34-12-12-23-02-12-23-02-12-23	1	0	0
	t529	04-34	1	0	0
	t774	07-23-12-34-34-12-12-12-23-02-12-23	0	1	0
	t1038	07-23-12-34-13-12-12-23-02-12-23	1	0	0
	t4802	07-23-12-34-34-12-12-23-23-02-12-23	0	0	1
CC20	t164	07-06-17-21-34-34-22-34	0	1	0
CC22	t005	26-23-13-23-31-05-17-25-17-25-16-28	0	1	0
	t310	26-23-31-05-17-25-17-25-16-28	0	1	0
	t852	07-23-13-23-31-05-17-25-17-25-16-28	0	1	0
	t8019	26-23-13-23-31-05-17-25-17-24-16-28	0	1	0
CC25	t349	04-21-12-17-20-17-12-12-17	1	0	0
CC30	t012	15-12-16-02-16-02-25-17-24-24	4	0	0
	t017	15-12-16-16-02-16-02-25-17-24-24	1	0	0
	t018[a]	15-12-16-02-16-02-25-17-24-24-24	1	1	2
	t021	15-12-16-02-16-02-25-17-24	2	0	0
	t338	15-21-16-02-25-17-24	1	0	0
	t8455	15-12-16-16-34-02-16-02-25-17-24-24	1	0	0
	t1076	04-12-25-22-34	1	0	0
	t1239	15-12-16-02-16-02-24-24-24	0	0	1
	t4516	15-12-16-24-24	1	0	0
CC45	t015[a]	08-16-02-16-34-13-17-34-16-34	4	4	1
	t050	08-16-02-16-34-34-17-34-16-34	1	0	0

Table 3 (continued)

Clonal complex	Spa type	Numerical code assigned to repeats	SANC($n=50$)	SAI($n=50$)	SFP($n=20$)
	t073	08-16-02-16-13-17-34-16-34	0	1	0
	t230	08-16-02-16-34	0	1	0
	t377	04-02-12-21-17-34-22-25	0	0	1
	t383	08-16-34-13-16-34	0	0	1
	t445	08-16-20-16-34-13-17-34-16-34	0	1	0
	t630	08-16-02-16-34-17-34-16-34	1	0	0
	t950	08-16-34-17-34-16-34	0	1	0
	t1270	09-34-34-34-17-34-16-34	0	0	2
	t1574	08-16-02-16-34-13-13-17-34-16-34	1	0	0
	t4460	08-16-02-16-17-34-16-34	0	1	0
	t5599	08-16-02-16-34-13-16-34-16-34	1	0	0
	t6969	08-16-13-17-34-16-34-34	0	0	1
	t8454	08-16-02-16-34-16-34-13-17-34-16-34	0	1	0
CC50	t246	04-17-23-24-20-17-25	0	1	0
	t8018	04-20-22-17	0	1	0
CC59	t216	04-20-17-20-17-31-16-34	2	6	0
	t270	14-44-13-12-17-17-17-17-23-18-17	1	0	0
	t437	04-20-17-20-17-25-34	0	1	0
CC78	t186[a]	07-12-21-17-13-13-34-34-33-34	0	1	0
	t912	08-12-17-13-13-34-13	0	0	1
	t1814	07-12-21-17-34-34-34-33-34	1	0	0
CC97	t267	07-23-12-21-17-34-34-34-33-34	0	1	0
	t276	15-12-16-02-16-02-25	0	1	0
	t359	07-23-12-21-17-34-34-33-34	1	0	0
CC101	t056	04-20-12-17-20-17-12-17-17	1	1	1
	t2888	04-20-12-17-13-17	0	1	0
CC121	t159	14-44-13-12-17-17-23-18-17	1	0	0
	t272	14-44-13-12-17-17-17-23-18-17	0	1	0
	t645	14-44-13-12-17-23-18-17	1	0	0
CC398	t011[b]	08-16-02-25-34-24-25	1	0	0
	t571	08-16-02-25-02-25-34-25	1	0	0

[a] spa types comprising mecA positive SAI isolates (SAI8: t954/CC5, SAI9: t018/CC30, SAI12: t015/CC45, SAI36: t186/CC78)

[b] spa type comprising the mecA positive isolate obtained from nasal colonization SANC11

associated with resistance to tetracycline (tetK/M) and methicillin (mecA). One SANC (SANC11) and four SAI isolates (SAI8, SAI9, SAI12, SAI36) possessed mecA. SANC11 was detected in a nasal swab from a female veterinarian aged 27 that could be assigned to ST398-MRSA-V ("Dutch Pig Strain", score: 93.1%). SAI8 was isolated from a skin lesion in a 58-year-old male patient suffering from sepsis. SAI9 was detected in a pharyngeal swab from a 76-year-old male patient and was assigned to ST36/39-MRSA-II, UK-EMRSA-16 (synonym to USA 200, Irish AR7.0, Canadian MRSA-4; score: 94.3%). SAI12 was isolated from a perineal/perianal swab of an 85-year-old male and was assigned to ST45-MRSA-IV, Berlin EMRSA (synonym to USA 600-MRSA-IV, score: 91.8%). SAI 36 was isolated from a 42-year-old female suffering from ulceration after a burn wound and could be assigned to CC78-MRSA-IV, WA MRSA-2 (score: 96.0%).

DNA microarray results for genes encoding superantigenic toxins are displayed in Table 6. We tested for genes encoding staphylococcal enterotoxins (entA-entJ), enterotoxin-like proteins (entK-entR, entU), as well as exfoliative toxins (entA/B/D), toxic shock syndrome toxin (tst-1), and panton valentine leukocidin (pvl). While entA-entD were found in isolates of all three sources, entE was not detected. SFP isolates were significantly more likely to possess enterotoxin A variant entA-320 than SANC ($p=0.005$) and SAI isolates ($p=0.002$). In comparison with SFP isolates, SANC isolates exhibited enterotoxin A variant entA-N315 in significantly higher ($p=0.042$) and entD in significantly lower numbers.

Table 4 Assignment to *agr* and *capsule* types based on DNA microarray analysis. The percentages represent the fragment of *S. aureus* nasal colonization (SANC), clinical cases of infection (SAI) and staphylococcal food poisoning (SFP) isolates, for which genes were determined to be present. Calculations include positive signals only, ambiguous signals were omitted

Group	Gene	Source		
		SANC (*n*=50)	SAI (*n*=50)	SFP (*n*=20)
agr types	*agr*I	36%[a SAI]	70%[a SANC]	55%
	*agr*II	28%	22%	25%
	*agr*III	28%[a SAI]	8%[a SANC]	20%
	*agr*IV	6%	12%	5%
capsule types	*capsule*-1	0%	0%	0%
	capsule-5	28%	26%	25%
	capsule-8	72%	74%	75%

[a] Result for this source differs significantly from the result calculated for one (source indicated) or both other sources of isolates investigated in this study (*p*<0.05)

We observed an even distribution of genes belonging to the enterotoxin gene cluster (*entG, entI, entM, entN, entO, entU*).

SFP isolates were shown to possess *entJ* and *entR* in significantly higher numbers than isolates obtained from

Table 5 Genes involved in antibiotic resistance. Percentages of *S. aureus* nasal colonization (SANC), clinical cases of infection (SAI) and staphylococcal food poisoning (SFP) isolates, for which genes were determined to be present based on DNA microarray analysis. Calculations include positive signals only, ambiguous signals were omitted

Gene	Affected antibiotic	Source		
		SANC(*n*=50)	SAI(*n*=50)	SFP(*n*=20)
mecA	Methicillin	2%	8%	0%
blaZ	Beta-lactam	74%	76%	85%
ermA	Macrolides, lincosamides, streptogramin	8%	6%	0%
ermB	Macrolides, lincosamides, streptogramin	2%	2%	5%
ermC	Macrolides, lincosamides, streptogramin	2%	0%	5%
linA	Lincosamide	0%	2%	0%
mrsA	Macrolides	0%	0%	0%
mefA	Macrolides	0%	0%	0%
mpbBM	Macrolides	0%	0%	0%
vatA	Streptogramin	0%	0%	0%
vatB	Streptogramin	0%	0%	0%
vga, b	Streptogramin	0%	0%	0%
vgaA	Streptogramin	0%	0%	0%
aacA-aphaD	Aaminoglycosides (gentamicin, tobramycin)	2%	2%	0%
aadD	Aminoglycosides (gentamicin, tobramycin)	0%	2%	3%
aphA	Aminoglycosides (gentamicin, tobramycin)	0%	2%	0%
sat	Streptothricin	0%	2%	0%
dfrA	Trimethoprim	0%	4%	0%
far	Fusidic acid	0%	0%	0%
mupR	Mupirocin	0%	0%	0%
tetK	Tetracycline	2%	8%	10%
tetM	Tetracycline	2%	4%	0%
cat	Chloramphenicol	0%	0%	0%
fexA	Chloramphenicol	0%	2%	0%
fosB	Fosfomycin, bleomycin	68%[a SAI]	38%[a SANC]	65%
vanA, Z	Vancomycin	0%	0%	0%
vanB	Vancomycin	0%	0%	0%
qacA, C	Unspecific efflux pump	0%	4%	5%

[a] Result for this source differs significantly from the result calculated for one (source indicated) or both other sources of isolates investigated in this study (p<0.05)

Table 6 Genes encoding super-antigenic toxins, such as genes coding for staphylococcal enterotoxins (*entA- entJ*) and enterotoxin-like proteins (*entK-entR, entU*), as well as exfoliative toxins (*etA/B/D*), toxic shock syndrome toxin (*tst*) and panton valentine leukocidin (*pvl*). Percentages of *S. aureus* nasal colonization (SANC), clinical cases of infection (SAI) and staphylococcal food poisoning (SFP) isolates, for which genes were determined to be present based on DNA microarray analysis. Calculations include positive signals only, ambiguous signals were omitted

Group	Gene	SANC(n=50)	SAI(n=50)	SFP(n=20)
Enterotoxins	entA	26%	20%	30%
	entA-320	6%	4%	30%[a]
	entA-N315	18%[a SFP]	16%	0%[a SANC]
	entB	8%	22%	5%
	entC	16%	26%	20%
	entD	2%[a SFP]	6%	15%[a SANC]
	entE	0%	0%	0%
	entG	58%	44%	50%
	entH	4%	4%	0%
	entI	64%	50%	50%
	entJ	2%[a SFP]	6%	15%[a SANC]
Enterotoxin-like proteins	entK	4%	18%[a]	0%
	entL	16%	26%	20%
	entM	68%	50%	50%
	entN	66%	50%	50%
	entO	64%	50%	45%
	entQ	4%[a SAI]	16%[a SANC]	0%
	entR	2%[a SFP]	6%	15%[a SANC]
	entU	46%	50%	50%
Exfoliative toxins	etA	2%	2%	5%
	etB	2%	0%	0%
	etD	2%	0%	0%
Toxic shock syndrome toxin	tst-1	12%	8%	15%
Panton valentine leukocidin	pvl	0%	2%	6%

[a] Result for this source differs significantly from the result calculated for one (source indicated) or both other sources of isolates investigated in this study (p<0.05)

nasal colonization (p=0.034 each). SAI isolates exhibited significantly higher numbers of *entQ* than SANC isolates (p=0.046) and significantly higher numbers of *entK* than both SANC (p=0.023) and SFP (p=0.040) isolates. Few isolates also possessed *tst-1*, *pvl*, and genes encoding exfoliative toxins, with no significant differences in prevalence among isolates of the three investigated sources.

DNA microarray results for genes encoding leukocidins, hemolysins and staphylokinase are depicted in Table 7. The genes were evenly distributed among isolates of all three

Table 7 Other virulence determinants, including genes encoding leukocidins, hemolysins, and staphylokinase. Percentages represent the fraction of *S. aureus* nasal colonization (SANC), clinical cases of infection (SAI) and staphylococcal food poisoning (SFP) isolates for which the genes were determined to be present based on DNA microarray analysis. Results depicted include positive signals only, ambiguous signals were not considered for the calculation

Gene	Protein	Source		
		SANC(n=50)	SAI(n=50)	SFP(n=20)
lukD	Leukocidin D	34%	54%	50%
lukE	Leukocidin E	28%[a SAI]	56%[a SANC]	35%
lukF	Leukocidin F/ hemolysin gamma (B)[1]	86%	96%	100%
lukS	Leukocidin S/ hemolysin gamma (C)[1]	78%	78%	85%
hla	Alpha toxin/ hemolysin alpha	94%	96%	95%
hlb	Beta toxin/ hemolysin beta	56%	62%	40%
hlgA	Gamma toxin/ hemolysin gamma (A)[1]	68%	100%[a]	80%
hld	Delta toxin/ hemolysin delta	100%	100%	100%
sak	Staphylokinase	72%	80%	60%

[a] Result for this source differs significantly from the result calculated for one (source indicated) or both other sources of isolates investigated in this study (p<0.05)

sources, with the exception of *lukE* and *hlgA*. SAI isolates possessed *lukE* significantly more frequently than SANC isolates ($p=0.005$), and *hlgA* significantly more often than both SANC ($p=0.000$) and SFP isolates ($p=0.017$).

Comparison of microarray profiles using the SplitsTree software resulted in no source-specific clusters, but a mixed distribution of isolates of all three sources (see Fig. 1).

Discussion

Screening nasal swabs for SANC isolates showed a nasal carriage rate of 38%. CC30 and CC45 represented the most common clonal complexes among nasal isolates investigated, comprising 24% and 16% of SANC isolates, respectively. These findings are consistent with a recent study conducted in Switzerland which observed a nasal carriage rate of *S. aureus* of 32% among healthy adults and reported CC30 and CC45 to be the most common clonal complexes among SANC

isolates, comprising 24% of nasal carriage isolates each [15]. It was reported that CC30 occurs at high frequencies and is stably maintained among human carriers worldwide [15–17]. A recent study conducted among asymptomatic carriers in Germany found CC8, CC15, CC30, and CC45 to be most common among asymptomatic carriers [18]. Among the tested SAI isolates, CC45 (20%) represented the dominant clonal complex, while a comprehensive Dutch study observed this clonal complex to be underrepresented among invasive strains [19]. A recent German study found CC8 and CC45 to be most common among *S. aureus* isolated from bone and joint infections [20]. The clonal complexes CC5 and CC30 that were also present among SAI isolates in our study. Isolates assigned to these clonal complexes were observed to significantly increase hematogenous complications in staphylococcal infections in humans [21]. Several clonal complexes found in our study among SFP isolates were also present among the investigated SANC and SAI isolates (CC8, CC15, CC30, CC45, CC78, CC101). SFP
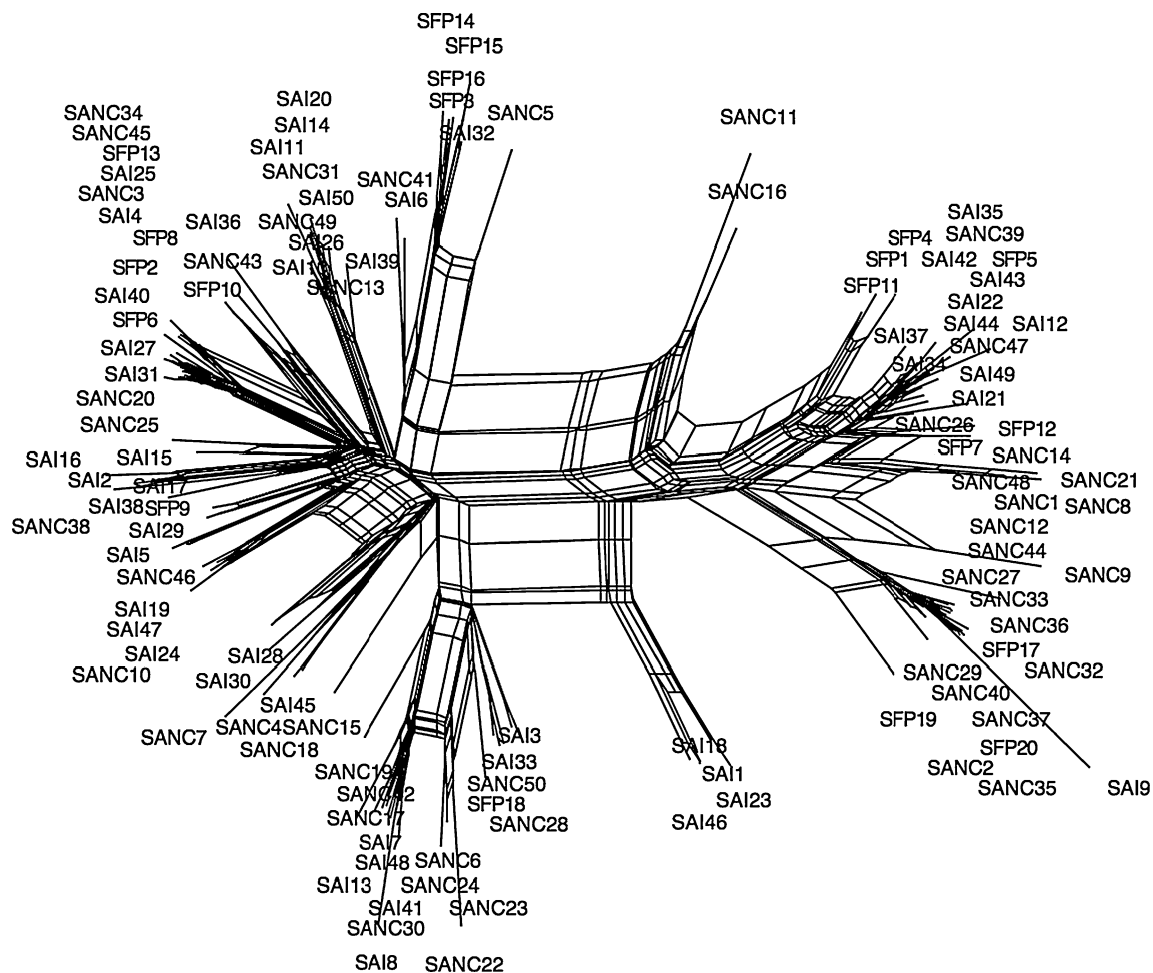

Fig. 1 SplitsTree showing similarity between gene profiles determined by DNA microarray analysis for 120 *S. aureus* isolates, comprising 50 isolates obtained from nasal colonization (SANC), 50 isolates collected from clinical cases of infection (SAI) and 20 isolates associated with staphylococcal food poisoning (SFP)

isolates were most frequently assigned to CC45 (30%), followed by CC8 (20%), CC15 (20%), and CC30 (15%). To the authors knowledge, there have been no previous studies on the distribution of clonal complexes among *S. aureus* isolates associated with outbreaks of SFP.

The *spa* types t008, t015, t018, t056, and t084 that we detected among SFP isolates were also present among SANC and SAI isolates investigated in our study. *Spa* types t008, t015, t056, and t084 were reported among methicillin-sensitive *S. aureus* causing infections in humans [22, 23] and *spa* type t018 was found in common MRSA clones in the United Kingdom and Denmark [24, 25].

DNA microarray profiling enabled the comparison of gene profiles of isolates from nasal colonization, clinical cases of infection and SFP. Interestingly, DNA microarray profiles of isolates from all three sources were rather similar. This is consistent with a recent study that found nasal carriage isolates and clinical isolates to be closely related [26]. Interestingly, especially few significant differences in prevalence rates were found when SFP and SAI isolates were compared.

Among each source of isolates investigated in this study, all *agr* types (*agrI-IV*) were found. The *agrIV* group was recently hypothesized to constitute a truly monophyletic group, while *agrI-III* might have evolved from several unrelated ancestors [10]. DNA microarray results in our study revealed a variety of isolates exhibiting differing virulence gene profiles that possessed *agrIV*. All isolates investigated in our study belonged to capsule type 5 or 8, which were reported to be the only capsular serotypes associated with human disease [27]. The spread of genes conferring resistance to antibiotic agents was corroborated by the antibiotic resistance determinants detected among the *S. aureus* investigated in our study. The most common resistance gene was *blaZ*, encoding penicillinase BlaZ, which enables hydrolysis of both methicillin and oxacillin, was high in isolates from all three sources (SANC: 74%, SAI: 76%, SFP: 85%). The detected prevalence rates for *blaZ* and *mecA* among SANC isolates (*blaZ*: 74%, *mecA*: 2%) are consistent with a recent German report on asymptomatic carriers, which found *blaZ* in 71% and *mecA* in 2% of staphylococcal isolates [18]. A study characterizing *S. aureus* from bone and joint infections detected *blaZ* in 65% and *mecA* in 6% of isolates, similar to the prevalence rates of *blaZ* and *mecA* genes among SAI isolates investigated in this study (*blaZ*: 76%, *mecA*: 8%) [20].

Four out of five *mecA* positive isolates detected in this study were obtained from clinical cases of staphylococcal infection. The MRSA isolate obtained from a nasal swab (SANC11) of a veterinarian working in equine practice belonged to *spa* type t011 and clonal complex CC398, which were also found among clinical MRSA isolates

collected from a human patient and several horses in a recent Finnish study [28].

Both tested variants of *entA* encoding enterotoxin A, the gene responsible for most cases of SFP, were detected among SANC and SAI isolates. Interestingly, all SFP isolates possessed the *entA-320* variant, which was first detected in a French field isolate in 2003 [29]. While prevalence rates of *entA* and *entC* detected among SANC isolates in this study were almost identical to those of a study conducted with nasal carriage isolates of restaurant workers in Kuwait city, we found lower prevalence rates of *entB*, *entD*, and *entE* [30]. While a German study reported similar rates of *entB* and *entC* among asymptomatic nasal carriers, slightly lower rates of *entA*, as well as higher rates of *entD* were found [18].

Comparison of microarray profiles using the SplitsTree software resulted in no source-specific clustering, but a mixed distribution of isolates of all three sources. In addition, in our study we found considerable overlap in *spa* types for SFP isolates with isolates collected from nasal colonization and clinical cases of infection. These results suggest contamination of foodstuff during preparation by food handlers that are colonized or infected by *S. aureus* represents a source of SFP.

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