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Incidence of *Staphylococcus aureus* and Analysis of Associated Bacterial Communities on Food Industry Surfaces

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Biofilms are a common cause of food contamination with undesirable bacteria, such as pathogenic bacteria. *Staphylococcus aureus* is one of the major bacteria causing food-borne diseases in humans. A study designed to determine the presence of *S. aureus* on food contact surfaces in dairy, meat, and seafood environments and to identify coexisting microbiota has therefore been carried out. A total of 442 samples were collected, and the presence of *S. aureus* was confirmed in 6.1% of samples. Sixty-three *S. aureus* isolates were recovered and typed by random amplification of polymorphic DNA (RAPD). Profiles were clustered into four groups which were related to specific food environments. All isolates harbored some potential virulence factors such as enterotoxin production genes, biofilm formation-associated genes, antibiotic resistance, or lysogeny. PCR-denaturing gradient gel electrophoresis (PCR-DGGE) fingerprints of bacterial communities coexisting with *S. aureus* revealed the presence of bacteria either involved in food spoilage or of concern for food safety in all food environments. Food industry surfaces could thus be a reservoir for *S. aureus* forming complex communities with undesirable bacteria in multispecies biofilms. Uneven microbiological conditions were found in each food sector, which indicates the need to improve hygienic conditions in food processing facilities, particularly the removal of bacterial biofilms, to enhance the safety of food products.

Poor hygiene practices in food processing plants may result in the contamination of food products with pathogens, which means a serious risk for the health of consumers. Moreover, the complete elimination of pathogens from food processing environments is a difficult task, in part because bacteria can attach to food contact surfaces and form biofilms, where they survive even after cleaning and disinfection (3, 46). Biofilms are the most common bacterial lifestyle in nature. After initial attachment of cells to a surface, they start to multiply and secrete a consistent matrix of extracellular polymeric substances (EPS) in which cells are wrapped. Biofilms are a serious problem in many food industry sectors. In dairy environments, the bacteria most commonly associated with food contact surfaces include *Enterobacter*, *Lactobacillus*, *Listeria*, *Micrococcus*, *Streptococcus*, *Bacillus*, *Staphylococcus*, and *Pseudomonas*. They can enter milk processing equipment by direct contact with contaminants in the dairy farm environment and also through water used in milking machines. Cleaning-in-place (CIP) procedures are usually employed in milk processing lines, but they have a limited effectiveness against residual microorganisms, which can regrow and form new biofilms (2). The major pathogens to be controlled in the meat industry include *Staphylococcus aureus*, *Salmonella* spp., *Campylobacter* spp., enterohemorrhagic *Escherichia coli* O157:H7, and *Listeria monocytogenes*. The best strategy for improving the safety of meat products is adequate hygiene and the application of antimicrobial intervention technologies at preharvest, postharvest, processing, storage, distribution, and consumption stages. Pathogenic bacteria such as *Vibrio* spp., *E. coli*, *Clostridium* spp., *L. monocytogenes*, and *S. aureus*, among others, can be also occasionally detected in fish products (19). Some of these bacteria can be present in the natural environment, but many others enter the food chain as a result of poor hygiene conditions during processing and storage.

S. aureus is one of the major bacterial agents causing food-borne diseases in humans. This microorganism can cause food poisoning through the production of enterotoxins (24). Humans are common asymptomatic carriers of enterotoxigenic *S. aureus* in nose, throat, and skin. Thus, food handlers can be an important source of food contamination. The ability to form biofilms allows *S. aureus* to survive in hostile environments such as food industry surfaces (13), and this enhances the recurrence of food contamination.

The widespread use of antibiotics has provoked an exponential increase in the incidence of antibiotic resistance in several bacterial groups in recent years. Thus, multidrug-resistant *S. aureus* strains are rather common in hospital settings and farms but have been also detected in food animals and in food like meat, milk and dairy products, and fishery products (23). The food chain is considered a potential route of transmission of antibiotic-resistant bacteria to humans.

The present work aimed to evaluate the hygienic conditions of food contact surfaces from the dairy, meat, and seafood industries by screening for the presence of *S. aureus*. Virulence determinants such as antibiotic resistance, enterotoxin-encoding genes, and biofilm-forming ability were analyzed in isolates. The lysogeny status was also assessed as some genes encoding virulent factors are located in phages. Additionally, associated microbial communities present in environmental samples in which *S. aureus* was detected were investigated by PCR-denaturing gradient gel electrophoresis (DGGE) for a better identification of microbial food safety risks in these food environments.

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TABLE 1 Primers and bacterial strains used in the PCRs carried out in this work

Gene or reaction	Control *S. aureus* strain	Primer	Primer sequence (5′–3′)	Amplicon size (bp)	Reference
16S rRNA gene		27FYM	AGAGTTTGATYMTGGCTCAG	1499	45
		1492R′	GGTTACCTTGTTACGACTT		
V2-V3 region of the 16S rRNA gene		F357-GC	CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGG GGGTACGGGAGGCAGCAG	161	29
		F357	TACGGGAGGCAGCAG		
		R518	ATTACCGCGGCTGCTGG		
RAPD-PCR		OPL5	ACGCAGGCAC		17
		RAPD5	AACGCGCAAC		
		P1	CCGCAGCCAA		
		ERIC2	AAGTAAGTGACTGGGGTGAGCG		41
		AP7	GTGGATGCGA		41
		S	TCACGATGCA		27
sea[a]	ATCC 13565	SEA3	CCTTTGGAAACGGTTAAAACG	127	1
		SEA4	TCTGAACCTTCCCATCAAAAAC		
seb[a]	ATCC 14458	SEB1	TCGCATCAAACTGACAAACG	477	
		SEB4	GCAGGTACTCTATAAGTGCCTGC		
sec[a]		SEC3	CTCAAGAACTAGACATAAAAGCTAGG	271	
		SEC4	TCAAAATCGGATTAACATTATCC		
sed[a]	ATCC 13565	SED3	CTAGTTTGGTAATATCTCCTTTAAACG	319	
		SED4	TTAATGCTATATCTTATAGGGTAAACATC		
see[a]	ATCC 27664	SEE2	TAACTTACCGTGGACCCTTC	178	
		SEE3	CAGTACCTATAGATAAAGTTAAAACAAGC		
seg[b]	ATCC 19095	SEG1	AAGTAGACATTTTTGGCGTTCC	287	32
		SEG2	AGAACCATCAAACTCGTATAGC		
seh[b]		SEH1	GTCTATATGGAGGTACAACACT	213	
		SEH2	GACCTTTACTTATTTCGCTGTC		
sei[b]		SEI1	GGTGATATTGGTGTAGGTAAC	454	
		SEI2	ATCCATATTCTTTGCCTTTACCAG		
icaA	ATCC 15981	icaA-F	CCTAACTAACGAAAGGTAG	1315	43
		icaA-R	AAGATATAGCGATAAGTGC		
icaD		icaD-F	AAACGTAAGAGAGGTGG	381	
		icaD-R	GGCAATATGATCAAGATAC		
bap	V329	sasp-6 m	CCCTATATCGAAGGTGTAGAATTGCAC	971	6
		sasp-7c	GCTGTTGAAGTTAATACTGTACCTGC		

[a] Primers were grouped in the same multiplex PCR.
[b] Primers were grouped in the same multiplex PCR.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. Bacterial strains used as positive controls for PCRs are listed in Table 1. *S. aureus* Sa9 (11) and *S. aureus* ATCC 15981, on one hand, and *Staphylococcus epidermidis* F12 (7), on the other, were used as positive and negative controls, respectively, for all biochemical tests (DNase activity, mannitol fermentation, and coagulase test) (see below). *S. aureus* ATCC 25923 was used as a control for antibiotic sensitivity. All strains were routinely cultured in tryptic soy broth (TSB) at 37°C or in tryptic soy agar (TSA). Bacterial stocks were stored at −80°C in TSB supplemented with glycerol (20% [vol/vol] final concentration).

Sampling design. A total of 442 samples were collected from different food contact surfaces in dairy, meat, and seafood industries (146, 124, and 172, respectively). Sampling was carried out in 18 visits from February 2010 to February 2011. Samples were taken aseptically from a surface covering approximately 100 cm^2 by using sterile sampling sponges moistened with LPT Neutralizing Broth (Laboratorios Microkit S.L., Madrid, Spain) and then transported at 4°C to the laboratory for immediate processing.

Microbiological analysis. Sampling sponges were mixed with 100 ml of peptone water and beat in a stomacher masticator (IUL Instruments, Barcelona, Spain). Subsequently, 0.1 ml of sample suspension was spread onto Baird-Parker (BP) agar supplemented with egg yolk tellurite emulsion (Biolife, Milan, Italy, and Scharlab, Barcelona, Spain). Plates were incubated at 37°C for 48 h. Isolation and identification of *S. aureus* were carried out by randomly picking several presumptive *S. aureus* colonies of each sample. Colonies were isolated by repeated subcultures, and then isolates were tested for DNase activity and mannitol fermentation (30). A coagulase test was also performed according to the supplier's instructions (Bactident Coagulase; Merck, Madrid, Spain). All putative *S. aureus* isolates were confirmed by PCR amplification and sequencing (see below) of the 16S rRNA gene (Table 1).

Total viable counts were obtained by plating appropriate 10-fold dilutions of sample suspensions on plate count agar (PCA; Scharlau, Barcelona, Spain), followed y incubation at 30°C for 48 to 72 h.

The presence of resident prophages in *S. aureus* strains was checked after mitomycin C induction as previously described (16). Antimicrobial susceptibility was determined by using the disc diffusion method and the concentrations specified by the CLSI (5). The following 10 antibiotics were used: trimethoprim-sulfamethoxazole (1.25/23.75 μg), rifampin (5 μg), oxacillin (1 μg), clindamycin (2 μg), erythromycin (15 μg), enrofloxacin (5 μg), vancomycin (5 μg), ciprofloxacin (5 μg), streptomycin (10 μg), and quinupristin-dalfopristin (15 μg) (Oxoid, Hampshire, United Kingdom). Slime production by *S. aureus* strains was determined by the Congo red agar (CRA) method, according to Freeman et al. (10).

DNA manipulations. DNA was extracted from putative *S. aureus* isolates by using a commercial GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich, Madrid, Spain) according to the manufacturer's recommendations. Total genomic DNA from positive samples for *S. aureus* was isolated with a QIAamp DNA stool minikit (Qiagen, GmbH, Hilden, Germany) following the optimized protocol (28) with an additional step of bacterial enzymatic lysis. This basically consisted of a previous treatment of the bacterial cell pellets with 30 mg/ml lysozyme (Sigma), 20 units of mutanolysin (Sigma), and 50 μg/ml of lysostaphin (Sigma) at 37°C for 60 min. PCRs were performed in a thermocycler (Bio-Rad, Hercules, CA) using a PureTaq Ready-To-Go PCR Beads kit (GE Healthcare, Munich, Germany) and the primers specified in Table 1. All PCR products were resolved by electrophoresis in 2% agarose gels, stained with ethidium bromide, and visualized under UV light with a Gel Doc 2000 documentation system equipped with Quantity One software (Bio-Rad Laboratories, Hercules, CA). A DNA ladder of 500 bp was included in all gels.

Automated sequencing of PCR products was performed using an ABI Prism gene sequencer (Applied Biosystems, Foster City, CA) at Secugen S.L. (Madrid, Spain). Nucleotide sequences were identified by comparison against sequences available in the GenBank database using the BLAST algorithm.

Genomic fingerprinting of *S. aureus* isolates. Random amplification of polymorphic DNA (RAPD) profiles was obtained for each isolate with primers OPL5, RAPD5, P1, ERIC2, AP7, and S (Table 1) by using a previously described method (21). RAPD-PCR band patterns were scanned and analyzed using the Pearson product moment correlation coefficient. Clustering analysis was carried out by the unweighted pair group method with arithmetic averages (UPGMA) (39).

DGGE analysis. The V2-V3 region of the 16S rRNA gene was amplified with the eubacterium-specific primers F357-GC and R518 (Table 1) using total genomic DNA from *S. aureus*-positive environmental samples as the template.

Reaction mixtures contained 0.25 μM each primer, 200 μM each deoxynucleoside triphosphate (Amersham Bioscience, Uppsala, Sweden), and 2.5 U of *Taq* polymerase (Eppendorf, Hamburg, Germany). PCR products were separated in 8% polyacrylamide gels using a 40 to 50% urea-formamide denaturing gradient. Electrophoresis was performed in a Dcode System apparatus (Bio-Rad) at 85 V in 0.5× Tris-acetate-EDTA (TAE) buffer at a constant temperature of 60°C during 16 h. The gels were visualized by ethidium bromide staining as described above.

The bands observed by DGGE were initially identified by comparison with those of a DGGE marker constructed using chromosomal DNA of pure cultures of the species *S. aureus*, *Staphylococcus sciuri*, *Enterococcus faecium*, *Enterococcus faecalis*, and *Macrococcus caseolyticus*, previously isolated from some of the samples of the study. DNA was obtained with a GenElute Bacterial Genomic DNA kit and used for PCR amplification using the same primers described above. The amplicons were purified using GenElute PCR Clean-Up columns (Sigma-Aldrich), and equal amounts were mixed to obtain the DGGE marker.

The dominant bands that did not match any of the bands present in the DGGE marker were excised from the acrylamide gels and used as templates for sequencing analysis. Excised DNA bands were resuspended in 100 μl of sterile ultrapure water and kept overnight at 4°C. Subsequently, eluted DNA was used to perform a secondary PCR amplification using primers F357 (without the GC clamp) and R518 (Table 1). The PCR products were purified using GenElute PCR Clean-Up columns and sequenced as above. Sequences were compared to those present in the GenBank database using the BLAST algorithm, and the identity of the isolates was determined on the basis of the highest scores. Only sequences with a percentage of similarity of 97% or higher were assigned to species level.

RESULTS

Identification of *S. aureus* from food contact surfaces and analysis of associated bacterial communities. A total of 442 samples were collected from food contact surfaces of three dairy, three meat, and two seafood industry sites (Table 2). Sampling was performed after application of standard cleaning and disinfection procedures. The presence of *S. aureus* in these samples was determined by plating on BP agar, followed by confirmation via biochemical tests. PCR amplification and sequencing of the 16S rRNA gene of all putative *S. aureus* isolates were also carried out for further confirmation. *S. aureus* was thus found to be present in 27 samples (4, 6, and 17 from dairy, meat, and seafood industry sites, respectively), which means a global incidence of 6.1%. Incidence rates of 2.73%, 3.22%, and 9.88% were found in dairy, meat, and seafood contact surfaces, respectively. A total of 63 isolates were recovered from these samples. Of these, 5 (named IPLA1 to IPLA5, where IPLA indicates Instituto de Productos Lácteos de Asturias) came from the dairy sector and 13 (named IPLA6 to IPLA18) were from meat contact surfaces, whereas 45 isolates (named IIM201 to IIM250, where IIM indicates Instituto de Investigaciones Marinas) were obtained from the seafood industry (Table 2).

Total bacterial counts on PCA were also determined to evaluate the bacterial load in the samples in which *S. aureus* was detected. Count levels ranged from 10^2 to 10^4 CFU/cm^2 in samples from the dairy industry, from 10^5 to 10^7 CFU/cm^2 on meat contact surfaces, and from 10^1 to 10^3 CFU/cm^2 in the seafood industry surfaces (Table 2). In general, total counts were highly correlated with the number of *S. aureus* bacteria, particularly in the dairy and fish industries. Thus, the highest contamination levels found in each food sector were correlated with the highest counts on BP.

The microbiota coexisting with *S. aureus* was identified by PCR-DGGE fingerprinting combined with sequence analysis of the dominant amplicons in 21 of 27 samples (4 from the dairy industry, 4 from the meat industry and 13 from the seafood industry). As shown in Fig. 1, a number of prominent bands, ranging from two to six, were observed in DGGE profiles. As expected, *S. aureus* (band 2) was present in all samples. *Leuconostoc citreum* (band 1) was the most common partner, being detected in 15 (71%) samples, which indicates a wide distribution of this species in these three food sectors and particularly in the dairy industry. The microbial diversity found in dairy samples was comprised also of *Enterococcus faecium* (band 4), *Pseudomonas* sp. (band 5), *Acinetobacter* (band 6), *Lactobacillus plantarum* (band 9), and *Macrococcus caseolyticus* (band 12). Regarding meat industry, it should be noted that *Acinetobacter* and *L. plantarum* were present in all samples, whereas *Morganella psychrotolerans* (band 7), *Serratia* sp. (band 14), *Streptococcus* sp. (band 15), and *Bacillus cereus* (band 16) were also detected in some cases. Despite the high number of samples collected in the seafood industry, the microbial diversity in DGGE profiles was lower than that observed in dairy and meat sectors although bacteria such as *Streptococcus uberis* (band 8), *Staphylococcus epidermidis* (band 3), *Pseudoalteromonas piscicida* (band 10), *Campylobacter* sp. (band 11), and *Psychrobacter* sp. (band 13) were found only in samples collected in this food environment.

Genetic diversity of isolates. To determine the genetic diversity of *S. aureus* isolates and explore its putative relationship with a specific food environment, all 63 isolates were analyzed by RAPD-PCR genotyping using six different primers (Table 1). On the basis of RAPD profiles, isolates were grouped into two main clusters (I and II) (Fig. 2). Each of these clusters was further subdivided into two subclusters (Ia, Ib, IIc, and IId). Each subcluster

TABLE 2 Sample description, total viable counts, and *S. aureus* counts[a]

Food industry (site no.)	Origin	Sample name	No. of CFU/cm^2 (unless otherwise noted) by plating method[b]		*S. aureus* strain(s)
			PCA	BP	
Dairy (1)	Receiving tank	A	7.8E3 ± 2.3E2	1.1E2 ± 4.2E1	IPLA1
		B	1.4E4 ± 2.4E1	1.4E2 ± 4.8E1	IPLA2
		C	9.3E2 ± 4.2E1	<10	IPLA3, IPLA4
		D	7.0E2 ± 6.9E1	<10	IPLA5
Meat (1)	Meat mincer	556	ND		IPLA6
		434	ND		IPLA7
	Stuffer	E	1.37E6 ± 1.4E5	1.7E3 ± 1.9E2	IPLA8, IPLA9, IPLA10, IPLA11, IPLA12
Meat (2)	Plastic curtain 1	F	1.1E7 ± 1.9E6	6.9E4 ± 3.3E2	IPLA13, IPLA14
Meat (3)	Plastic curtain 2	G	5.7E4 ± 9.8E3	1.9E3 ± 1.3E2	IPLA15, IPLA16
	Cutting knife	H	3.2E5 ± 4.2E4	1.2E2 ± 4.6E1	IPLA17
					IPLA18
Seafood (1)	Forklift truck 1	Zcom.3b	1.4E5 ± 5.0E4	4.7E4 ± 1.7E4	IIM201, IIM202, IIM203, IIM204
	Sanitary access	I	1.2E2 ± 2.1E2	1.2E2 ± 1.2E2	IIM207, IIM208
	Forklift truck 2	Zcom.3	6.4E4 ± 3.0E4	7.1E3 ± 3.7E3	IIM209, IIM210, IIM211, IIM212,
	Floor 1	J	<10	<10	IIM214
	Floor 2	K	5.0E1 ± 2.3E1	<10	IIM215
	Hopper	L	9.3E1 ± 3.2E1	<10	IIM216
	Conveyor belt	1A.5a	2.3E1 ± 1.5E1	<10	IIM217
	Waste pipe	M	3.1E3 ± 2.6E2	3.1E3 ± 1.2E2	IIM219
	Residues*	N	1.9E2 ± 7.4E1*	<10*	IIM221
Seafood (2)	Unknown	2	ND		IIM222, IIM223, IIM224, IIM225, IIM226, IIM227
Seafood (3)	Conveyor belt 1	O	1.9E3 ± 8.8E2	1.6E2 ± 1.8E2	IIM228, IIM229, IIM230, IIM231, IIM232, IIM233, IIM250
	Forklift truck	P	4.8E3 ± 3.1E2	5.2E2 ± 2.9E2	IIM234, IIM235, IIM236
		Q	5.6E2 ± 7.9E1	1.8E2 ± 1.2E1	IIM237
		R	6.1E2 ± 1.2E2	3.0E2 ± 9.5E1	IIM238, IIM239, IIM240
	Brushing equipment	S	4.4E1 ± 1.8E1	3.2E1 ± 1.1E1	IIM241, IIM242
	Floor	T	8.5E2 ± 3.1E2	4.5E1 ± 2.7E1	IIM243, IIM244
	Conveyor belt 2	U	1.1E2 ± 9.6E1	3.8E1 ± 2.2E1	IIM245, IIM246, IIM247, IIM248 IIM249,

[a] Only data for *Staphylococcus aureus*-containing samples are included. *, results expressed as CFU/ml.
[b] ND, not determined.

nearly matched the origin of the isolates. Cluster I comprised most isolates from the seafood industry (with a similarity percentage of 67%). Cluster II, with a 62% of similarity with cluster I, included all other isolates from the seafood sector as well as all those from the dairy and meat industries. In spite of their different origins, all dairy and meat isolates were grouped into subcluster IId and showed over 71% similarity. In contrast, no isolate from the seafood industry was included in this subcluster.

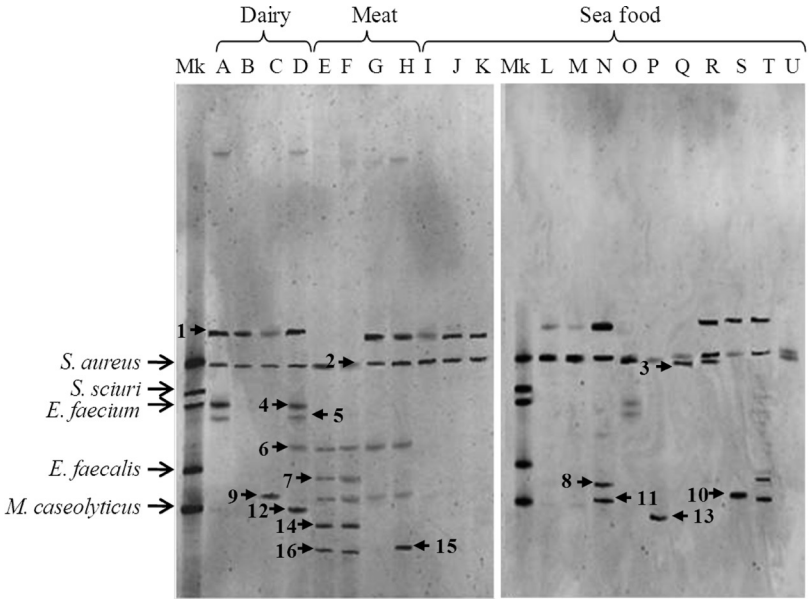
On the basis of these results, a threshold value of 95% was set so that all patterns with a similarity higher than this value were considered identical. In this regard, seafood isolates (45) resulted in 18 RAPD-PCR patterns, meat isolates (13) shared 9 patterns, and dairy isolates (5) had 3 different patterns. All isolates sharing the same pattern were considered to be clones, i.e., genetically closely related strains.

Presence of potential virulence factors in *S. aureus* strains. One isolate representative of each of the 30 different RAPD-PCR profiles was then selected and tested for the presence of enterotoxin-encoding genes, potential biofilm production, and lysogeny. Staphylococcal enterotoxin (SE) genes were detected by two multiplex PCRs for *sea-see* genes, on one hand, and *seg-sei* genes, on the other. Overall, 27 strains (90%) were positive for the *sea*, *seg*, *seh*, or *sei* gene (Table 3). The presence of *sea* (63%), *seg* (40%), and *sei* (77%) genes was widespread in the isolates from all food sectors. Particularly, isolates grouped in subclusters Ia and IIc (all from the seafood industry) showed the highest frequency of *sei* genes (data not shown). In contrast, no isolate of subcluster Ia harbored the *seh* gene though this gene was restricted to meat and seafood environments (17%). A total of 20 (66%) isolates harbored between two and four SE genes and were, therefore, potential producers of more than one enterotoxin. The genes *seb*, *sec*, *sed*, and *see* were not detected in any of the isolates. Some non-enterotoxin-producing strains (IPLA7, IPLA16, and IPLA18) were isolated from the meat environment.

The ability of these isolates to form biofilms was assessed by visual inspection of slime production on CRA plates. All isolates formed black colonies, which are considered to be representative of strong biofilm formers. PCR amplification of some genes involved in biofilm formation (*icaA*, *icaD*, and *bap*) was also performed. All isolates were positive for the *icaA* and *icaD* genes, whereas the *bap* gene was not detected in any isolate (Table 3).

Lysogeny was also tested in the 30 strains representative of each different RAPD-PCR profile. The presence of prophages was detected in 18 strains (60%). None of the other strains (two strains from the dairy industry, six from the meat environment, and four from the seafood industry) released phages able to infect any of the strains tested in the study. Although lysogeny was widespread in isolates from all food environments, it is remarkable that the highest percentage was observed in those from the seafood sector (Table 3).

Antimicrobial susceptibility. The same 30 *S. aureus* isolates were also assayed for susceptibility to 10 antibiotics commonly



Band	Organism	Incidence in the samples (%)			
		Dairy	Meat	Sea food	Total
1	*Lc. citreum*	100	50	69	71
2	*S. aureus*	100	100	100	100
3	*S. epidermidis*	-	-	23	14
4	*E. faecium*	50	-	-	10
5	*Pseudomonas sp.*	50	-	-	10
6	*Acinetobacter sp.*	25	100	-	24
7	*M. psychrotolerans*	-	50	-	10
8	*Str. uberis*	-	-	15	10
9	*Lb. plantarum*	25	100	-	24
10	*P. piscicida*	-	-	8	5
11	*Campylobacter sp.*	-	-	15	10
12	*M. caseolyticus*	25	-	-	5
13	*Psychrobacter sp.*	-	-	8	5
14	*Serratia sp.*	-	50	-	10
15	*Streptococcus sp.*	-	25	-	5
16	*B. cereus*	-	50	-	10

FIG 1 DGGE profiles of microbial populations coexisting with *Staphylococcus aureus* on food industry surfaces using amplicons of the V3 variable region of the bacterial 16S rRNA gene. Bands are identified in the table at right. Mk: DGGE identification marker. *Lc*, *Leuconostoc*; *Lb*, *Lactobacillus*; *Str*, *Streptococcus*.

used in medical and veterinary practice. A total of 21 strains (70%) were susceptible to all the antimicrobials tested. However, intermediate resistance was observed in strains isolated from the three food sectors (Table 3). Intermediate resistance was observed for streptomycin (eight isolates) and oxacillin, enrofloxacin, and ciprofloxacin (one isolate each) (Table 3).

DISCUSSION

The pathogenicity of food-borne *S. aureus* is associated with the ability of some strains to produce enterotoxins (24). The resistance to antimicrobials, particularly to β-lactam antibiotics, has also raised high concern as an emerging problem in the food environment (23). Adhesion and biofilm formation are also important virulence factors in *S. aureus* since they promote the colonization of food environments (3). In fact, biofilms formed on food processing surfaces enhance the tolerance to disinfectants, thereby increasing the risk of cross-contamination of food. Of note, biofilm formation by *S. aureus* can be enhanced by some processing conditions used in the food industry, such as a suboptimal growth temperature or the combined presence of salt and glucose (37).

The present study provides information about the incidence of *S. aureus* in the dairy, meat, and seafood industries. *S. aureus* was recovered from 27 out of 442 samples, giving an overall incidence of 6.1%. *S. aureus* had already been detected in a higher proportion (11.7%) from food contact surfaces of meat processing facilities (14). Another previous study (33) reported a still higher incidence (15.50%) in equipment of a pork processing setting; however, sampling was not carried out after disinfection of surfaces but, rather, after 5 h of work.

The contamination level of food contact surfaces with *S. aureus* suggests that the handling of livestock as well as cleaning and disinfection of food industry facilities must be improved. In this respect, it must be pointed out that all isolates showed the potential ability to form biofilms, which may allow them to adhere to different food contact surfaces. These biofilms could be a potential source of food contamination, likely due to an unsatisfactory application of disinfectants against biofilm-associated cells (42).

All *S. aureus* strains isolated in this study seemed to be reliant on polysaccharide intercellular adhesin (PIA) production for biofilm formation as they harbored *icaA* and *icaD* genes, whereas the *bap* gene associated with *ica*-independent biofilm formation was not detected (31). However, bacterial biofilms are generally composed of several different species, among which pathogenic or spoilage bacteria can be included. *S. aureus* was found to be forming mixed biofilms with other bacteria in all food environments. The microorganisms coexisting with *S. aureus* in the food industry surfaces were identified by PCR-DGGE in order to detect any other food safety risk. They were mainly of environmental origin, most likely carried by incoming raw food ingredients. Thus, consortia were specific to each food environment, with the exception of *L. citreum*, which was present in all food sectors. Some bacterial amplicons belonged to genera commonly found in soil and water (*Pseudomonas* sp., *Acinetobacter* sp., *Psychrobacter* sp., and *Serratia* sp.) including endospore-forming bacteria such as *B. cereus*.

It is also worth noting the presence of DNA from common pathogenic bacteria such as *B. cereus*, *S. uberis*, or members of the genus *Campylobacter* in some samples. The presence of these bacteria on food contact surfaces must be considered a cause of concern as they also can contaminate food products processed in these facilities. *E. faecium* is commonly found in a large number of traditional cheeses, but the presence of several putative virulence factors which promote proliferation in the human body and provoke infection has increased the concern about these bacteria (8). Two samples from the seafood industry included *Campylobacter* sp., a genus frequently isolated from poultry products and occasionally from mussels and other shellfish. Seagulls feeding close to seafood waters are presumably the source of this contamination (40). Finally, *Bacillus cereus* is recognized as a serious problem and an important contaminant in food industry settings, such as red

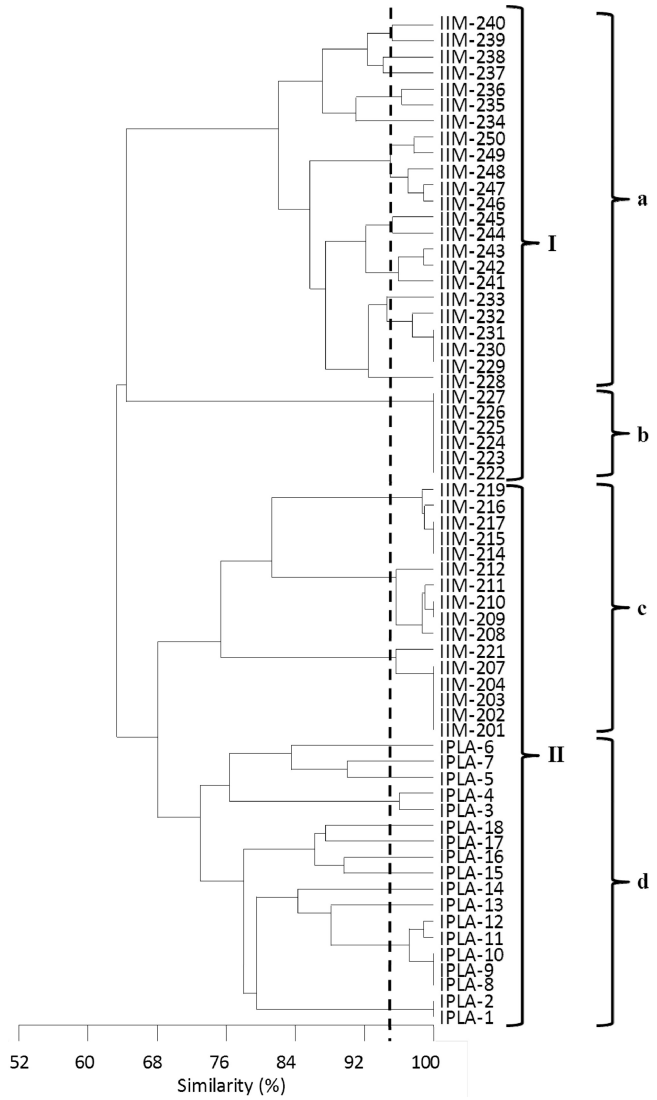


FIG 2 Dendrogram obtained by cluster analysis of RAPD-PCR profiles of *Staphylococcus aureus* isolates from food industry surfaces, using the UPGMA method and the Pearson product moment correlation coefficient. The dashed line represents the threshold similarity value of 95% set for discriminating strains.

meat processing plants, where it has been routinely isolated from different surfaces of food production plants (20).

Bacteria that can be involved in food spoilage were also detected. This was the case of *L. citreum*, routinely found in cheeses, which was isolated from all three food industry sectors. This species has been identified as a source of spoilage in cooked meat products (18) and vacuum-packaged fish products (25). The widespread presence of *L. citreum* on food contact surfaces can be explained by its ability to produce a variety of extracellular glucans which can be involved in biofilm formation (22). *L. plantarum*, which is usually found in several fermented foods (dairy, meat, and vegetable products), was also detected in samples from dairy and meat environments. This microorganism can also cause spoilage of meat products, but, most important, it can form mixed biofilms with pathogenic bacteria such as *L. monocytogenes* (42). Another spoilage bacterium, *M. psychrotolerans*, was detected on meat contact surfaces. This bacterium can produce toxic concentrations of histamine even when food products are stored chilled (9).

The study of genetic relationships among the isolates from each food industry could provide interesting data about contamination sources. RAPD-PCR analysis showed a clonal distribution of isolates, which is likely related to the contamination source. The remarkable genetic relationship between dairy and meat strains (subcluster IId) allowed us to speculate about an animal origin for these strains. *S. aureus* is a major component of the microbiota of animal skin, from where it can spread to food products or processing facilities in both the dairy and meat industries. In contrast, the genetic difference observed among isolates from the seafood industry might be explained by contamination that has taken place during handling and processing, with human carriers as the major original source (38). The distribution of *S. aureus* in the food environment may cause food contamination, which implies some risk of staphylococcal food poisoning in dairy, meat, and fish products, all of which support the growth of *S. aureus*.

Clear genetic differences were observed among isolates from the seafood industry and those from dairy and meat environments. These differences were found to be correlated with the presence of some virulence factors, with the highest incidence of *sei* genes and lysogeny showed by isolates from the seafood industry. Clear genetic differences were also observed among isolates from the seafood industry as a function of sample origin, with isolates from each facility being grouped in different subclusters (Ia, Ib, and IIc). Antibiotic resistance was much more frequent in strains from the dairy industry than in those from the meat and seafood environments. It is likely that they had received more exposure to some antibiotics (used in veterinary practices) than isolates from the seafood industry.

Most strains (90%) were found to be potentially enterotoxigenic and thus pathogenic as they carried at least one SE gene

TABLE 3 Presence of virulence factors in *S. aureus* strains from different food environments

Industry	No. of strains	Frequency of the indicated characteristic (% of strains)															
		Enterotoxin gene								Intermediate resistance to antibiotics[a]					Biofilm formation-associated gene		
		sea	*seb*	*sec*	*sed*	*see*	*seg*	*seh*	*sei*	OX	ENR	CIP	STR	Lysogeny	*icaA*	*icaD*	*bap*
Dairy	3	100					33		67	33			67	60	100	100	
Meat	9	22					44	33	33		11	11	33	54	100	100	
Seafood	18	78					39	6	100				17	91	100	100	
Total	30	63					40	13	77	3	3	3	27	81	100	100	

[a] OX, oxacillin (1 μg); ENR, enrofloxacin (5 μg); CIP, ciprofloxacin (5 μg); STR, streptomycin (10 μg).

which could be a source of staphylococcal food poisoning. At present, nine different serological types of SEs (SEA to SEE and SEG to SEJ) have been proven to have emetic activity (35). Classical staphylococcal enterotoxins (SEA to SEE) have been reported to cause 95% of staphylococcal food poisoning. Among them, SEA is the most common in staphylococcus-related food poisoning (35). In this work, strains from the dairy and fish industry showed the highest incidence of the *sea* gene. Therefore, the presence of the *sea* gene in numerous isolates implies a high risk for consumers. Of note, most strains were multi-SE carriers. This is the case for nine strains (one from the dairy industry and eight from the seafood environment) that harbored three SE genes and for one strain (IPLA17, from the meat industry) that carried four SE genes. A high incidence of *seg-sei* genes was also found. It had been previously reported that the *sei* gene was common in isolates from subclinical bovine mastitis (15) and retail meats (36). In this study, this gene was also present in all strains from the seafood industry. However, SEG and SEI have been considered to play a minor role in food poisoning. In contrast, a previous report showed that a significant proportion of isolates from staphylococcal food poisoning incidents in South Korea had *seg-sei* genes, either alone or with other SE genes (4).

The lysogeny status demonstrated by 60% of the strains represents a possible additional health risk factor. Bacteriophages play an important role in the pathogenicity of *S. aureus* either by carrying accessory virulence factors, such as Panton-Valentine leukocidin (PVL) (encoded by the *luk*-PV operon), staphylokinase (encoded by *sak*), enterotoxin A (encoded by *sea*), and exfoliative toxin A (encoded by *eta*), or by interrupting chromosomal virulence genes, such as those for β-hemolysin (*hlb*) and lipase (*geh*). In addition, phages facilitate the adaptation of the pathogen to host conditions. Our data support previous studies indicating a widespread distribution of prophages in *S. aureus* strains from different origins (12).

S. aureus has developed multidrug resistance worldwide. This is recognized as an environmental hazard to the food supply and to human health, but there are wide variations in incidence as a function of different factors. In this study, all strains were found to be susceptible to a number of antimicrobials used in food production animals or human medicine, such as trimethoprim-sulfamethoxazole, erythromycin, clindamycin, rifampin, and vancomycin.

The presence of methicillin-resistant *S. aureus* (MRSA) is of particular concern. MRSA strains have been found in food-producing animals (23) and different foods (34). In this study, no strains showed resistance to ß-lactams, but one strain isolated from the dairy environment (IPLA3) showed intermediate resistance to the oxacillin, a result that was confirmed by the amplification of the *mecA* gene (data not shown). Intermediate resistance to the aminoglycoside streptomycin was also observed in eight strains from all three food environments. Additionally, one isolate from the meat processing industry showed intermediate resistance to the fluoroquinolones ciprofloxacin and enrofloxacin, which are commonly used against infections causing heavy losses in cattle, swine, poultry, and fish.

These results agree with previous reports in which the incidence of antibiotic resistance among *S. aureus* strains isolated from food was not remarkably high, excluding penicillin (26). In contrast, a number of studies found that *S. aureus* from different foods showed relatively high incidence rates of resistance to different antibiotics (44). Interestingly, a study carried out in a nearby geographical region (34) reported that 38% of isolates were resistant to oxacillin, but only 0.68% showed the presence of the *mecA* gene.

In conclusion, this study has shown that hygiene practices used in the dairy, meat, and seafood industries did not allow pathogenic and other undesirable bacteria to be completely removed. In fact, any of the food contact surfaces support growth of not only of *S. aureus* but also other microorganisms, meaning that any of them are suitable for biofilm development. Of note, enterotoxigenic *S. aureus* strains were found to be present in each of these food sectors. The incidence was relatively low in the dairy and meat industries, but it would have been highly advisable that they were not present to prevent food contamination from occurring. When present, food can be contaminated, and there is some risk of staphylococcal food poisoning. In the case of the seafood industry, the incidence of enterotoxigenic *S. aureus* strains was much higher, and the risk of food poisoning was therefore much higher too. Coagulase-positive staphylococci (CAS; mainly *S. aureus*) are used as a process hygiene criterion in some dairy products and seafood. In addition, food industry surfaces seemed to be a reservoir for other food pathogenic bacteria as well as for some food spoilage microorganisms coexisting with *S. aureus*. In turn, the presence of *S. aureus* in mixed-species biofilms could also enhance the colonization and persistence of this bacterium in the food environment. These results therefore point toward the need to improve hygiene conditions during the production of food.

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