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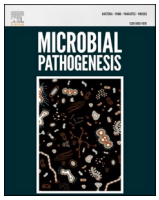

Livestock associated *Staphylococcus aureus* in cystic fibrosis patients in Spain: Detection of MRSA and MSSA CC398

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ABSTRACT

Purpose: Staphylococcus aureus is one of the most prevalent pathogens in cystic fibrosis (CF), being of special relevance those methicillin-resistant (MRSA). The livestock-associated (LA)-MRSA lineage CC398 is an emerging problem, specially related to pig-farming (PF) environments. The objective was to characterize the *S. aureus* isolates recovered from CF-patients in a Spanish hospital located in a region with high-PF activity.

Methods: Forty-two isolates were obtained (January–October/2022) and characterised (one/patient). The antimicrobial resistance phenotype/genotype was evaluated by Microscan/PCR. The presence of virulence and Immune Evasion Cluster (IEC) genes as well as the *agr* type was determined by PCR. MLST and *spa*-typing were studied by PCR-sequencing.

Results: Nine of the 42 isolates were MRSA (21.4 %), and 8 of them multidrug resistant (MDR). Among MRSA, 6 *spa*-types were detected, assigned to CC1, CC5, CC8, CC30, and CC398. Four MRSA isolates belonged to the lineage CC398-t011-IEC negative (animal adapted-clade, LA-MRSA). The remaining 33 isolates were methicillin-susceptible (MSSA), of 26 *spa*-types and associated with 11 CCs (predominant: CC5, CC30, and CC398). Seven MSSA isolates were of the lineage CC398 (*spa*-types t034, t108, t571, t20352); four of them were IEC-positive and *erm*(T)-positive (t571, and t20352, human-adapted CC398 clade), being IEC-negative the remaining three. The *tst* and *eta/etb* genes were identified in 12 and 2 isolates, respectively (none CC398). Small-colony-variants were demonstrated in 9 isolates (two CC398, both MDR).

Conclusion: The lineage CC398 was very frequent among CF-patients (26.2 %), both among MSSA and MRSA. The emergence of LA-MRSA-CC398 in CF-patients requires monitorization, especially in hospitals of high-PF-regions.

1. Introduction

Cystic fibrosis (CF) is the most prevalent inherited disease in the Caucasian population [1]. The most severe symptoms are related to the respiratory tract, however, other organs such as the liver, pancreas or secretory cells can also be affected [2]. CF is known to be caused by mutations in the regulator gene that encodes the CFTR protein [1]. These mutations alter ion and water transport across epithelial membranes, producing sputum accumulation and a dysregulated immune system response. Patients with CF suffer from recurrent bacterial airway infections, which worsen their quality of life and cause severe damages to their lungs.

Among the bacterial species most frequently associated with infections in these patients, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most prevalent ones [3]. *S. aureus* is the most common pathogen in paediatric CF-patients, with the highest frequency between

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11 and 17 years old [4]. Important adaptative changes have been found in several bacterial species isolates obtained in different phases throughout persistent CF infections, this is the case of the phenotype known as small-colony-variant (SCV). In *S. aureus*, these SCV colonies can be differentiated because they are small, non-pigmented and non-hemolytic [5]. *S. aureus* SCVs are characterised by slow growth and repression of virulence factors, as well as increased adherence, invasiveness and intracellular persistence. The clinical impact of SCVs is increased because some of them have the ability to revert to the wild type phenotype [6]. Moreover, these SCV isolates have higher antibiotic resistance rates and are associated with advanced stages of lung disease [7].

Over the past few years, the prevalence of infections caused by methicillin-resistant *S. aureus* (MRSA) has been increasing in CF-patients [4]. During decades, hospital-associated (HA-) or community-associated (CA-) clonal lineages of MRSA have been frequently found in CF patients in different parts of the world [8,9]. Recently, livestock-associated (LA) MRSA isolates have been also detected in CF-patients in some studies in Austria, Boston, Brazil, Belgium, France, and Germany [2,10–13]. LA-MRSA is frequently detected in livestock animals (especially pigs) or in humans with high exposure to these animals. Among clonal lineages associated with livestock animals, CC398 is highly relevant. CC398 has been identified in MRSA and MSSA isolates and it has been hypothesized the presence of two main clades (and different subclades). LA-MRSA-CC398 -known as "animal-adapted clade"- in addition to the methicillin resistance gene *mecA*, usually carries tetracycline resistance *tet* genes, frequently belong to *spa*-types t011 or t034 and lack the genes of the human immune evasion cluster (IEC, *scn* gene is marker of this system). On the other hand, the livestock-independent (LI)-MSSA-CC398–"human-adapted clade"- contain the IEC genes, belong to different *spa*-types (t571, among others), and usually shows an erythromycin/clindamycin inducible resistance phenotype due to the *erm*(T) gene [14]. LA-MSSA-CC398 (IEC-negative) have also been sporadically described (*spa* t011, t034, among others). In recent years, LI-MSSA-CC398 has become more prevalent in hospital settings, and it has been associated, in some cases, with invasive infections [14].

Currently, although there is some information about the prevalence of *S. aureus* in CF-patients in our country [15], there is not much data on the predominant clonal lineages. Moreover, the transmission of livestock associated clonal lineages of *S. aureus* on CF-patients and their impact is still little known and requires more information. This study aims to know the main clonal lineages of *S. aureus* in CF-patients in a Spanish hospital located in an area of high pig-farming (PF) activity, as well as the possible emergence of LA-MRSA, determining the antimicrobial resistance phenotype/genotype, the virulence content, and performing the molecular typing of *S. aureus* isolates obtained from CF-patients.

2. Materials and methods

2.1. Isolates included in the study

A total of 42 isolates (1 isolate/patient) were included in this study. These isolates were recovered from respiratory samples of CF-patients in the Universitary Hospital Miguel Servet of Zaragoza (Spain), during January–October 2022.

2.2. Culture and isolates identification

Brain Heart Infusion agar medium (BHI; Condalab, Madrid, Spain) was used for growth of all isolates. Isolates were identified by MALDI-TOF mass spectrometry (matrix-assisted laser desorption-ionization time-of-flight) using the standard protein extraction protocol recommended by manufacturers (Bruker Daltonik, Bremen, Germany). Detection and growth of SCV, characterization of pigmentation (gray or yellow), and the haemolytic activity were studied in Columbia Blood Agar (Biomerieux, Marcy-l'Étoile, Francia) and/or Chocolate Agar (Thermo Fisher Scientific, Nuevo Hampshire, US). The haemolytic zone was analysed after incubating in Columbia Blood Agar plates for 24 h at 37 °C as previously indicated [5]. Isolates were considered β-toxin positive when double haemolytic zone around the colonies was visible.

2.3. Antimicrobial resistance phenotype and genotype

Antimicrobial susceptibility testing of 19 antibiotics (penicillin, oxacillin, cefoxitin, tetracycline, erythromycin, clindamycin, gentamicin, tobramycin, amikacin, chloramphenicol, linezolid, ciprofloxacin, trimethoprim-sulfamethoxazole, mupirocin, daptomycin, vancomycin, teicoplanin, ceftaroline, and quinupristin-dalfopristin) was evaluated by Microscan system. The results were interpreted according to the European Committee of Antimicrobial Susceptibility Testing criteria [16].

The presence of 22 resistance genes (*blaZ*, *mec*A, *tet*(K), *tet*(L), *tet*(M), *erm*(A), *erm*(B), *erm*(C), *erm*(T), *msr*(A), *mph*(C), *lnu*(A), *lnu*(B), *vga*(A), *lsa*(B), *aac* (6′)-Ie-*aph* (2″)-Ia, *ant* (4′)-Ia, *aph* (3')-IIIa, *dfr*(A), *dfr*(D), *dfr* (G), *dfr*(K)) was tested by PCR (Supplementary Table 1). Positive and negative controls were used in all PCRs. As positive controls, DNA from positive isolates of the University of La Rioja collection, which had been sequenced in previously studies, was utilized. As negative control, a sample with water instead of DNA was used.

2.4. Molecular typing

Molecular characterization of the *S. aureus* isolates was performed by different methods. In all isolates, the determination of the *spa*-type was performed by PCR and sequencing, and the obtained sequences were analysed using Ridom Staph Type software (Ridom GmbH, Münster, Germany) (Supplementary Table 1). Determination of *agr* allotypes was also carried out by PCR for all of them (Supplementary Table 1). MLST (Multi Locus Sequence Typing) was performed in a total of 30 selected isolates (one strain per *spa* type and all those strains that presented any of the virulence genes analysed). For the remaining isolates, the presumptive clonal complexes (CCs) associated with MLST were assigned according to the *spa*-type detected.

2.5. Virulence factors

The human Immune Evasion Cluster (IEC) genes (*scn*, *chp*, *sak*, *sea*, and *sep*) were investigated by PCR (Supplementary Table 1), using *scn* gene as marker of IEC system. According to the IEC genes detected, it could be classified into seven IEC types (A to G). The presence of the genes encoding the virulence factors Panton-Valentine leucocidin (*luk*F/S-PV), toxic shock syndrome toxin (*tst*), and exfoliative-toxins A (*eta*), B (*etb*) and D (*etd*), was studied by PCR (Supplementary Table 1).

2.6. Statistical analysis

Statistical analysis was performed using R and RStudio software version 4.3.1. The association between the age (paediatric/adults) and multi-resistance phenotype (presence/absence), the age (paediatric/adults) and the colony size (normal/SCV), and multi-resistance phenotype (presence/absence) and the colony size (normal/SCV) was determined using the Chi-square test. A p-value of $\leq$0,05 was considered to be statistically significant.

3. Results

3.1. Characteristics of CF patients with S. aureus infections

A total of 82 CF-patients was screened for the presence of *S. aureus* during the study period (January–November 2022). *S. aureus* was detected in 53 patients, and 42 isolates (one per patient) were available for this study. Among these 42 patients, 27 % of them showed the

homozygous F508del in the CFTR gene. Patients ranged in age from 4 months to 49 years, 26 (61.9 %) of patients were male and 16 females (38.1 %). The prevalence of *S. aureus* colonization/infection was 64.6 %. Twenty-eight isolates were obtained from paediatric patients (≤14 years-old), being the remaining 14 isolates from adult patients. The age and gender distribution of patients is shown in Fig. 1. All isolates were obtained from respiratory specimens of the patients, 14 were from sputum samples and 28 were from deep throat swab samples.

Sixteen patients were coinfected with other microorganisms, in 8 cases with *P. aeruginosa.* Moreover, *S. aureus* and *P. aeruginosa* coinfections were detected in patients that were ≥10 years old. In the other nine cases, other microorganisms like *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Burkholderia cenocepacia, Candida albicans*, *Enterobacter cloacae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Penicillium* spp, *Scopulariopsis brevicaulis*, and filamentous fungus were detected.

3.2. Phenotypic characteristics of the S. aureus isolates

Ten different phenotypes were identified among the 42 *S. aureus* isolates according to the following characteristics: size of the colony (normal-size/SCV), pigmentation (yellow/gray), haemolysis (haemolytic/nonhaemolytic), and presence of β-toxin (positive/negative) (Table 1). Phenotype 1 (normal-size, gray, nonhaemolytic, β-toxin-negative) and phenotype 4 (normal-size, yellow, nonhaemolytic, β-toxin-negative) were the most frequently detected phenotypes. In 9 patients, SCVs morphology was detected (21.4 %). There was a significant association between the age of the patients and the presence of the SCV phenotype (p value = 0.01670), being more frequent to detect SCV in adults.

3.3. Antimicrobial resistance phenotype and genotype

Among the 42 isolates, resistance to antimicrobial agents was found in all isolates except one which remained susceptible to all the antibiotics tested. Twenty-two isolates were multi-drug resistant (MDR, resistant to at least three different classes of antimicrobial agents). A high number of isolates presented resistance to penicillin (39/42, 92.8 %), 42.8 % of isolates showed erythromycin-clindamycin inducible resistance, 23.8 % were resistant to gentamicin, tobramycin and amikacin, and 16.7 % presented resistance to tetracycline. Nine MRSA isolates were identified (21.4 %), being 8 of them MDR. There was no significant association between the presence of SCV and MDR (p value = 0.9569), and between the age of the patients and MDR (p value = 0.6622). Table 2 shows the antimicrobial resistance range detected among the 42 *S. aureus* isolates. All of them showed susceptibility for linezolid, chloramphenicol, daptomycin, vancomycin, teicoplanin, ceftaroline, and quinupristin-dalfopristin.

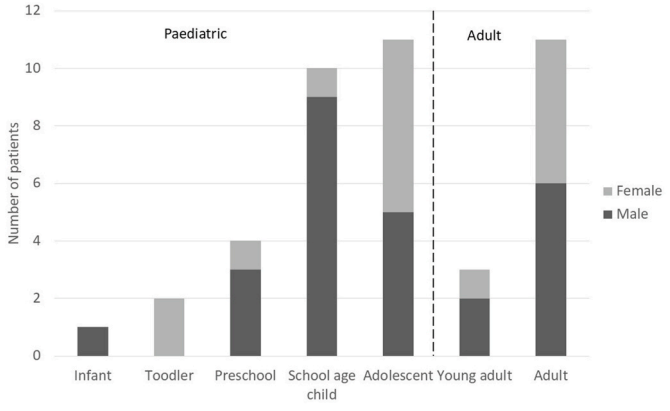

Fig. 1. Distribution of CF patients carrying S. *aureus* isolates, according to gender and age. Infant (up to one year old), toodler (1–3 years-old), preschool (3–6 years-old), school age child (6–12 years-old), and adolescent (12–14 years-old); adult patients were separated between young adult (14–18 years-old) and adult (older than 18 years-old).

Table 1
Phenotypic characteristics detected in the 42 *S. aureus* isolates recovered from CF patients.

Phenotype	Size[a]	Pigmentation[b]	Hemolysis[c]	β-toxin[d]	Nº of isolates
1	N	G	nh	–	11
2	N	G	h	–	6
3	N	G	h	+	2
4	N	Y	nh	–	7
5	N	Y	h	–	7
6	N	Y	h	+	5
7	SCV	G	nh	–	1
8	SCV	G	h	+	1
9	SCV	Y	nh	–	1
10	SCV	Y	h	–	1

[a] Size: N, normal; SCV, small colony variant.
[b] Pigmentation: G, gray; Y, yellow.
[c] Hemolysis: nh, nonhemolytic; h, haemolytic.
[d] β-toxin: +, positive; -, negative.

The antimicrobial resistance mechanisms detected for each antibiotic were the following ones (gene/nº of isolates) (Table 3): penicillin (*blaZ*/39), cefoxitin (*mecA*/9), erythromycin-clindamycin (*erm*(A)/8, *erm*(C)/7; *erm*(T)/6), erythromycin (*msr*(A)/5; *mph*(C)/4), clindamycin (*vga*(A)/6), tetracycline (*tet*(K)/7; *tet*(M)/6), gentamicin-tobramycin (*aac* (6′)-le-*aph* (2″)-la/9, *ant* (4’)-Ia/9), and amikacin (*aph* (3′)-lll/10).

3.4. Molecular characterization of S. aureus isolates

The *S. aureus* isolates were assigned to 11 MLST clonal complexes, being the most common CC398 (26.2 %), CC30 (21.4 %), CC5 (14.3 %), CC45 (9.5 %), and CC121 (7.1 %) (Table 3). The remaining CCs (CC1, CC8, CC10, CC15, CC22 and CC97) showed a frequency of less than 5 %. MLST was performed in thirty representative isolates and a total of 18 STs were detected, being ST398 and ST30 the most commonly found. A high diversity of *spa*-types was detected, with the identification of 30 different *spa*-types. The lineages MRSA-t011-CC398 (9.5 %), MSSA-t230-CC45 (6.7 %), and MSSA-t571-CC398 (6.7 %) were the most frequently *spa*-CC types detected in this study. A new *spa*-type ascribed

Table 2
Antimicrobial resistance detected among the 42 *S. aureus* isolates.

Antibiotics	MRSA[a] Nº (%)	MSSA[b] Nº (%)	All isolates Nº (%)
Penicillin	9 (100.0 %)	30 (90.9 %)	39 (92.9 %)
Oxacillin	9 (100.0 %)	0 (0.0 %)	9 (21.4 %)
Erythromycin	1 (11.1 %)	3 (9.1 %)	4 (9.5 %)
Erythromycin-clindamycin[Ind]	6 (66.7 %)	12 (36.4 %)	18 (42.9 %)
Tetracycline	5 (55.6 %)	2 (6.1 %)	7 (16.7 %)
Gentamicin-tobramycin-amikacin	4 (44.4 %)	5 (15.2 %)	9 (21.4 %)
Tobramycin-amikacin	0 (0.0 %)	1 (3.0 %)	1 (2.4 %)
Trimethoprim-sulfamethoxazole	0 (0.0 %)	3 (9.1 %)	3 (7.1 %)
Mupirocin[c]	0 (0.0 %)	1 (3.0 %)	1 (2.4 %)
Ciprofloxacin	7 (77.8 %)	7 (21.2 %)	14 (33.3 %)
Mdr	8 (88.9 %)	14 (42.4 %)	22 (52.4 %)
Susceptible	0 (0.0 %)	1 (3.0 %)	1 (2.4 %)
Total	9	33	42

[Ind]Erythromycin clindamycin inducible resistance phenotype.
Nº, number of isolates
[a] MRSA, methicillin-resistant *S. aureus*.
[b] MSSA, methicillin-susceptible *S. aureus*.
[c] Mupirocin 256 mg/L.

Table 3
Molecular characteristics of the *S. aureus* isolates from CF-patients.

	Clonal Complex[a]	Sequence Type[b]	*spa*-type (Nº of isolates)	Nº of SCV	IEC	*agr*-type	Virulence genotype	Resistance phenotype[c,d]	Resistance genotype[a]
MRSA	CC398	ST398	t011 (4)	1	–	I	–	PEN, OXA, ERY[3], CLI^{Ind3}, TET, GEN[3], TOB[3], AMK[3], CIP[3]	*blaZ*, *mecA*, *erm*(A)[3], *erm*(T)[1], *vga*(A)[2,] *tet*(K), *tet*(M),*aac* (6′)-le-*aph* (2″)-la[3], *ant* (4′)-Ia[2], *aph* (3′)-lll[3]
	CC5	ST5	t002 (1)	–	–	II	–	PEN, OXA	*blaZ*, *mecA*
		ST6	t304 (1)	–	D	I	–	PEN, OXA, ERY, CIP	*blaZ*, *mecA*, *msr*(A), *mph*(C)
	CC1	ST1	t321 (1)	–	B	III	–	PEN, OXA, ERY, CLI^{Ind}, CIP	*blaZ*, *mecA*, *erm*(A), *erm*(C)
	CC8	ST4224	t1476 (1)	–	B	I	–	PEN, OXA, ERY, CLI^{Ind}, TET, GEN, TOB, AMK, CIP	*blaZ*, *mecA*, *erm*(C), *erm*(T), *tet*(K), *aac* (6′)-le-*aph* (2″)-la, *ant* (4′)-Ia, *aph* (3′)-lll
	CC30	ST30	t342 (1)	–	E	III	–	PEN, OXA, ERY, CLI^{Ind}, TET, CIP	*blaZ*, *mecA*, *erm*(C), *vga*(A), *tet*(K)
MSSA	CC398	ST398	t034 (1)	–	–	I	–	PEN, TET, TOB, AMK	*blaZ*, *tet*(M), *ant* (4′)-Ia, *aph* (3′)-lll
			t108 (2)	1	–	I	-	PEN, ERY, CLI^{Ind1}, TET[1], SXT[1], CIP[1]	*blaZ*, *mrs*(A)[1], *mph*(C)[1], *erm*(C), *tet*(M)[1], *dfr* (A)[1]
			t571 (3)	–	C	I	–	PEN, ERY, CLI^{Ind}, GEN[1], TOB[1], AMK[1], MUP[1]	*blaZ*, *erm*(C)[1], *erm*(T), *vga*(A)[1], *aac* (6′)-le-*aph* (2″)-la[1], *ant* (4′)-Ia[1], *aph* (3′)-lll[1]
			t20352 (1)	–	C	I	–	PEN, ERY, CLI, GEN, TOB, AMK	*blaZ*, *erm*(T), *aac* (6′)-le-*aph* (2″)-la, *ant* (4′)-Ia, *aph* (3′)-lll
	CC30	ST30	t012 (2)	1	–	III	–	PEN, ERY[1], CLI[1], SXT[1], CIP[1]	*blaZ*, *erm*(A)[1], *dfr*(A)[1]
			t021 (1)	–	B	III	*tst*	PEN, ERY, CLI^{Ind}	*blaZ*, *erm*(A), *vga*(A)
			t342 (1)	–	A	III	*tst*	PEN, ERY, CLI^{Ind}	*blaZ*, *erm*(A)
			t4446 (1)	1	B	III	*tst*	PEN, GEN, TOB, AMK	*blaZ*, *aac* (6′)-le-*aph* (2″)-la, *ant* (4′)-Ia, *aph* (3′)-lll
			t17051 (1)	–	A	III	*tst*	PEN	*blaZ*
			t21127 (1)	1	–	III	*tst*	PEN	*blaZ*
		ST1564	t884 (1)	–	B	III	*tst*	PEN, GEN, TOB, AMK, CIP,	*blaZ*, *aac* (6′)-le-*aph* (2″)-la, *ant* (4′)-Ia, *aph* (3′)-lll
	CC5	ST5	t045 (1)	–	–	NT	–	CIP	–
			t306 (1)	1	–	II	*tst*	ERY	*msr*(A), *mph*(C)
		ST6	t304 (1)	–	D	I	–	PEN	*blaZ*
		ST148	t5299 (1)	–	–	NT	–	PEN, ERY, CLI, TET, GEN, TOB, AMK, SXT, CIP	*blaZ*, *erm*(C), *tet*(K), *tet*(M), *aac* (6′)-le-*aph* (2″)-la, *aph* (3′)-lll, *dfr*(G)
	CC45	ST1871	t230 (3)	1	B	I	*tst*[1]	PEN, ERY[1], CLI^{Ind1}	*blaZ*, *erm*(C)
		ST15	t605 (1)	–	B	II	–	PEN, ERY, CLI, CIP	*blaZ*, *msr*(A), *vga*(A)
		ST45	t026 (1)	–	B	I	–	PEN	*blaZ*
	CC121	ST2959	t1994 (2)	1	B	IV	*eta*, *etb*[1]	PEN	*blaZ*
		ST121	t272 (1)	–	C	IV	–	PEN	*blaZ*
	CC1	ST1	t922 (1)	–	–	III	*tst*	PEN	*blaZ*
	CC8	ST4252	t008 (1)	–	B	I	–	PEN	*blaZ*
	CC10	ST19	t240 (1)	–	D	II	*tst*	PEN, ERY	*blaZ*, *msr*(A), *mph*(C)
	CC22	ST22	t130 (1)	–	B	I	*tst*, *eta*	PEN	*blaZ*
	CC97	ST97	t521 (1)	–	B	I	–	–	–
	–	ST509	t375 (1)	1	–	III	*tst*	PEN, ERY, CLI^{Ind}	*blaZ*, *erm*(A)

^{Ind}Erythromycin clindamycin inducible resistance phenotype.
NT, No typeable
[a] CC was assigned according to the ST determined and/or *spa* type detected.
[b] MLST was performed in one strain per *spa* type and all those strains that presented any of the virulence genes analysed.
[c] A number in superscript reflects the number of isolates that have the characteristic when not all isolates of the group have it.
[d] PEN, penicillin; OXA, oxacillin; ERY, erythromycin; CLI, clindamycin; TET, tetracycline; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; CIP, ciproflozaxin; SXT, trimethoprin-sulfamethoxazole; MUP, mupirocin.

to CC30 was registered as t21127 in Ridom Spa Server (t21127: r15-r12-r16-r02-r34-r02-r25-r17-r24-r24). Moreover, *agr*-typing was also performed. The most detected ones were *agr*-type I (47.6 %), and *agr*-type III (28,6 %). The other *agr*-types II and IV were detected in 5 (11.9 %) and 3 (7.1 %) isolates, respectively.

Nine isolates were MRSA and belonged to CC398-t011 (n = 4), CC5-t002/t304 (n = 2), CC1-t321 (n = 1), CC8-t1476 (n = 1), and CC30-t342 (n = 1) (Table 3).

For the virulence content, two MSSA isolates were positives for *eta* and/or *etb* genes (CC121-ST2959-t1994, CC22-ST22-t130) and the *tst* gene was detected in 12 MSSA isolates (CC1-ST1, CC10-ST10, CC22-ST22, CC30-ST30, CC45-ST1871 and ST509). None isolate was positive for the Panton-Valentine leucocidin (PVL)-toxin genes. The presence of the *scn* gene (IEC marker) was detected in 25 isolates. The IEC-negative isolates were associated with CC398, CC5, CC30, and CC1 (Fig. 2). The IEC-type B was the most frequent and was detected in 16 isolates (Table 3).

The SCV morphology was identified in 9 isolates, being two of them of the lineage CC398 (one MRSA-t011 with MDR phenotype and the

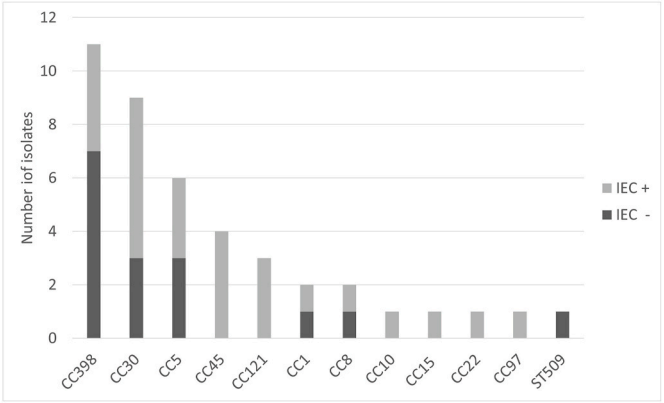

Fig. 2. Distribution of Immune Evasion Cluster (IEC) according to MLST clonal complexes. CC, Clonal Complex; ST, Sequence Type.

other MSSA-t108). The remaining 7 MSSA isolates with SCV morphology were associated with 4 CCs (CC5, CC30, CC45, and CC121) (Table 3).

Eleven of the 42 *S. aureus* isolates (26.1 %) were ascribed to CC398 lineage, and four of them were MRSA with *spa*-type t011 (lacked the *scn* gene and carried the *tet*(M) gene). The seven CC398-MSSA isolates, corresponded to 4 *spa*-types: t571 (n = 3), t108 (n = 2), and t034 and t20352 (one isolate each). The 4 isolates of the *spa*-types t571 and t20352 carried the IEC system (type C) and also the gene *erm*(T) responsible for ERY^{R}-CLI^{Ind} resistance phenotype and were tetracycline-susceptible. The other 3 MSSA-CC398 isolates (t034, and t108) did not present the IEC system and two of them were tetracycline-resistant (*spa*-types t034, t108).

4. Discussion

It is known that patients with CF develop recurrent infections during their lifetime, and as they grow up, the organisms identified in their airways change [4]. *S. aureus* is one of the most commonly detected microorganisms in the early years of children with CF, with a prevalence exceeding 50 % in patients under 2 years of age, and 80 % in early adolescence [17]. In our study, *S. aureus* was detected in 64.6 % of CF-patients being 62.2 % paediatrics. Patients with MRSA isolates have higher rates of hospitalisation and higher mortality because these patients are characterised by lower lung function [17]. Centres with higher MRSA rates are those with older CF patients [12]. Our MRSA isolates were detected in 9 patients (21.4 %), all of them from paediatric patients except one that came from a young adult. However, it must be considered that almost of CF patients of our studied hospital were paediatric (28/42).

There is a phenotypic change in *S. aureus* isolates to adapt and survive in the respiratory tract, which is known as Small Colony Variant (SCV). SCV confers adaptive advantages such as increased antibiotic resistance and persistence [18]. Nine SCV *S. aureus* isolates were detected among the 42 isolates (21.4 %). A slightly higher percentage than those found in other works in CF-patients in Belgium [12], the USA [19], Austria [20], Czeck Republic [21], and Spain [22]. Previous studies have shown that SCV and especially SCV-MRSA isolates are highly resistant to trimethoprim/sulfamethoxazole, an antibiotic frequently used for the treatment of lung infections in CF-patients [19]. In our case, only one of the SCV isolates exhibited resistance to trimethoprim/sulfamethoxazole. It is important to remark that many times it is difficult to detect this type of phenotype since these SCV isolates can change to wild type while they are manipulated in the microbiology lab [23].

The occurrence of multidrug-resistant *S. aureus* isolates is one of the major problems that CF-patients must face. In this study, 52.4 % of *S. aureus* isolates were MDR; most of them were resistant to β-lactams, macrolides and lincosamides in combination with other antibiotic families such as tetracycline or aminoglycosides. As the age of patients increases, the number of MDR isolates increases, in the same way that co-infection with *P. aeruginosa* [24]. However, no significant difference was detected in the association between age and MDR.

A high diversity of clonal complexes was found, being CC398 (26.2 %), CC30 (21.4 %), CC5 (14.3 %), CC45 (9.5 %), and CC121 (7.1 %) the most frequently detected. This is in accordance with the results of other studies in CF-patients [2,12]. The emergence of LA-MRSA isolates in CF-patients is of concern and may suggest that contact with farm animals (especially pigs) or with humans in contact with pig farms can be a risk for these patients. A previous multicentre study performed in 20 Spanish hospitals demonstrated that the prevalence of LA-MRSA-CC398 at hospital level is significatively associated with the density of pig farming in the region in which the hospital is located [25]. The hospital in which the present study was performed is in a region of high pig farming density, and LA-MRSA-CC398 could represent 7.2 % of all MRSA recovered from clinical samples in that hospital [25].

Eleven isolates (26.1 %) belonging to CC398 were detected, 4 of them MRSA-CC398-t011. All MRSA-CC398-t011 isolates were tetracycline resistant and lacked IEC genes. Previous studies have already shown that methicillin and tetracycline resistance and the absence of IEC genes are markers of the CC398 animal-adapted clade [26]. Similar results have been observed in other countries. In Belgium and Germany MRSA-CC398-t011 was also detected in CF patients [12,13]. In Germany [13] and in Brazil [11] MRSA CC398-t034 isolates were detected. In our study, the *spa*-type t034 was only found in one MSSA isolate. Our 3 MSSA-CC398-t571 isolates and one MSSA-CC398-t20352 isolate presented an erythromycin resistance-clindamycin inducible resistance phenotype, carried the *erm*(T) gene, and were IEC-positive of type C, typical characteristics of the human-adapted clade [12]. Nevertheless, the MSSA-CC398 of *spa*-types t034 and t108 isolates lacked the IEC genes and were positive for the gene *tet*(M) gene, characteristic of the animal-adapted clade [27].

IEC-negative *S. aureus* isolates belonging to other CCs (non CC398, such as CC1, CC5 and CC30) were also detected; the lack of the IEC system is frequently found in isolates of animal origin, but in a low percentage in isolates of human origin. These clonal lineages have already been previously associated with some livestock animals [28].

In addition to livestock clonal lineages, the presence of important hospital-associated (HA) or community-associated (CA) clones must be also monitored, being remarkable the detection of MRSA CC5, CC8 or CC30 [2,10,29]. Moreover, virulence factors also have an important influence on *S. aureus* infections. PVL toxin is associated with skin and soft tissue infections, but also with necrotising pneumonia. In this study, PVL toxin was not detected in any of the *S. aureus* isolates. This is in line with other CF studies in which the persistence of this toxin was very low or absent [2,30]. The *tst* gene was detected in 26.1 % of the isolates belonging to diverse CCs (CC1-ST1, CC10-ST10, CC22-ST22, CC30-ST30, CC45-ST1871 and ST509). Other studies carried out in CF-patients have already detected high percentages of this gene in their isolates [3]. Moreover, one MSSA-CC121-ST2959-t1994 carried out both *eta* and *etb* genes and two CC121-ST2959-t1994/CC22-ST22-t130 isolates were positive for *eta* gene.

5. Conclusions

In conclusion, MRSA and MSSA CC398 isolates were frequently identified among CF patients showing characteristic of both human- and animal-adapted clades (and subclades). The emergence of LA-MRSA clonal lineages in CF-patients should be monitored and their epidemiology analysed.

CRediT authorship contribution statement

Paula Eguizábal: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Ana Isabel López-Calleja:** Writing – review & editing, Resources, Formal analysis. **Antonina Arias:** Writing – review & editing, Resources, Formal analysis. **Irene Antoñanzas-Torres:** Writing – review & editing, Resources, Formal analysis. **Allelen Campaña-Burguet:** Writing – review & editing, Methodology. **Carmen González-Azcona:** Writing – review & editing, Methodology. **Agustí Martínez:** Writing – review & editing, Formal analysis. **Carlos Martin-de Vicente:** Writing – review & editing, Resources, Formal analysis. **Inés Herrero:** Writing – review & editing, Resources, Formal analysis. **Antonio Rezusta:** Writing – review & editing, Resources, Formal analysis. **Carmen Torres:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Carmen Lozano:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Ethical approval

This study was approved by the ethical committee of the Hospital Universitario Miguel Servet of Zaragoza, Spain (PI 22–308).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.micpath.2024.107016.

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