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Research Article

RESEARCH ARTICLE

Diversity of species and antimicrobial resistance determinants of staphylococci in superficial waters in Spain

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One sentence summary: A high diversity of genetic lineages of *Staphylococcus aureus* and of multidrug resistant coagulase-negative staphylococci has been detected in superficial waters. Aquatic environments are a source of staphylococci of ecological and clinical relevance.

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ABSTRACT

The objectives were to determine the presence and diversity of staphylococcal species in surface waters in La Rioja region (Spain), and to characterize recovered isolates. Staphylococci were detected in 42 of 47 evaluable samples, and 72 isolates were obtained, of which 13 were coagulase-positive (CoPS) and 59 were coagulase-negative (CoNS). Twelve CoPS were identified as *S. aureus* and typed as follows (number of strains): t002/t502/ST5 (four), t10668/ST425 (one), t10712//ST1643 (one), t843/ST130 (one), t10855/ST2461 (one), t3369/ST2657 (one), t1166/ST133 (one), t8083/ST2049 (one) and t045/ST2460 (one); and one as *S. pseudintermedius* ST147. Virulence genes *tst*, *cna* and *lukS/F-I* were detected, and one strain showed the immune evasion cluster type F. Regarding CoNS, 12 different species were recovered (number of strains): *S. epidermidis* (11), *S. vitulinus* (10), *S. sciuri* (nine), *S. fleurettii* (seven), *S. lentus* (six), *S. simulans* (five), *S. xylosus* (four), *S. chromogenes* (two), *S. hominis* (two), and *S. equorum*, *S. succinus* and *S. warneri* (one each). Fourteen CoNS isolates presented a multidrug resistance phenotype, with the following resistance genes: *blaZ*, *mecA*, *fusB*, *fusC*, *erm*(C), *mph*(C), *erm*(A), *msr*(A)/(B), *mph*(C), *ant*(4′)-*Ia*, *tet*(K), *tet*(L), cat_{pc194} and *str*. The high diversity of staphylococcal species, as well as multiple resistance and virulence genes, highlights the importance of surface waters as a temporary reservoir and source of transmission.

Keywords: *Staphylococcus*; *S. aureus*; *S. pseudintermedius*; coagulase-negative; surface-water; antibiotic resistance; virulence

INTRODUCTION

The emergence and spread of antimicrobial-resistant bacteria is an important public health problem. Moreover, antimicrobial resistance is a widespread and global phenomenon that affects not only clinical isolates causing infections, but also non-pathogenic bacteria in very different ecosystems (Allen *et al.* 2010). The natural environment has been described as a vehicle by which transmission of antimicrobial-resistant bacteria could occur (Huijbers *et al.* 2015), and resistance genes can be transferred to pathogenic or potentially pathogenic bacteria with important implications (Taylor, Verner-Jeffreys and Baker-Austin 2011). In fact, antimicrobial-resistant bacteria and resistance genes are regarded as environmental pollutants (Martinez 2009).

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Staphylococcus spp. are reported as normal microbiota of mammals and birds, although they may behave as opportunistic pathogens that can cause minor and severe infections. Species of this ubiquitous genus can be classified as coagulase-positive *Staphylococcus* (CoPS) or coagulase-negative *Staphylococcus* (CoNS). Among CoPS, the two most relevant species are *S. aureus*, an important pathogen of humans and animals, and *S. pseudintermedius*, a relevant pathogen in veterinary medicine, especially in dogs and cats, that also has been detected in different animal species and in humans (Paul *et al.* 2011; Kjellman *et al.* 2015). Some CoNS species, like *S. epidermidis* or *S. haemolyticus*, are also gaining interest due to their increased detection as responsible agents of infections (Becker, Heilmann and Peters 2014). In addition, methicillin-resistant *Staphylococcus* (MRS), associated to the expression of the *mecA* gene (and less frequently to the *mecC* gene), represent an important clinical problem.

Different studies have correlated bacterial density and anthropogenic activities. *Staphylococcus* have been detected in some studies of wastewater and recreational water showing the contribution of effluent wastewaters to surface waters (Alexander *et al.* 2015; Gómez *et al.* 2016), and revealing *S. aureus* as a possible source of skin infections in recreational waters (Charoenca and Fujioka 1995). Other studies have correlated the number of bathers in recreational waters and the presence of *S. aureus* in these waters (Plano *et al.* 2013), which may be another source of staphylococci in the environment. However, little is known about the *Staphylococcus* spp. in other environmental compartments. Regarding surface fresh waters and CoPS, *S. aureus* has been detected on different occasions (Heß and Gallert 2014a; Skariyachan *et al.* 2015), including in one case methicillin-resistant *S. aureus* (Porrero *et al.* 2014). Regarding CoNS, 15 species have been described in river water (Heß and Gallert 2014a,b; Sood *et al.* 2014; Wendlandt *et al.* 2015), including the new species *S. argensis* in the Argen river (Heß and Gallert 2015). Moreover, three species of CoPS and 11 of CoNS were detected in a polluted stream (Basso *et al.* 2014). Nevertheless, few studies carried out the molecular characterization of isolates in relation to molecular typing (Porrero *et al.* 2014) or antimicrobial resistance and virulence genes (Basso *et al.* 2014; Porrero *et al.* 2014; Heß and Gallert 2014a; Seyedmonir, Yilmaz and Icgen 2015; Wendlandt *et al.* 2015). For this reason, the aim of this study was to determine the presence and diversity of staphylococcal species in surface waters of La Rioja (northern Spain), as well as to perform the molecular and antimicrobial resistance characterization of the obtained isolates.

MATERIAL AND METHODS

Sample collection

Sixty-two surface water samples were collected from the major hydrographic basins of La Rioja region (northern Spain), during the period February–April 2012 (sampling points are shown in Supplementary Fig. S1). Samples included were as follows: 38 lotic waters (26 rivers, 10 ditches and two streams); and 24 lentic waters (nine water reservoirs, five fountains, eight ponds and two troughs). Specific data from each collected sample are included in Supplementary Table S1.

Sterile water sampling bottles (500 mL) dosed with sodium thiosulfate (10 mg) (Gosselin) were used to collect the water. The samples were directly transported under refrigeration conditions and conserved at 4°C until processing, no longer than 24 h.

Staphylococci isolation and identification

A total of 250 mL of each water sample was filtered through a cellulose nitrate 0.45 μm pore membrane filter (Biotech). The filter was subsequently placed in a tube with 5 mL of brain–heart-infusion broth (Difco) supplemented with 6.5% sodium chloride, and incubated at 37°C for 24 h. After that, aliquots of 100 μL were seeded on mannitol–salt–agar plates (Becton, Dickinson and Company) and on oxacillin resistance screening agar base plates (Oxoid) supplemented with oxacillin (2 mg L^{-1}), to recover *Staphylococcus* and MRS, respectively. Plates were incubated at 37°C for 24–48 h.

Up to 10 suspected staphylococcal colonies were recovered per sample, and initially identified by conventional microbiological methods (colony morphology, Gram staining and DNase test). Nevertheless, only isolates showing different antimicrobial resistance phenotypes of each species and of each sample were included in this study.

Identification of *S. aureus* and *S. (pseud)intermedius* was carried out by amplification of the species-specific *nuc* gene as previously described (Lautz *et al.* 2006). In order to discriminate between *S. intermedius* and *S. pseudintermedius*, a PCR restriction fragment length polymorphism of the *pta* gene with the MboI endonuclease (Biolabs) was conducted (Bannoehr *et al.* 2009). Amplification and sequencing of the *sodA* gene (Poyart *et al.* 2001) was performed for CoNS identification.

Antimicrobial susceptibility testing and detection of antimicrobial resistance genes

Susceptibility to 15 antimicrobial agents was studied by the agar disk diffusion method. The antimicrobials tested were the following: penicillin, cefoxitin, gentamicin, tobramycin, kanamycin, streptomycin, ciprofloxacin, clindamycin, erythromycin, tetracycline, trimethoprim/sulfamethoxazole, chloramphenicol, mupirocin, fusidic acid and linezolid. The method and breakpoints recommended by the Clinical and Laboratory Standards Institute (http://clsi.org, 2015) were used for all antimicrobials except for streptomycin, fusidic acid and mupirocin, where the Société Française de Microbiologie guidelines were followed (www.sfm.asso.fr, 2013). The double disk diffusion test was performed to detect inducible clindamycin resistance.

Detection of 34 antimicrobial resistance genes (*mecA*, *mecC*, *blaZ*, *tet*(K), *tet*(M), *tet*(L), *tet*(O), *aac*(6′)-*aph*(2″), *fusB*, *fusC*, *erm*(A), *erm*(B), *erm*(C), *erm*(F), *erm*(T), *msr*(A), *msr*(B), *mph*(C), *ant*(4′)-Ia, *lnu*(A), *lnu*(B), *cfr*, *ant*(6)-Ia, *ant*(3″)(9), *str*, *dfr*(A), *dfr*(D), *dfr*(K), *dfr*(G), *mupA*, *mupB*, cat_{pc194}, cat_{pc221} and cat_{pc223}) was performed by PCR (Cuny *et al.* 2011; Gómez-Sanz *et al.* 2011; Desroches *et al.* 2013; Gómez *et al.* 2016). The *mecC* gene was tested in all CoPS isolates (regardless of the cefoxitin-resistant phenotype) and the *mecA* gene in all staphylococci. The amplicon was sequenced in all cases of cefoxitin-susceptible phenotype/*mecA* genotype discrepancy.

Virulence genotype and detection of immune evasion cluster genes

The presence of the genes encoding exfoliative toxins (*eta*, *etb* and *etd*), the leukocidin *lukS/F-PV* and *lukS/F-I*, the toxic-shock syndrome toxin (*tst*), and the collagen adhesin (*cna*) was studied by PCR in CoPS (Gómez-Sanz *et al.* 2011; Gómez *et al.* 2014).

Additionally, the detection of genes of the immune evasion cluster (IEC) system (*scn*, *chp*, *sak*, *sea* and *sep*), which enables the

Table 1. Type of the 47 evaluable superficial waters analyzed, and characteristics of staphylococci obtained.

Type of samples	Number of samples	Number of samples with CoPS/number of isolates	Number of samples with CoNS/number of isolates	Number of samples with CoPS and CoNS
Lotic water samples				
River and stream	23	5/5	18/27	3
Ditch	10	3/3	7/9	1
Lentic water samples				
Water reservoir	5		4/9	
Fountain/Trough	6	2/3	4/10	
Pond	3	2/2	3/4	2
Total	47	12/13	36/59	6

classification into different IEC types, was performed in these isolates (van Wamel *et al.* 2006).

Molecular typing of CoPS isolates

The recovered *S. aureus* isolates were characterized by *spa* typing (www.ridom.com), *agr* typing (Shopsin *et al.* 2003) and multilocus sequence typing (MLST) to determine the sequence type (ST) and clonal complex (CC) (www.mlst.net). On the other hand, *spa* typing (Moodley *et al.* 2009) and MLST according to a previous proposal (Bannoehr *et al.* 2007) were carried out for *S. pseudintermedius*.

RESULTS AND DISCUSSION

Staphylococcus isolates recovered

Bacterial growth was detected in all water samples analyzed. Nevertheless, only 47 samples were evaluable and have been considered in this work (Supplementary Table S1); the remaining 15 samples (10 lentic and five lotic water samples) showed overgrowth with other invasive microorganisms that prevented recovery of staphylococci. A previous study already showed the problems of *Staphylococcus* isolation in samples with high microbial loads and they used acriflavine to minimize the problem, which affected some species of the genus (Davis, Farrah and Wilkie 2006). Other alternatives should be evaluated to facilitate staphylococci recovery.

Staphylococcus spp. were detected in 42 (30 lotic and 12 lentic water samples) of the 47 samples (89.4%). According to these results, staphylococci are frequently detected bacteria in surface waters, which could be considered a vehicle of bacterial transmission to other origins. Indeed, some studies have shown that staphylococci can survive in water environments for several days, and contaminated water could act as a temporary reservoir and source of transmission of these antimicrobial-resistant bacteria (Tolba *et al.* 2008).

Seventy-two staphylococcal isolates (13 CoPS and 59 CoNS) from the 42 positive samples were included in the study and further characterized (one to four different isolates per positive water sample). CoPS were detected in 12 of the 47 evaluable samples (25.5%); CoNS were detected in 36 of these 47 samples (76.6%). Both CoPS and CoNS were obtained in six samples (12.8%) (Table 1 and Supplementary Table S1).

Two species were identified among CoPS (12 *S. aureus* and one *S. pseudintermedius*); 12 different species were identified among CoNS (number of strains): *S. epidermidis* (11), *S. vitulinus* (10), *S. sciuri* (nine), *S. fleurettii* (seven), *S. lentus* (six), *S. simulans* (five), *S. xylosus* (four), *S. chromogenes* (two), *S. hominis* (two), *S. equorum* (one), *S. succinus* (one) and *S. warneri* (one); this showed a high variability of staphylococcal species. Noted that most species isolated have cattle and rodents as animal hosts, but others such as *S. aureus*, *S. epidermidis*, *S. hominis*, *S. simulans* and *S. warneri* are predominant in humans and primates (Becker, Heilmann and Peters 2014). It might be of interest to conduct studies on the density of animals and/or humans who have contact with these waters to make correlations. To our knowledge, this is the first detection of the *S. pseudintermedius* (CoPS), and *S. equorum* and *S. succinus* (CoNS) in water samples.

Characterization of CoPS

Table 2 shows the characteristics of the *S. aureus* and *S. pseudintermedius* isolates included in this study.

All CoPS were negative for *mecA* and *mecC* genes and were susceptible to all antimicrobials tested, except for penicillin. Penicillin resistance was found in 50% of *S. aureus* isolates and in the *S. pseudintermedius* strain, and was associated with presence of the *blaZ* gene. Penicillin resistance is common in both species, and the presence of resistance to other antimicrobials is often associated with resistance to methicillin (Kadlec and Schwarz 2012). Regarding the molecular typing of *S. aureus*, 10 different *spa* types were identified among *S. aureus* isolates (number of isolates): t002 (three), and t045, t502, t843, t3369, t8083, t1166, t10668, t10712 and t10855 (one each). The last three *spa* types were first described in this study. MLST rendered the following results (number of strains, associated clonal complex (CC)): ST5 (four, CC5), ST130 (one, CC130), ST133 (one, CC133), ST425 (one, CC425), ST1643 (one, group 72), ST2049 (one, group 56), and three new ST corresponding to the numbers ST2460 (one, singleton), ST2461, a single-locus-variant of ST425 (one, CC425), and ST2657 (one, singleton). These results show the great variability present in the environment as well as the wide spread of *S. aureus*. To our knowledge, there is only one previous study of clonal lineages in *S. aureus* in environmental freshwater (Porrero *et al.* 2014). Most *S. aureus* in our study were ascribed to CC5 (33%), which is one of the biggest clonal complexes associated with the hospital environment and animals (Argudín *et al.* 2009; McCarthy, Lindsay and Loeffler 2012). Lineages CC130 and CC133 are related primarily to ruminant animal clones (Smyth *et al.* 2009; Holmes and Zadoks 2011). MRSA-ST425 strains, containing the new *mecC* gene, have been associated with livestock and humans (Holmes and Zadoks 2011). Additionally one *mecC*-MRSA-ST425 strain was previously detected in a river in Spain (Porrero *et al.* 2014), whereas our CC425 and CC130 isolates were negative for the *mecC* gene. One MSSA strain was typed as ST1643, being the type previously described in MSSA implicated in a skin lesion of a boar (Meemken

Table 2. Characteristics of the 13 CoPS recovered from water samples in this study.

Strain	Water sample[a]	Species	Molecular typing			Immune evasion cluster	Phenotype of resistance[f]	Resistance gene	Virulence gene
			spa	*agr*	ST/CC				
C5794	3/B	*S. aureus*	t502	II	ST5/CC5	*sak*, *sep*	PEN	*blaZ*	
C5843	45/A	*S. aureus*	t002	II	ST5/CC5	*chp*, *sak*, *sep*	PEN	*blaZ*	
C5850	52/A	*S. aureus*	t002	II	ST5/CC5	*scn*, *chp*, *sak*, *sep* (type F)	PEN	*blaZ*	
C5813	16/A	*S. aureus*	t002	II	ST5/CC5	*chp*, *sak*, *sep*	PEN	*blaZ*	
C5797	5/B	*S. aureus*	t10668[b]	II	ST425/CC425		Susceptible		*cna*
C5796	5/B	*S. aureus*	t10712[b]	II	ST1643/group 72		Susceptible		
C5802	8/A	*S. aureus*	t843	III	ST130/CC130		PEN	*blaZ*	
C5804	11/A	*S. aureus*	t10855[b]	II	ST2461[d]/CC425		Susceptible		*tst*, *cna*
C5840	40/A	*S. aureus*	t3369	II	ST2657[d]/SN[e]	*sep*	Susceptible		
C5858	58/A	*S. aureus*	t1166	I	ST133/CC133		Susceptible		
C5834	34/B	*S. aureus*	t8083	IV	ST2049/group 56		Susceptible		*cna*
C5864	61/A	*S. aureus*	t045	II	ST2460[d]/SN[e]	*sak*, *sep*	Susceptible		*cna*
C5829	32/B	*S. pseudintermedius*	NT[c]	*agrD*1	ST147		PEN	*blaZ*	*lukS/FI*

[a]Number of the water sample (Supplementary Table S1). A, lotic water; B, lentic water.
[b]New *spa* type.
[c]NT, non-typeable.
[d]New sequence type.
[e]SN, singleton.
[f]PEN, penicillin.

et al. 2013). Moreover, another strain presented the ST2049, a lineage previously identified in an MRSA isolated from recreational beach water (Roberts, Soge and No 2013). The four *agr* types were detected, *agr* type II being the predominant one (nine strains). The four strains typed as CC5 and the strains with *spa* t3369 and t045 harbored some of the genes of the IEC system. Only one strain of CC5 contained the *scn* gene and could be ascribed to IEC type F (*scn-chp-sak-sep*), pointing to a possible human origin; the other five *S. aureus* strains could not be ascribed to any IEC type according to the classification of van Wamel *et al*. (2006). One strain presented the *tst* and the *cna* genes, and three strains only the *cna* gene. The detection of these virulence determinants among our *S. aureus* isolates, which could be transferred and disseminated, is of interest.

Concerning the unique *S. pseudintermedius* isolate recovered in this study, the detection of the *spa* gene was negative, *agr* type corresponded to *agrD*1, and the strain belonged to ST147. Methicillin-susceptible *S. pseudintermedius* shows more clonal diversity than methicillin-resistant isolates in which clonal lineage ST71 seems to be predominant in Europe (Kjellman *et al*. 2015). This strain showed the presence of the *lukS/F-I* leukocidin gene that could be an important determinant for the severity of the infection as opportunistic pathogens.

Antimicrobial resistance of CoNS

Table 3 shows the phenotypes and genotypes of antimicrobial resistance of the 59 CoNS isolates obtained from water samples.

Regarding β-lactam antimicrobials, 18 of the 59 isolates carried the *mecA* gene (30.5%), although only 12 of them exhibited cefoxitin resistance by disk diffusion test; the remaining six *mecA*-positive isolates showed a cefoxitin-susceptible phenotype (three *S. vitulinus*, two *S. fleurettii* and one *S. sciuri*, species included in the *S. sciuri* group). The presence of *mecA*-positive CoNS has been previously described in recreational waters, and in community and hospital wastewater (Börjesson *et al*. 2009; Fogarty *et al*. 2015), as well as in other surface waters (Seyedmonir, Yilmaz and Icgen 2015). All seven *S. fleuretti* isolates harbored the *mecA* gene, associated on two occasions with a cefoxitin-susceptible phenotype. It is known that the *mecA* gene is ubiquitously present in the *S. sciuri* group, although is not always associated with cefoxitin resistance (Becker, Heilmann and Peters 2014); it is noteworthy that species *S. fleurettii* has been considered the most probable origin of the *mecA* gene (Tsubakishita *et al*. 2010). On the other hand, 29 CoNS were penicillin resistant (49.2%); of these, 11 strains presented only the *blaZ* gene, 11 strains the *mecA* gene, three strains the *blaZ* and *mecA* genes, and four strains none.

Seventy-six per cent of isolates showed resistance to at least one of the 13 non-β-lactams tested, and multidrug resistance phenotypes (resistance to three or more antimicrobial families) were found in 23.7% of the isolates. These results contrasted with CoPS isolates, which were quite susceptible to the antimicrobials tested. The patterns of resistance to non-β-lactam antimicrobials were very heterogeneous, underlining the high frequency of resistance to fusidic acid (68.1%), where two isolates harbored the *fusB* or *fusC* gene (*S. epidermidis* and *S. hominis*, respectively). CoNS also showed resistance to erythromycin (25.4%) and/or clindamycin (6.7% inducible, 8.5% resistant and one strain resistant to clindamycin but susceptible to erythromycin). Seven of the eight erythromycin–clindamycin-resistant isolates harbored the *erm*(C), *mph*(C), and/or *erm*(A) genes, and seven additional erythromycin-resistant isolates showed the *msr*(A)/(B) and/or *mph*(C) genes. Tetracycline resistance was found in different species (15.3%), and in all isolates associated to the *tet*(K) gene and in one isolate to the *tet*(L) gene. Resistance to the remaining antimicrobials tested showed lower prevalence. Tobramycin and mupirocin resistance was detected in *S. epidermidis*, harboring the *ant*(4′)-*Ia* gene in all tobramycin-resistant isolates and the *mupA* gene in one of the two resistant *S. epidermidis*. Two isolates showed resistance to chloramphenicol, the cat_{pc194} gene being detected in one of them, and two isolates exhibited resistance to streptomycin, with the *str* gene identified in one of the two isolates. All CoNS were susceptible to gentamicin, kanamycin, ciprofloxacin, or linezolid, and 12 of them were susceptible to all of the antimicrobials tested. With the exception of fusidic acid resistance, these percentages are lower than those previously described in other studies of aquatic

Table 3. Antimicrobial resistance of the 59 CoNS recovered from water samples in this study.

Species	Number of strains	β-Lactams		Non-β-lactams	
		Phenotype of resistance[a]	Resistance genes[b]	Phenotype of resistance[a]	Resistance genes[b]
S. epidermidis	1	PEN, **FOX**	*blaZ*, ***mecA***	ERY, CLI, FUS, MUP, STR	*erm*(C), *fusB*, *mupA*
	1	PEN, **FOX**	*blaZ*, ***mecA***	TET, ERY, CLI[i]	*tet*(L), *erm*(C)
	4	PEN[4]	*blaZ*[4]	ERY[4], TOB[4]	*msr*(A)/(B)[4], *ant*(4′)*Ia*[4]
	3	PEN[3]	*blaZ*[2]	ERY[2], TOB[2]	*msr*(A)/(B)[2], *ant*(4′)*Ia*[2]
	2	PEN[2]	*blaZ*[2]	MUP[1]	
S. vitulinus	2	PEN[1]	***mecA***[2]	FUS[2]	
	2	Susceptible		TET[2], FUS[1]	*tet*(K)[2]
	1	Susceptible	***mecA***	Susceptible	
	2	Susceptible		FUS[2]	
	3	Susceptible		Susceptible	
S. sciuri	1	PEN, **FOX**	***mecA***	FUS	
	1	PEN	***mecA***	FUS	
	1	Susceptible		ERY, CLI, STR, FUS, CHL	*erm*(C), *str*
	1	PEN	*blaZ*	ERY	*msr*(A)/(B), *mph*(C)
	5	PEN[1]		CLI[1], FUS[5]	
S. fleurettii	1	PEN, **FOX**	***mecA***	ERY[I], CLI, FUS	*mph*(C)
	4	PEN[4], **FOX**[4]	***mecA***[4]	FUS[4]	
	2	Susceptible	***mecA***[2]	FUS[2]	
S. lentus	1	PEN, **FOX**	***mecA***	ERY, CLI[i], TET, FUS	*erm*(A), *mph*(C), *tet*(K)
	1	PEN, **FOX**	***mecA***	ERY, CLI[i], FUS	*mph*(C)
	3	PEN[1]		TET[1], FUS[3]	*tet*(K)[1]
	1	Susceptible		Susceptible	
S. simulans	3	PEN[1]	*blaZ*[1]	TET[1], FUS[1]	*tet*(K)[1]
	2	Susceptible		Susceptible	
S. xylosus	1	PEN, **FOX**	***mecA***	FUS	
	1	PEN	*blaZ*	TET, FUS	*tet*(K)
	2	PEN[1]		ERY[1], CLI[i,1], FUS[2]	
S. chromogenes	2	Susceptible		Susceptible	
S. hominis	1	PEN, **FOX**	*blaZ*, ***mecA***	ERY, CLI[i], TET, FUS, CHL, SXT	*erm*(C), *tet*(K), *fusC*, cat_{pc194}
	1	Susceptible		Susceptible	
S. equorum	1	Susceptible		Susceptible	
S. succinus	1	Susceptible		TET	*tet*(K)
S. warneri	1	Susceptible		Susceptible	

[a]If the number of strains in the group is more than one, the number of strains resistant to a specific antimicrobial agent is indicated by the superscript shown in brackets. CLI, clindamycin; CHL, chloramphenicol; ERY, erythromycin; FOX, cefoxitin; FUS, fusidic acid; i, intermediate; I, inducible; MUP, mupirocin; PEN, penicillin; STR, streptomycin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; TOB, tobramycin. The 12 cefoxitin-resistant isolates are shown in bold.
[b]If the number of strains in the group is more than one, the superscript enclosed in brackets indicates how many strains harbored a specific resistance gene. The 18 *mecA* detected are in shown in bold.

environments (Basso *et al.* 2014; Sood *et al.* 2014; Heß and Gallert 2014a). A high variety of resistance genes were detected in our study [*fusB*, *fusC erm*(C), *mph*(C), *erm*(A), *msr*(A)/(B), *mph*(C), *ant*(4′)-*Ia*, *tet*(K), *tet*(L), cat_{pc194}, and *str*], although in some strains other mechanisms responsible for the resistance might be implicated.

CONCLUSION

A great diversity of staphylococcal species was detected in this study in superficial waters in Spain, including diverse genetic lineages of *S. aureus* of potential human and animal origins and CoNS with high content of antimicrobial resistance genes. Monitoring the evolution of staphylococcal species and their molecular characteristics in the environment as a potential reservoir and vehicle for transmission to other ecosystems could help in understanding the changing epidemiology of *Staphylococcus* infections in humans and animals for effective intervention strategies.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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Conflict of interest. None declared.

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