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Characterization of staphylococci in urban wastewater treatment plants in Spain, with detection of methicillin resistant *Staphylococcus aureus* ST398☆

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ABSTRACT

The objective of this study was to determine the prevalence of *Staphylococcus* in urban wastewater treatment plants (UWTP) of La Rioja (Spain), and to characterize de obtained isolates. 16 wastewater samples (8 influent, 8 effluent) of six UWTPs were seeded on mannitol-salt-agar and oxacillin-resistance-screening-agar-base for staphylococci and methicillin-resistant *Staphylococcus aureus* recovery. Antimicrobial susceptibility profile was determined for 16 antibiotics and the presence of 35 antimicrobial resistance genes and 14 virulence genes by PCR. *S. aureus* was typed by *spa*, *agr*, and multilocus-sequence-typing, and the presence of immune-evasion-genes cluster was analyzed. *Staphylococcus* spp. were detected in 13 of 16 tested wastewater samples (81%), although the number of CFU/mL decreased after treatment. 40 staphylococci were recovered (1–5/sample), and 8 of them were identified as *S. aureus* being typed as (number of strains): *spa*-t011/*agr*-II/ST398 (1), *spa*-t002/*agr*-II/ST5 (2), *spa*-t3262/*agr*-II/ST5 (1), *spa*-t605/*agr*-II/ST126 (3), and *spa*-t878/*agr*-III/ST2849 (1). *S. aureus* ST398 strain was methicillin-resistant and showed a multidrug resistance phenotype. Virulence genes *tst*, *etd*, *sea*, *sec*, *seg*, *sei*, *sem*, *sen*, *seo*, and *seu,* were detected among *S. aureus* and only ST5 strains showed genes of immune evasion cluster. Thirty-two coagulase-negative *Staphylococcus* of 12 different species were recovered (number of strains): *Staphylococcus equorum* (7), *Staphylococcus vitulinus* (4), *Staphylococcus lentus* (4), *Staphylococcus sciuri* (4), *Staphylococcus fleurettii* (2), *Staphylococcus haemolyticus* (2), *Staphylococcus hominis* (2), *Staphylococcus saprophyticus* (2), *Staphylococcus succinus* (2), *Staphylococcus capitis* (1), *Staphylococcus cohnii* (1), and *Staphylococcus epidermidis* (1). Five presented a multidrug resistance phenotype. The following resistance and virulence genes were found: *mecA*, *lnu*(A), *vga*(A), *tet*(K), *erm*(C), *msr*(A)/(B), *mph*(C), *tst*, and *sem*. We found that *Staphylococcus* spp. are normal contaminants of urban wastewater, including different lineages of *S. aureus* and a high diversity of coagulase-negative species. The presence of multiple resistance and virulence genes, including *mecA*, in staphylococci of wastewater can be a concern for the public health.

1. Introduction

The extended use of antimicrobials for prophylaxis or treatment of human or animal infections, as well as the use in the past of these agents as animal growth promoters (now banned in EU), or the use in agriculture, have caused the spread of resistant bacteria or their resistance genes in different ecosystems, including the environment. The widespread use of these agents may act as selective pressure on natural bacteria population becoming an important determinant in resistance maintenance, development and dissemination. Moreover, some antimicrobials are very stable which might become in a long-term persistence of these active compounds (McArdell et al., 2003; Miao et al., 2004). On the other hand, some authors have proposed that the origin of virulence determinants could probably reside in the environmental microbiota (Martínez, 2013; Søborg et al., 2013).

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Conventional Urban Wastewater Treatment Plants (UWTP) could not completely remove antimicrobials or microorganisms. Previous studies have indicated that UWTP might be a vehicle for the dissemination of antimicrobials and antimicrobial resistant bacteria in the environment, mainly by effluent water or sludge (Kim and Aga, 2007; Rizzo et al., 2013). Additionally, water and sludge are reused in different ways, and the spread of resistant and virulent bacteria are of particular concern. The evaluation of an efficient treatment is important, and it is generally based on monitoring conventional water quality parameters that include heterotrophic bacterial counts and the abundance of coliforms.

Staphylococcus spp. are ubiquitous bacteria reported as normal microbiota of the mucous membranes and of the skin of mammals and birds; however, it may behave as opportunistic pathogens that can cause minor and severe infections. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important concerns in public health and it constitutes a serious threat. Although *S. aureus* is the most relevant species in the genus, coagulase-negative *Staphylococcus* (CoNS) is gaining interest due to its increased detection as responsible agents of infections (Becker et al., 2014).

Most studies on characterization of resistant bacteria in UWTPs are focused on fecal pollution indicator bacteria. Nevertheless, some studies have reported the presence of *S. aureus* (Porrero et al., 2014), MRSA (Börjesson et al., 2010; Kumar et al., 2015; Rosenberg Goldstein et al., 2012), or CoNS (Faria et al., 2009; Heβ and Gallert, 2014) isolates in UWTP or have reported the presence of antimicrobial resistance genes (Börjesson et al., 2009; Colomer-Lluch et al., 2014). In all of them, the presence of *S. aureus* after treatment seems to be low; however, MRSA is considered an emerging contaminant in water environments, and the fact that the isolates from wastewater can be more virulent and multidrug resistant has been speculated (Börjesson et al., 2010).

The aim of this study was to determine the prevalence of *Staphylococcus* in UWTPs of La Rioja (Spain), before and after treatment, as well as to perform the molecular characterization of the obtained isolates.

2. Material and methods

2.1. Sample collection

Sixteen water samples were collected during December-2012 to February-2013 from six different UWTPs (8 at influent points and 8 at effluent points) in La Rioja region (Logroño 2/2; Cornago 2/2, Aldeanueva de Ebro 1/1, Alfaro 1/1, Rincón de Soto 1/1, and Torrecilla en Cameros 1/1). Sample collection was performed in two different shots, but influent and effluent samples of each UWTP were recovered in the same day. In two of the UWTPs (Logroño and Cornago), samples were collected from both shots. Wastewater samples were obtained in sterile glass bottles (500 mL), and directly transported under refrigeration conditions to the microbiology laboratory for analysis.

2.2. Staphylococci colony count, isolation and identification

Different aliquots of wastewater samples were seeded in mannitol-salt-agar (MSA, BD, France) plates and oxacillin-resistance-screening-agar-base (ORSAB, Oxoid, England) plates supplemented with oxacillin (2 mg/L), for recovery of staphylococci and methicillin-resistant staphylococci (MRS), respectively. Plates were incubated at 37 °C for 48 h. *Staphylococcus* isolates that were grown in MSA plates were counted (CFU/mL).

In parallel, an aliquot of 100 μL of wastewater samples were pre-enriched by inoculation into brain heart infusion (BHI) broth (BD, France) with 6.5% NaCl and incubated at 37 °C for 24–48 h. After that, aliquots were inoculated in MSA and ORSAB media.

Up to six colonies per wastewater sample with staphylococci morphology were recovered, and initially identified by microbiological conventional methods (Gram staining, coagulase and DNase test). Identification was carried out by amplification of the species-specific *nuc* gene for *S. aureus* (Sasaki et al., 2010), and by amplification and sequencing of the *sodA* gene for CoNS (Poyart et al., 2001). Only strains showing different phenotypes of antimicrobial resistance of each species and of each sample were further characterized.

2.3. Antimicrobial susceptibility testing and detection of antimicrobial resistance genes

Susceptibility to penicillin, oxacillin, cefoxitin, kanamycin, gentamicin, tobramycin, streptomycin, tetracycline, chloramphenicol, erythromycin, clindamycin, ciprofloxacin, linezolid, trimethoprim/sulfamethoxazole, mupirocin, and fusidic acid, was performed by disk-diffusion method (CASFM, 2010; EUCAST, 2014). Cefoxitin resistance was used as phenotypic marker of methicillin resistance.

Detection of 35 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), *msr*(A), *msr*(B), *mph*(C), *aph*(3′)-IIIa, *ant*(4′)-Ia, *lnu*(A), *lnu*(B), *lnu*(D), *lnu*(C), *vga*(A), *cfr*, *ant*(6)-Ia, *ant*(3″)(9), *str*, *dfr*(A), *dfr*(D), *dfr*(K), *dfr*(G), cat_{pc194}, cat_{pc221}, and cat_{pc223}] was performed by PCR (Benito et al., 2014; García-Álvarez et al., 2011; Gharsa et al., 2012; Lozano et al., 2012).

2.4. Virulence genotype and detection of immune evasion cluster genes

The presence of the genes encoding enterotoxins (*sea*, *seb*, *seg*, *sei*, *sem*, *sen*, *sec*, *seu*, and *seo*), exfoliative toxins (*eta, etb*, and *etd*), the Panton Valentine Leukocidin (PVL, *luk*F/S), and the toxic-shock syndrome toxin (*tst*) was studied by PCR (Benito et al., 2014; Gharsa et al., 2012).

Furthermore, the detection of genes of the immune evasion cluster system (IEC) [staphylococcal complement inhibitor (*scn*), chemotaxis inhibitory protein (*chp*), staphylokinase (*sak*), enterotoxin A (*sea*) or enterotoxin P (*sep*)] which enables the classification into different IEC types (A–G), was performed in *S. aureus* isolates (van Wamel et al., 2006).

2.5. Molecular typing of S. aureus isolates

All *S. aureus* isolates obtained were characterized by *spa*-typing (www.ridom.com) and *agr* typing, as previously described (Shopsin et al., 2003). One isolate of each *spa*-type was selected for molecular characterization by multilocus sequence typing (MLST) (www.mlst.net).

3. Results

3.1. Staphylococcus recovered from wastewater

Staphylococcal isolates were detected in 13 of the 16 tested wastewater samples (81%), corresponding to 6 influent and 7 effluent samples. Colony counts performed of samples directly seeded in MSA plates revealed an average of staphylococci in influent samples of 6.8×10^2 CFU/mL versus 8×10^1 CFU/mL in effluent samples.

Forty *Staphylococcus* spp. isolates, 21 of them in influent samples [3 *S. aureus* y 18 CoNS] and 19 in effluent samples [5 *S. aureus* y 14

CoNS] were obtained. *S. aureus* isolates were recovered only when a previous pre-enrichment in BHI growth with NaCl was performed. Nevertheless, CoNS were detected with and without previous pre-enrichment process. No other coagulase-positive species was detected in addition to *S. aureus*.

3.2. Molecular characterization of S. aureus isolates from wastewater

Table 1 shows the characteristics of the eight *S. aureus* isolates that were obtained from influent and/or effluent samples of four UWTPs. All eight *S. aureus* isolates were submitted to *spa*-typing and they corresponded to (number of strains): *spa*-t002 (2), *spa*-t011 (1), *spa*-t605 (3), *spa*-t878 (1), and *spa*-t3262 (1). MLST-typing performed in one strain of each *spa*-type rendered the following results: ST398 (*spa*-t011), ST5 (*spa*-t002), ST5 (*spa*-t3262), ST126 (*spa*-t605) and the new sequence type ST2849 (*spa*-t878). The new ST2849 presented the new allelic combination: *arcC*-3, *aroE*-1, *glpF*-1, *gmk*-15, *pta*-100, *tpi*-1, *yqiL*-10. Seven of *S. aureus* presented *agr*-type II, and one *agr*-type III.

Only the ST398 *S. aureus* was methicillin-resistant (C6857) and carried the *mecA* gene. This strain was obtained from an effluent water sample and presented a multidrug resistance phenotype (MDR phenotype is defined as non-susceptibility to at least one agent in three or more antimicrobial families), being resistant to the following non-beta-lactam antimicrobials (resistance gene detected): clindamycin [*lnu*(B)], ciprofloxacin, tetracycline [*tet*(M), *tet*(K)], and aminoglycosides [*aph*(3′)-IIIa, *aac*(6′)-*aph*(2″)].

Regarding methicillin-susceptible *S. aureus* isolates (MSSA), three strains exhibited penicillin resistance and harbored the *blaZ* gene and four showed susceptibility to all antimicrobials tested. Three MSSA isolates, belonging to clonal complex (CC) 5, were ascribed to IEC type F. The remaining *S. aureus* lacked the IEC system. Nevertheless, one *S. aureus* strain with ST2849-*spa*-t878 could not be ascribed to an IEC type, but harbored the *chp* and *sak* genes of this cluster. Interestingly, this strain ST2849 presented the genes encoding the toxic-shock syndrome toxin, and the gene of the exfoliative toxin D. The presence of different enterotoxin genes was detected in four MSSA: *sea*, *sec*, *seg*, *sei*, *sem*, *sen*, *seo*, and *seu*.

3.3. Characterization of CoNS isolates from wastewater

Species detected by sequencing of *sodA* gene within the 32 CoNS isolates were as follows (number of strains): *Staphylococcus equorum* (7), *Staphylococcus vitulinus* (4), *Staphylococcus lentus* (4), *Staphylococcus sciuri* (4), *Staphylococcus fleurettii* (2), *Staphylococcus haemolyticus* (2), *Staphylococcus hominis* (2), *Staphylococcus saprophyticus* (2), *Staphylococcus succinus* (2), *Staphylococcus capitis* (1), *Staphylococcus cohnii* (1), and *Staphylococcus epidermidis* (1). Table 2 shows the characteristics of these isolates. Nine of them showed susceptibility to all antimicrobials tested (28.1%), five presented a multidrug resistance phenotype (15.6%, 3 *S. lentus*, 1 *S. sciuri*, and 1 *S. hominis*), and the remaining 18 showed resistance to at least one of the antimicrobials. Resistance to the following antimicrobials was observed (number of strains): cefoxitin (4), chloramphenicol (1), ciprofloxacin (1), clindamycin (3), erythromycin (5), fusidic acid (15), tetracycline (6), penicillin (13), streptomycin (1), and trimethoprim/sulfamethoxazole (1).

Four CoNS isolates (three *S. lentus* and one *S. sciuri*) showed cefoxitin-resistance, harbored the *mecA* gene and were considered as methicillin resistant isolates. In addition, other three cefoxitin-susceptible CoNS isolates also carried the *mecA* gene (one *S. sciuri* andtwo *S. fleurettii*) (Table 2). The *lnu*(A) gene was detected in one *S. lentus* strain and the *vga*(A) gene in one *S. hominis* strain, both clindamycin-resistant. All tetracycline resistant isolates harbored the *tet*(K) gene. The genes *erm*(C), *msr*(A)/(B), and *mph*(C) were identified in erythromycin resistant strains.

CoNS isolates presented few virulence genes. Nevertheless, one *S. equorum* strain contained the *tst* gene and one *S. hominis* the *sem* gene.

4. Discussion

In this study, during wastewater treatment, the number of CFU decreased approximately in one $\log_{10}$/mL on staphylococci counts. It seems to agree with previous results that show a drop in the number of *Staphylococcus* found after treatment (Börjesson et al., 2009; Rosenberg Goldstein et al., 2012). Under environmental stress conditions caused by the wastewater treatment, the population of viable *Staphylococcus* might be also reduced by entering in a viable but unculturable state (Masmoudi et al., 2010). Most of effluent samples of UWTP were positive for staphylococci detection in our study (87.5%), indicating that treated wastewater can contribute to the dissemination of *Staphylococcus* and their resistance genes to the environment. Moreover, an additional treatment or adjustment of treatment procedures could be necessary, especially if the water will be reused.

As far as we know, there are very few studies in which the characterization of *S. aureus* in wastewater has been performed. Until now, different clonal complexes (CC5, CC8, CC22, CC30, CC45,

Table 1
Characteristics and origin of the eight *S. aureus* strains included in this study.

Strain	UWTP sample[a]	Molecular typing				IEC[c]	Resistance phenotype[d]	Resistance genes	Virulence genes
		spa	*agr*	ST	CC				
C6857	C-E	t011	II	ST398	CC398		PEN-OXA-FOX-CLI-CIP-TET-KAN-GEN-TOB-STR	*mecA*-*lnu*(B)-*tet*(M)-*tet*(K-*aph*(3')-IIIa-*aac*(6')-*aph*(2")	
C6872	AN-I	t002	II		CC5	Type F	Susceptible		*sea-sem-seu*
C6853	A-E	t002	II	ST5	CC5	Type F	PEN	*blaZ*	*sea-seg-sei-sem-sen-seo-seu*
C6884	C-E	t3262	II	ST5	CC5	Type F	PEN	*blaZ*	*sea-seg-sem-sen-seo-seu*
C6856	C–I	t605	II	ST126	CC126		Susceptible		
C6862	L-I	t605	II		CC126		Susceptible		
C6889	L-E	t605	II		CC126		PEN	*blaZ*	
C6865	L-E	t878	III	ST2849 [b]	CC779	*sak-chp*	Susceptible		*tst-etd-sec*

[a] UWTP-sample: the location of the UWTP (A, Alfaro; AN, Aldeanueva; C, Cornago; L, Logroño) is indicated as well as the type of sample (I, influent; E, effluent).
[b] New allelic combination (*arcC*:3; *aroE*:1; *glpF*:1; *gmk*:15; *pta*:100; *tpi*:1; *yqiL*:10).
[c] IEC: Immune Evasion Cluster genes. Type F: presence of *scn-chp-sak-sep* genes.
[d] PEN, penicillin; OXA, oxacillin; FOX, cefoxitin; CLI, clindamycin; CIP, ciprofloxacin; TET, tetracycline; KAN, kanamycin; GEN, gentamicin; TOB, tobramycin; STR, streptomycin.

Table 2
Characteristics and origin of the 32 coagulase-negative *Staphylococcus* strains included in this study.

Strain	Species	UWTP-sample[a]	Resistance phenotype[b]	Resistance genes	Virulence genes
C6870	*S. equorum*	AN-I	Susceptible		
C6877	*S. equorum*	C-I	Susceptible		
C6879	*S. equorum*	C-I	Susceptible		
C6883	*S. equorum*	C-E	Susceptible		
C6876	*S. equorum*	AN-E	Susceptible		
C6885	*S. equorum*	L-I	Susceptible		
C6864	*S. equorum*	L-E	TET	*tet*(K)	*tst*
C6867	*S. vitulinus*	AN-I	Susceptible		
C6886	*S. vitulinus*	L-I	Susceptible		
C6860	*S. vitulinus*	L-I	FA		
C6878	*S. vitulinus*	C-I	FA		
C6855	*S. lentus*	C-I	PEN-FOX	*mecA*	
C6890	*S. lentus*	T-I	PEN-FOX-STR-FA	*mecA-str*	
C6880	*S. lentus*	C-I	TET-CLI-SXT-FA	*tet*(K)-*lnu*(A)-*dfr*(A)	
C6861	*S. lentus*	L-I	PEN-FOX-ERY-CLI-CIP-CHL-FA	*mecA-erm*(C)-*mph*(C)-cat_{pc221}	
C6854	*S. sciuri*	C-I	FA		
C6888	*S. sciuri*	L-E	FA		
C6874	*S. sciuri*	AN-E	PEN-FA	*mecA*	
C6881	*S. sciuri*	C-E	PEN-FOX-TET-FA	*mecA- tet*(K)	
C6871	*S. fleurettii*	AN-I	PEN-FA	*mecA*	
C6873	*S. fleurettii*	AN-E	PEN-FA	*mecA*	
C6858	*S. haemolyticus*	L-I	PEN-ERY	*blaZ-msr*(A)/(B)	
C6850	*S. haemolyticus*	A-E	PEN-ERY	*blaZ-msr*(A)/(B)-*mph*(C)	
C6851	*S. hominis*	A-E	ERY-CLI	*vga*(A)-*msr*(A)/(B)-*mph*(C)	*sem*
C6863	*S. hominis*	L-E	PEN-ERY-TET-FA	*blaZ-msr*(A)/(B)-*tet*(K)-*fus*(B)	
C6887	*S. saprophyticus*	L-I	TET-FA	*tet*(K)	
C6882	*S. saprophyticus*	C-E	TET-FA	*tet*(K)	
C6852	*S. succinus*	A-E	Susceptible		
C6875	*S. succinus*	AN-E	PEN		
C6866	*S. capitis*	RS-E	PEN	*blaZ*	
C6859	*S. cohnii*	L-I	FA		
C6869	*S. epidermidis*	AN-I	PEN	*blaZ*	

[a] UWTP-sample: the location of the UWTP (A, Alfaro; AN, Aldeanueva; C, Cornago; L, Logroño; RS, Rincón de Soto; T, Torrecilla) is indicated as well as the type of sample (I, influent; E, effluent).
[b] PEN, penicillin; FOX, cefoxitin; ERY, erythromycin; CLI, clindamycin; FA, fusidic acid; CIP, ciprofloxacin; CHL, chloramphenicol; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; STR, streptomycin.

CC59, CC88, and CC130) have been identified in wastewater (Börjesson et al., 2010; Porrero et al., 2014). The possible origin of these isolates might be either from human or animal residues. In our study, we have found some *spa*-types (t011, t605, t878, and t3262), that to our knowledge, have not been described in wastewater. Two of the lineages found, t011-CC398 and t605-CC126, have been associated with livestock and ruminants respectively (Peeters et al., 2015; Silva et al., 2013), t878-CC779 is a nosocomial-associated lineage (Kinnevey et al., 2014), and t002/t3262 belongs to CC5, which is one of the most prevalent CCs world-wide (Deurenberg and Stobberingh, 2008). Interestingly, the *spa*-type t002 was detected previously in wastewater in Northern Europe (Börjesson et al., 2010).

The clonal lineage MRSA-CC398 has been described in animal species, mainly in pigs, and in people in contact with these animals (Fluit, 2012). Remarkably, the MRSA CC398 isolate was detected in this study, which showed a multi-drug resistance phenotype, in an effluent sample of an UWTP of Cornago. It is interesting to note that Cornago surroundings is one of the most populated areas of pig farms in La Rioja. Moreover, it has been reported the detection of MRSA-ST398, mostly in patients related to farm animals ($p < 0.05$) in a hospital located in a close region (Benito et al., 2014).

On the other hand, all resistance genes in *S. aureus* were found in isolates of effluent samples. Previous reports demonstrated that there is an increase of multidrug resistance after conventional treatment of wastewater (Alouache et al., 2014; Czekalski et al., 2012). It can be the result of a co-selection of different antimicrobial resistance genes by different chemical water compounds, such as heavy metals, different enzymes for biodegradation process, or directly by the presence of antimicrobials (Gillings et al., 2014). All our *S. aureus* isolates lacked of the IEC system except those belonging to lineage CC5 that were classified into IEC type-F, which points to a possible human origin because the genes that conform this system, allow the evasion of the first barriers of the human immune defenses. In addition to that, these CC5 isolates presented a high number of enterotoxin genes. *S. aureus* is supposed to be one of the main causes of foodborne illness (Normanno et al., 2007), and the presence of isolates harboring enterotoxin genes is highly relevant.

Most of tested wastewater samples contained CoNS (75%), and a wide variety of species were demonstrated. Although there are some previous studies that describe the presence of CoNS in wastewater samples, only some of these have identified them at the species level (Faria et al., 2009; Heβ and Gallert, 2014).

Fourteen CoNS isolates obtained in the present study belonged to the *S. sciuri* group, which includes five species, four of which were detected in our study (*S. sciuri*, *S. lentus*, *S. vitulinus*, and *S. fleurettii*). Members of this group are normally found in healthy animals (Nemeghaire et al., 2014), and in particular, *S. sciuri* is the most primitive *Staphylococcus* species, and is spread out in environment. Four of our *S. sciuri* group isolates were methicillin resistant and harbored the *mecA* gene. Moreover, this gene was also found in three methicillin susceptible strains (one *S. sciuri*, and two *S. fleurettii*). An interesting feature of species belonging to the *S. sciuri* group is that they carry homologues of *mecA* gene in their chromosomal DNA. These homologues have a similarity of 80% with

the gene *mecA* responsible for methicillin resistance (Monecke et al., 2012; Nemeghaire et al., 2014), although they do not confer this resistance phenotype. Indeed, *S. fleurettii* seems to be the common ancestor of *mecA* genes in this group and *mecA* gene conferring resistance in other *Staphylococcus* spp. (Tsubakishita et al., 2010), and future studies should focused in the characterization of polymorphisms in this gene.

The species *S. equorum*, *S. succinus*, and *S. saprophyticus* belong to the *S. saprophyticus* group. *S. saprophyticus* is one of the most common causatives of human urinary tract infections, though it can be found in the gastrointestinal microbiota of livestock animals. Moreover, *S. equorum* and *S. succinus* have been associated with fermented foods (Becker et al., 2014).

Six CoNS isolates belonged to the *S. epidermidis* group, in which we can find *S. haemolyticus*, *S. hominis*, *S. capitis*, and *S. epidermidis*. It is interesting to remark that, normally, the most common species that cause infections among CoNS is *S. epidermidis*, followed by *S. hominis*, *S. haemolyticus*, and *S. capitis* (Becker et al., 2014).

Finally, we have identified one *S. cohnii* isolate. There are few reports about this species and most of them are associated with nosocomial infections (Chen et al., 2015), though *S. cohnii* has been also found in animals (Sousa et al., 2014).

This study showed a high diversity of resistance genes among CoNS, highlighting the resistance to macrolides and the presence of genes as *lnu*(A), *vga*(A), and cat_{pc221}. Although fusidic acid resistance was raised in CoNS (46.9%), it seems not to be associated with the encoding genes *fus*(B) or *fus*(C), just in one strain the *fus*(B) gene has been found. The finding of multiple antimicrobial resistances in CoNS is worrying, because the genes responsible for these resistance phenotypes can be transferred to other pathogenic species. Dates showed that species specificity in this genus is not high, making *Staphylococcus* spp. an efficient donors and recipients of resistance genes between different ecosystems (Nemeghaire et al., 2014). In addition, the capacity of *S. aureus* to transfer antimicrobial resistance genes in wastewater has been previously observed (Ohlsen et al., 2003).

Our results agree with the fact that CoNS isolates possess fewer aggressive virulence properties than *S. aureus*. Indeed, only one *S. equorum* presented the *tst* gene and one *S. hominis* the enterotoxin gene *sem*. However, most of the mechanisms of virulence in CoNS are undiscovered, and it is increasing their medical impact.

5. Conclusion

In summary, *Staphylococcus* spp. is very frequently detected in UWTP, even in effluent samples, and it has been reported for the first time the presence of MRSA-ST398 in wastewater. Different clonal complexes associated with hospital settings have been found. The presence of multiple resistance genes in CoNS, including *mecA*, as well as *tst* gene and enterotoxin genes in *S. aureus* of wastewater origin can have impact in public health.

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