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Genotypes, exotoxin gene content and antimicrobial resistance in *Staphylococcus aureus* recovered from foods and food-handlers

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ABSTRACT

Staphylococcal food poisoning, one of the most common food-borne diseases, results from ingestion of one or more staphylococcal enterotoxins (SEs) performed by *Staphylococcus aureus* in foods. In the present study, 64 *S. aureus* isolates recovered from foods and food handlers, associated or not with food-poisoning outbreaks in Spain, were investigated. They were assigned to 31 strains by *spa* typing, MLST, exotoxin gene content, and antimicrobial resistance. The strains belonged to ten clonal complexes (CCs): CC5 (29.0%), CC30 (25.8%), CC45 (16.1%), CC8, CC15 (two strains each), CC1, CC22, CC25, CC59, and CC121 (one each). They contained haemolysin genes (90.3%); *lukED* (77.4%); exfoliatin genes: *eta, etd* (6.5% each) and *etb* (3.2%); *tst* (25.8%); and enterotoxin or enterotoxin-like genes or clusters: *sea* (38.7%), *seb* (12.9%), *sec* (16.1%), *sed-selj±ser* (22.9%), *selk-selq* (6.5%), *seh*, *sell*, *selp* (9.7% each), *egc1* (32.3%), and *egc2* (48.4%). The number of *se*/*sel* genes ranged from 0 to 12. All isolates carrying *tst*, and most isolates with genes encoding classical enterotoxins (SEA, SEB, SEC and SED), expressed the corresponding toxin(s). Two CC5 isolates from hamburgers (t002/ST5, t2173/ST5) were methicillin resistant and harboured SCC*mec* IVd. Six (19.4%) were mupirocin resistant and one (t120/ST15) from a food handler carried *mupA* (MIC 1250 μg/ml). Resistances to ampicillin (*blaZ*; 61.3%), erythromycin (*ermA-ermC/ermC*; 25.8%), clindamycin (*msrA-msrB/msrB*; 16.1%), tetracycline (*tetK*; 3.2%), and amikacin-gentamicin-kanamycin-tobramycin (*aacA+aphD-aphA*; 6.5%) were also observed. The presence of *S. aureus* with an important repertoire of virulence and resistance determinants in the food chain represents a potential health hazard for consumers and deserves further observation.

Keywords: food-borne pathogens, enterotoxins, antimicrobial resistance, MRSA, mupirocin resistance.

INTRODUCTION

Staphylococcal food poisoning (SFP) is a common intoxication which results from consumption of foods containing sufficient amounts of one or more preformed enterotoxin(s) (3, 29). Symptoms have a rapid onset, and comprise nausea and vomiting, with or without diarrhea (43). The illness is usually self-limiting but occasionally it can be severe enough to warrant hospitalization (33). Real incidence of SFP is underestimated, mainly due to misdiagnosis and because sporadic episodes and minor outbreaks are not reported. Despite this, 29 outbreaks were communicated in 2008 in the European Union, 414 persons were affected and 26 required hospitalization (17). With regard to Spain, 431 outbreaks have been recorded during the 1994-2008 period (10, 17, 21, 32). Four of them occurred in Asturias (a Northern Spanish region), at three different restaurants in 2002 (31), and at an elderly nursing home in 2006, and the associated isolates were examined in this work.

Staphylococcus aureus, the casual agent of SFP, is a common commensal of the skin and mucosal membranes. Food handlers carrying enterotoxin-producing strain(s) in their noses or hands are regarded as the main source of food contamination, via manual contact or through respiratory secretions (40). *S. aureus* enterotoxins (SEs) belong to the broad family of pyrogenic toxin superantigens, and have emetic activity (6, 18). Classical SEs (SEA, SEB, SEC, SED and SEE) have been discovered in studies of *S. aureus* involved in SFP outbreaks, and are classified in distinct serological types (15). Related toxins that lack emetic activity or have not been tested for it are designated as staphylococcal enterotoxin-like (SEls) (30). All known *se* and *sel* genes are located on mobile genetic elements, including νSaβ genomic island, which carries the enterotoxin gene clusters known as *egc1* (*seg*, *seln*, *sei*, *selm*, *selo*; νSaβ type I), *egc2* (*seg*, *seln*, *selu*, *sei*, *selm*, *selo*; νSaβ type III) and variants therein (5, 13, 42); *S. aureus*

pathogenicity islands (SaPIs, *etd*, *tst*, *seb*, *sec*, *sell*, *selq*, *selk*; 34); prophages (*lukPV*, *eta*, *sea*, *see*, *selp*, *selk*, *selq*; 19), and plasmids (*etb*, *seb*, *sed*, *selj*, *ser*, *ses*, *set*; 8, 36).

The changing epidemiology of *S. aureus* over the last decades, including the emergence of the novel methicillin resistant *S. aureus* (MRSA) ST398 clone linked to food production animals (livestock associated MRSA), highlights the necessity of monitoring the *S. aureus* clones which are circulating through the food chain (27). *S. aureus* substantially contributes to the virulence and resistance gene pool of Gram positive bacteria and the molecular analysis of the circulating strains could help to understand the exchange of such genes between bacterial species and clones (1). In this study, we examined a collection of *S. aureus* recovered in the Spanish Region of Asturias from manually handled foods and food-handlers, associated or not with SFP outbreaks recorded in the region. The objectives were i) to establish the genetic backgrounds of the food-related isolates and to compare them with isolates that have been circulating in human carriers or causing disease in hospitals of Asturias; ii) to investigate the distribution of exotoxin genes in food-related isolates, and the production of *tst* and classical enterotoxins; and iii) to evaluate their resistance properties, paying particular attention to the presence of MRSA.

MATERIALS AND METHODS

Bacterial strains. The *S. aureus* isolates analyzed in this study (64 isolates) have been collected from food handlers (14 isolates) and manually handled foods (50 isolates) in Asturias (Table 1). Thirty-five isolates were recovered at the Public Health Laboratory (LSP) of Asturias from foods and employees during three poisoning outbreaks (outbreaks 1 to 3) which occurred at different restaurants were included in the study. These isolates had been partially characterized in a previous study (31). New isolates (29 isolates) were collected from foodstuff associated with a fourth outbreak which took place at an elderly nursing home; from a variety of foods within the "Security Food Program" of the "Agency for Environmental Health and Consumption" of Asturias at the LSP, and from healthy carriers attending a teaching course for food handlers. The new isolates were biochemically confirmed as *S. aureus* (API Staph system; bioMerieux), and tested for haemolytic (sheep blood agar; Oxoid, Madrid, Spain), thermonuclease (DNase test agar; Oxoid) and coagulase (Slidex Staph Plus; bioMerieux, Marcy-l'Etolie, France) activities, and production of classical enterotoxins was determined by reverse passive latex agglutination using the SET-RPLA commercial kit (Oxoid), according to the manufacturer's recommendations. All isolates were also tested for TSST-1, using the TST-RPLA kit (Oxoid).

Antimicrobial susceptibility testing. All isolates were tested for antimicrobial susceptibility by the disk diffusion method, using Mueller-Hinton agar and commercially available discs (Oxoid). The antimicrobial agents used were ampicillin, penicillin, oxacillin, methicillin, gentamicin, amikacin, kanamycin**,** tobramycin, tetracycline, erythromycin, clindamycin, chloramphenicol, ciprofloxacin, moxifloxacin, rifampicin, linezolid, vancomycin, tigecycline, mupirocin, trimethoprim and trimethoprim-sulfamethoxazole. Minimal inhibitory concentrations (MICs) for

mupirocin (GlaxoSmithKline, United Kingdom) and vancomycin (Laboratorios Normon SA, Madrid, Spain) were determined by the agar dilution method in Mueller-Hinton (Oxoid, Madrid, Spain), with concentrations ranging from 0 to 1500 µg/ml and from 0 to 4 µg/ml, respectively. Results were scored according to the Clinical and Laboratory Standards Institute (12), or categorized as low (MIC 8 to 64 mg/l) and high (≥ 512 µg/ml) resistance in the case of mupirocin (37). *S. aureus* NCTC 8325 was included as control. To estimate the rate of inducible lincosamide resistance, the double-disk diffusion test was performed as reported (41).

PCR amplification. Genomic DNA was purified using the phenol-chloroform and proteinase K (50 µg/ml; Roche Diagnostics, Spain) method (39), preceded by a lysis step with lysostaphin (0.02 µg/ml, Sigma, St. Louis, USA). SCC*mec* typing in MRSA strains was carried out as described by Zhang *et al*. (46). In addition, all isolates were tested by simplex PCR amplifications (Tables S1 and S2, supplementary material) of genes which confer resistance to ampicillin-penicillin (*blaZ*), methicillin-oxacillin (*mecA* and SCC*mec* type), macrolides (*msrA, msrB*), lincosamides (*linA/linA'*), macrolides-lincosamides-streptogramins B [*ermA, ermB, ermC*], tetracyclines [*tet*(K), *tet*(L), *tet*(M), *tet*(O)], aminoglycosides (*aacA+aphD, aadD*, *aphA*), phenicols (*cat*::pC194, *cat*::pC221, *cat*::pC223, *fexA*) and mupirocin (*mupA*). The isolates were also tested by simplex PCR for virulence determinants encoding haemolysins (*hla, hlb, hld, hlg, hlg*-variant), leukotoxins (*lukED, lukM, lukPV*), exfoliatins (*eta, etb, etd*), toxic shock syndrome toxin (*tst*), SEs (*sea, seb, sec, sed, see, seg, seh, sei, ser, ses, set*), and SEls (*selj, selk, sell, selm, seln, selo, selp, selq, selu*), and for the type of accessory gene regulatory system *agr* (*agrI, agrII, agrIII, agrIV*). Markers for the νSaβ genomic islands (*splF*, *bsaB*) and SaPIs (*ear*) were also screened. The *sea* to *see*, *selj* and *seg* to *sei* genes have previously been tested in isolates associated with outbreaks 1 to 3 (31), but

all other genes were screened in the present work. The presence/absence of different types of *egc* clusters, νSaβ genomic islands, SaPIs, prophages, and plasmids was inferred on the basis of positive PCR results for relevant gene combinations (3).

Typing techniques. All isolates were subjected to staphylococcal protein A (*spa*) typing, and at least one representative of each *spa* type was also analyzed by multilocus sequence typing (MLST) (16, 20). Genomic DNA, extracted as described previously, was used in PCR amplifications for the *spa* (*spa* typing), *arcC, aroE, gplF, gmk, pta, tpi* and *yqiL* (MLST) genes. PCR products generated by both techniques were purified with the GFX PCR DNA Gel Band Purification kit (GE Healthcare, Madrid, Spain), and sequenced at Macrogen Inc (Seoul, Korea). The Ridom Spa Server (http://spaserver.ridom.de/) and the MLST website (http://www.mlst.net) were used to assign *spa* types and sequence types (ST), respectively. Isolates were grouped into a single CC when at least five of the seven housekeeping genes included in the MLST scheme were identical (16).

Strain assignment and frequencies determination. Isolates with the same *spa*-type, ST, *agr*-type, virulence profile and resistance profile were considered as a single strain. Percentages reported on the Results and Discussion sections were calculated with respect to the total number of strains (31 strains; Table 1).

RESULTS

Exotoxin gene profiles and association with mobile genetic elements. As shown in Table 1, the majority of the strains included in this study contained haemolysin genes (90.3%; two to five *hl* genes) and the *lukED* leukotoxin gene (77.4%); less strains carried *tst* (25.8%), *eta*, *etd* (6.5% each) or *etb* (3.2%); and none of them tested positive for *lukPV* or *lukM*. One or more genes for classical SEs were detected in 54.8% of the

strains: *sea* (38.7%), *seb* (12.9%), *sec* (16.1%) and *sed* (22.6%). Other enterotoxin genes, including *selj* (22.6%), *selk-selq* (6.5%), *seh*, *sell*, *selp* (9.7% each), and *ser* (16.1%), were also represented, while *see*, *ses* and *set* were absent. Most strains (80.7%) harbored *egc1* (32.3%) or *egc2* (48.4%). In all, the number of enterotoxin genes ranged from 0 (four strains) up to twelve (one strain), with an average of six.

Markers for νSaβ (*splF*, *bsaB*) and SaPIs (*ear*) were found in 35.5%, 48.4% and 9.7% of the strains, respectively (Table 1). Different combinations of *lukED, egc*-genes, *splF* and *bsaB* support the occurrence of νSaβ type I (*splF-lukED-egc1*; six strains), type II (*bsaB-splF-lukED*; one strain), and type III (*splF-egc*2; one strain). Additional combinations, like *splF-bsaB-lukED-egc1* (one strain), *lukED-egc2-splF* (four strains), *lukED-egc1* (three strains), *lukED-egc*2 (seven strains), *splF-lukED* (one strain), *splF-bsaB* (one strain), *lukED* alone (one strain), and *egc2* alone (three strains), suggest deletions in known types of νSaβ or the existence of new variants. On the other hand, detection of *ear* together with certain *se*/*sel* genes is consistent with the presence of SaPI3 (*ear-seb-selk-selq*; one strain), SaPI5 (*ear-selk-selq*; one strain), or SaPImw2 (*ear-sec-sell*; three strains). In other cases, the presence of *se*/*sel* genes could be presumptively associated with prophages, which are known to carry *sea* (12 strains), *selp* (three strains), or with plasmids where *sed/selj* ± *ser* have been located (seven strains).

Expression of *tst* and classical *se* genes. In all *tst*- *sec*- and *sed*-positive strains (eight, five and seven, respectively), the encoded toxins could be immunologically detected, while 58.3% and 50% the strains carrying *sea* or *seb* expressed the corresponding toxin, respectively. In four strains, production of two classical enterotoxins (SEA plus SEC, SEA plus SED, and SEB plus SEC) was detected, and TSST-1 was expressed together with SEA, SED, and SEA plus SED.

Antimicrobial drug resistance. Nine out of the 31 strains (29.0%) were susceptible to all tested compounds, and none of them were resistant to chloramphenicol, ciprofloxacin, moxifloxacin, rifampicin, linezolid, vancomycin, tigecycline, trimethoprim and trimethoprim-sulfamethoxazole. The remaining strains were resistant to three or more (19.4%; here considered as multiresistant), two (6.5%) or one (45.2%) classes of antimicrobials. The frequencies of individual resistances (in parenthesis) were: ampicillin-penicillin (61.3%), oxacillin-methicillin (6.5%), erythromycin (25.8%), clindamycin (19.4%), tetracycline (3.2%), amikacin-gentamicin-kanamycin-tobramycin (6.5%), and mupirocin (19.4%). The MICs for mupirocin were 32 µg/ml (three strains from food handlers associated with outbreak 2), 64 µg/ml (two strains from hamburgers), and 1250 µg/ml (one strain from a food handler in outbreak 1). As shown in Table 1, all strains resistant to ampicillin-penicillin carried the *blaZ* gene which encodes a β-lactamase, and in most of them (63.2%) the gene was associated with transposon Tn*552*, as demonstrated by PCR amplification of a region spanning between *blaZ* and the tranposase gene of Tn*552*. The two oxacillin-methicillin resistant strains (MRSA), both from hamburgers, carried SCC*mec* IVd. The genes implicated in erythromycin resistance together with inducible resistance to clindamycin were *ermC* (three strains) or *ermA* and *ermC* (two strains), while resistance to only erythromycin was associated with the presence of *msrB* or *msrA* and *msrB* (one and two strains, respectively). The gene *aphA* was found in the two aminoglycoside resistant strains together with *aacA+aphD* or *aadD*, and the plasmid associated *tetK* gene was responsible for tetracycline resistance (one strain). The strain with high level resistance to mupirocin (MIC 1250 µg/ml) carried *mupA*, while the low level resistant strains lacked this gene.

Clones and clonal complexes. The strains were assigned to 21 *spa* types and 15 STs, and distributed among 10 CCs (Tables 1 and 2). The most frequent CCs were CC5 (29.0%), CC30 (25.8%) and CC45 (16.1%). CC8, CC15 (two strains each), CC1, CC22, CC25, CC59 and CC121 (one strain each) were also represented, but the emerging CC398 was not detected. The four groups of *agr* system were found in the food-related strains (Table 1): *agrI* (16.1%), *agrII* (29.0%), *agrIII* (32.3%) and *agrIV* (22.6%), and a strong correlation between different CCs and certain *agr* groups was observed: CC1-*agrIII*, CC5-*agrII*, CC15-*agrII*, CC22-*agrI*, CC30-*agrIII*, CC45-*agrI* and *agrIII*, CC121-*agrIV* (38, 45). The *agrIV* group previously reported in CC5 and CC25 in Asturias (2, 4) was here found in CC5, CC8, CC25 and CC59 isolates. The two MRSA strains from hamburgers, acquired at a local supermarket, had different *spa* types (t002 and t2173), but both were ST5 (CC5) and had the *agrII* system.

DISCUSSION

The presence of staphylococcal species in foods is well documented, but information on the *S. aureus* lineages which are circulating through the food chain is still limited. In the present study, 64 food-related isolates were assigned to 31 strains by molecular typing techniques, in conjunction with virulence and resistance properties. These strains belonged to ten CCs, and all except one (CC22) were also represented in isolates from hospitals and healthy carriers recovered in the same geographical region, which are consistent with epidemiological data from Spain (2, 4, M. A. Argudín, M. C. Mendoza, and M. R. Rodicio, unpublished data). In addition, the most frequent CCs in food-related isolates (CC5, CC30 and CC45) were also the most frequent in isolates from healthy carriers of the region, and the three included potential outbreak strains. Strain 3, which was collected from different foods served at the outbreak 1 restaurant, was

t050/ST45 (CC45), carried the *sec* gene and expressed the toxin. This strain was not found in two food-handlers working at the same restaurant, although both were *S. aureus* carriers. Strain 8 (t701/ST6; CC5; positive for SEA/*sea*; and resistant to several antimicrobials) was recovered from stuffed crab and from two food-handlers associated with outbreak 3, being the probable cause of the outbreak, as already proposed by Martín et al. (31). Strain 8 was also present in vegetables from the outbreak 2 restaurant, but a total of six strains (strains numbered from 4 to 9), belonging to four CCs, were recovered from foods and food-handlers at this restaurant. In addition, all except one of these strains carried at least one enterotoxin gene, and most produced SEA or SEC. With regard to outbreak 4, a single isolate (strain 15) is available. It was recovered from Russian salad served at an elderly nursing home, was positive for *sea*/SEA, and belonged to t012 (ST30; CC30).

SEA followed by SED, are the enterotoxins most frequently associated with SFP, although outbreaks caused by SEB, SEC, SEE and SEH have also been reported (3, 29). In the food-related strains characterized in this study, *sea*, *seb*, *sec*, *sed* and *seh* genes, but not *see*, were detected, and SEA, SEB, SEC and SED, for which commercial detection systems are available, were expressed in most strains carrying the genes. The relationship between other SEs or SEls and SFP is poorly understood, partly due to the unavailability of commercial kits for detection. In the present study, genes belonging to the *egc* were very common, appearing in more than 80% of the strains, either alone or in combination with other enterotoxin genes. A high incidence of *egc* genes in food-borne *S. aureus* was also reported by other authors (7, 9, 22, 28, 35). Accordingly, the pathogenic role of the *egc* enterotoxins may be underestimated.

Like in *S. aureus* from other sources, resistance to ampicillin-penicillin was high (61.3%) in food-related isolates tested in this study, and the responsible gene, *blaZ*, was

often associated with Tn*552* (23). The frequency of other resistances, including oxacillin (methicillin; 6.5%), erythromycin (25.8%), clindamycin (19.4%), gentamicin-tobramycin (6.5%), was lower than that reported in Spanish hospitals (2, 4, 14). This is in agreement with data from Kerouanton *et al*. (25), showing that strains from SFP outbreaks were more susceptible to antimicrobials than human clinical isolates. However, the frequency of resistance to mupirocin (19.4%) was higher than that reported for nosocomial *S. aureus* in Spain (8.9%; 14), although only strain 2 from a food handler, harbored the *mupA* gene which confers high level resistance. Mupirocin is the antibiotic of choice for eradication of *S. aureus* carriage, and resistance has been implicated in failure of the treatment (37). The two MRSA detected (strains 26 and 27) belonged to CC5 and carried SCC*mec* IVd. This subtype of SCC*mec* is uncommon in Spain, and was only associated with CC8 (44). Both strains were recovered from hamburgers acquired at a local supermarket, and produced SED (strain 26) or SEA plus SED (strain 27). The presence of these strains in hamburgers is of note since MRSA, a highly relevant nosocomial- and community-acquired pathogen (11), has also been incriminated in SFP outbreaks (24, 25, 26).

In conclusion, the molecular epidemiology study conducted shows the presence of different *S. aureus* strains containing important resistance and virulence determinants in foods and food handlers. These strains encode not only classical and novel enterotoxin genes, but also major virulence factors like exfoliative toxins and TSST-1. The presence in the food chain of these isolates represents a potential health hazard for consumers and deserves further attention.

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TABLE 1. Features of food-related *S. aureus* isolates analyzed in this work.

Source (N)[a]	Strain[b]	Toxins[c]	Virulence profile	Resistance profile[d]	CC[e]	*spa* type	MLST
O1-FH1 (2)	1	na	*hla/b/d/g-lukED-egc1-splF-agrIII*	AMP / *blaZ*::Tn*552*	CC30	t021	ST30
O1-FH2	2	na	*hla/d/gv-lukED-splF-agrII*	MUP / *mupA*	CC15	t120	ST15
O1-Cooked lamb (5)	3	SEC	*hla/d/g-lukED-sec/l-egc1-ear-agrI*	AMP / *blaZ*::Tn*552*	CC45	t050	ST45
O1- Shellfish salad (5)	3	SEC	*hla/d/g-lukED-sec/l-egc1-ear-agrI*	AMP / *blaZ*::Tn*552*	CC45	t050	nd
O1- Paella (4)	3	SEC	*hla/d/g-lukED-sec/l-egc1-ear-agrI*	AMP / *blaZ*::Tn*552*	CC45	t050	nd
O1-Cream-cake (2)	3	SEC	*hla/d/g-lukED-sec/l-egc1-ear-agrI*	AMP / *blaZ*::Tn*552*	CC45	t050	nd
O2-FH3	4	na	*hla/d/g/gv-eta-agrIII*	AMP-[ERY-CLI^{I}]-MUP / *blaZ*::Tn*552*-[*ermA-ermC*]	CC1	t177	ST3
O2-FH4	5	TSST-SEA	*hla/d/g/gv-eta-lukED-tst-sea-egc2-splF-agrIII*	AMP-[ERY-CLI^{I}]-MUP / *blaZ*::Tn*552-ermC*	CC30	t012	nd
O2-FH5	6	TSST	*lukED-tst-seh-egc2-splF-agrIII*	AMP-[ERY-CLI^{I}]-MUP / *blaZ*::Tn*552-ermC*	CC30	t136	ST34
O2-Cream cake	7	na	*hla/b/d/g/gv-lukED-seh-egc2-agrIV*	AMP-ERY / *blaZ*-[*msrA-msrB*]	CC5	t616	ST1619
O2-Vegetables (2)	8	SEA	*hla/b/d/gv-sea-bsaB-splF-agrIV*	AMP-[ERY-CLI^{I}]-TET / *blaZ*::Tn*552-ermC-tetK*	CC5	t701	ST6
	9	SEC	*hla/b/d/g/gv-lukED-sec-egc1-ear-agrI*	AMP/*blaZ*	CC22	t790	ST217
O2-Beef	9	SEC	*hla/b/d/g/gv-lukED-sec-egc1-ear-agrI*	AMP/*blaZ*	CC22	t790	ST217
O3-FH6	8	SEA	*hla/b/d/gv-sea-bsaB-splF-agrIV*	AMP-[ERY-CLI^{I}]-TET / *blaZ*::Tn*552-ermC-tetK*	CC5	t701	nd
O3-FH7	10	na	*lukED-sek/q-ear-bsaB-splF-agrIV*	AMP / *blaZ*	CC8	t008	ST8
O3-FH8	11	SEB	*hla/d/gv-etd-lukED-seb-egc1-ear-bsaB-splF-agrIV*	AMP / *blaZ*::Tn*552*	CC25	t7125	ST26
O3-FH9	8	SEA	*hla/b/d/gv-sea-bsaB-splF-agrIV*	AMP-[ERY-CLI^{I}]-TET / *blaZ*::Tn*552-ermC-tetK*	CC5	t701	nd
O3-Stuffed crab	8	SEA	*hla/b/d/gv-sea-bsaB-splF-agrIV*	AMP-[ERY-CLI^{I}]-TET / *blaZ*::Tn*552-ermC-tetK*	CC5	t701	nd
O3-Shellfish salad (2)	12	na	*hla/d/gv-lukED-egc1-splF-agrII*	-	CC5	t1560	ST146
	13	na	*hla/b/d/gv-lukED-egc1-splF-agrII*	AMP / *blaZ*	CC5	t1560	nd
O3-Cream-cake (2)	14	na	*hla/b/d/gv-lukED-egc1-splF-agrII*	-	CC5	t1560	nd
	13	na	*hla/b/d/gv-lukED-egc1-splF-agrII*	AMP / *blaZ*	CC5	t1560	nd
O4-Russian salad	15	TSST-SEA	*hld/g-tst-sea-egc2-agrIII*	AMP / *blaZ*::Tn*552*	CC30	t012	nd
Cake	16	(-)-(-)-SED	*hla/b/d/g/gv-lukED-etd-sea/b/d/j/p/r-egc2-ear-agrII*	ERY / *msrB*	CC5	t002	nd
Cake	17	(-)-(-)-SED	*hla/b/d/g/gv-lukED-sea/b/d/j/p-egc2-agrIV*	AMP / *blaz*	CC8	t008	nd
Cake	18	SED	*hla/d/gv-lukED-sed/j/p-egc1-ear-splF-agrII*	ERY / *msrA-msrB-linA/linA'*	CC5	t002	ST5
Stuffed eggs	19	(-)-SEC	*hla/b/d/g/gv-lukED-sea/c/l-egc2-ear-agrI*	AMP-[ERY-CLI^{I}] / *blaZ*::Tn*552*-[*ermA-ermC*]	CC45	t015	ST45

TABLE 1 (continuation). Features of food-related *S. aureus* analyzed in this work.

Source (N)[a]	Strain[b]	Toxins[c]	Virulence profile	Resistance profile[d]	CC[e]	*spa* type	MLST
Sponge cake (5)	20	SEA-SEC	*hld/g-lukED-sea/c/l-egc2-ear-agrI*	AMP / *blaZ*::Tn*552*	CC45	t015	nd
Sponge cake (5)	21	TSST-SEA	*hld/g-lukED-tst-sea-egc2-agrIII*	AMP / *blaZ*::Tn*552*	CC30	t840	ST30
Swiss roll (5)	22	(-)-SEB-SEC	*hla/b/d/g/gv-sea/b/c/k/q-egc2-ear-agrIV*	-	CC59	t216	ST59
Cheese	23	na	*agrIII*	-	CC30	t012	nd
Hamburger	24	TSST-(-)-SED	*hla/b/d/g/gv-lukED-tst-sea/d/j/r-egc2-ear-agrI*	-	CC45	t015	nd
Hamburger	25	TSST-SEA-SED	*hla/b/d/g/gv-lukED-tst-sea/d/j/r-egc1-agrIII*	AMP / *blaZ*::Tn*552*	CC45	t604	ST546
Hamburger	26	SED	*hla/b/d/gv-lukED-sed/j/r-egc1-splF-agrII*	[AMP-OXA]-[AMK-GEN-KAN-TOB]-MUP / [*blaZ*-SCC*mec* IVd]-[*aacA+aphD-aphA*]	CC5	t002	ST5
Hamburger	27	SEA-SED	*hla/b/d/g/gv-lukED-sea/d/j/r-egc2-splF-agrII*	[AMP-OXA]-CLI-[AMK-GEN-KAN-TOB]-MUP / [*blaZ*-SCC*mec* IVd]-nd-[*aadD-aphA*]	CC5	t2173	ST5
TC-FH10	28	TSST	*hla/b/d/g-lukED-tst-egc2-splF-agrIII*	-	CC30	t166	ST34
TC-FH11	29	na	*hla/d/gv-lukED-ear-agrII*	-	CC15	t3474	ST15
TC-FH12	30	na	*hla/b/d/gv-etb-egc2-agrIV*	-	CC121	t272	ST51
TC-FH13	31	TSST	*hla/b/d/g-tst-seh-egc2-splF-agrIII*	-	CC30	t166	nd

[a]The order corresponds to the year of isolation. Thirty six isolates from foods (26 isolates) and food handlers (FH; 10 isolates) were recovered during four poisoning outbreaks. Three of them (O1 to O3) occurred in different restaurants during 2002 (31), and the fourth (O4) happened in an elderly nursing home in 2006; 24 isolates were collected from non-outbreak associated foods during 1997, 2006 and 2007; four isolates were recovered from the nasal cavities of healthy carriers attending a teaching course (TC) for food handlers in 2008. N, number of isolates when more than one.

[b]The 64 food-borne isolates were assigned to 31 strains.

[c]Production of "classic" staphylococcal enterotoxins (SEA, SEB, SEC and SED) and TSST-1 determined by reverse passive latex agglutination in this or a previous study (31).

[d]Resistance phenotypes and genotypes were determined. The antimicrobial agents tested were ampicillin-penicillin (AMP), oxacillin-methicillin (OXA), gentamicin (GEN), amikacin (AMK), kanamycin (KAN), tobramycin (TOB), tetracycline (TET), erythromycin (ERY), clindamycin (CLI; being CLI^{I}, inducible), chloramphenicol, ciprofloxacin, moxifloxacin, rifampicin, linezolid, vancomycin, tigecycline, mupirocin (MUP), trimethoprim and trimethoprim-sulfamethoxazole.

[e]CC, clonal complex according to MLST and supported by *spa* typing.

nd, not determined, na, not applicable, (-), negative.

TABLE 2. Clonal complexes, sequence types and *spa*-types found in food-related *S. aureus* strains.

CC (N/%)[a]	ST (n)	*spa* type (n)	Origin (n)
CC5 (9/29.0)	ST5 (3), ST6, ST146, ST1619	t002 (3), t616, t701 (1), t1560 (3), t2173	O2-F (2), O3-F/FH (3), F (4)
CC30 (8/25.8)	ST30 (2), ST34 (2)	t012 (3), t021, t136, t166 (2), t840	O1-FH, O2-FH (2), O4-F, F (4)
CC45 (5/16.1)	ST45 (2), ST546	t015 (3), t050, t604	O1-F, F (4)
CC8 (2/6.5)	ST8	t008 (2)	O3-FH, F
CC15 (2/6.5)	ST15 (2)	t120, t3474	O1-FH, TC-FH
CC1 (1/3.2)	ST3	t177	O2-FH
CC22 (1/3.2)	ST217	t790	O2-F
CC25 (1/3.2)	ST26	t7125	O3-FH
CC59 (1/3.2)	ST59	t216	F
CC121 (1/3.2)	ST51	t272	TC-FH

CC, clonal complex; ST, sequence type; N, number of strains; n, number of strains when more than one; O1-O4, food poisoning outbreaks; F, foods; FH, food handlers; TC, teaching course.