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Nasal carriage of coagulase positive staphylococci in patients of a Primary-Healthcare-Center: genetic lineages and resistance and virulence genes



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ABSTRACT

Introduction: *Staphylococcus aureus* and *Staphylococcus pseudintermedius* are highly important due to their capacity for producing diseases in humans and animals, respectively. The aim of the study was to investigate and characterize the coagulase positive *Staphylococcus* (CoPS) carriage in a Primary Healthcare Center population.

Methods: Nasal swabs were obtained from 281 non-infectious patients. The CoPS isolates recovered were typed, and their resistance phenotype and genotype, as well as their virulence profiles, were analyzed.

Results: CoPS isolates were recovered from 56/281 patients (19.9%). Fifty-five were *S. aureus* (19.6%), 54 were methicillin susceptible (MSSA) and one was methicillin resistant (MRSA). The remaining isolate was *S. pseudintermedius* (0.4%). A high diversity of *spa*-types ($n=40$) was detected, with 6 of them being new ones. The multi-locus-sequence-typing of 13 MSSA and one MRSA selected isolates was performed and the STs detected were: ST8, ST15, ST30, ST34, ST121, ST146, ST398, ST554, ST942, ST2499, and ST2500 (the last two STs being new). One MSSA isolate was typed as t1197-ST398-(Clonal complex)CC398. The MRSA isolate was typed as t002-ST146-CC5-SCCmec-IVc, and exhibited a multiresistance phenotype. The detected resistances were: penicillin (76%), macrolides (7%), tetracycline (7%), trimethoprim-sulfamethoxazole (7%), quinolones (7%), and lincosamides (5%). Five isolates contained *lukF/lukS*-PV genes, 17 *tst* gene, one *eta* gene, and two *etb* gene. The *S. pseudintermedius* isolate presented a new *spa*-type (t57) (belonging to a new ST180) and the genes *lukS/F-I*, *siet*, *se-int*, and *expB*.

Conclusions: A high genetic diversity of *S. aureus* was detected. Mention must be made of the identification of MSSA CC398 and *S. pseudintermedius* isolates in two patients, one of them with animal contact. The detection of the genes *lukF/lukS*-PV and *tst* should be noted.

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Staphylococcus coagulasa positiva en muestras nasales de pacientes de un centro de atención primaria: líneas genéticas y contenido en genes de resistencia y de virulencia

RESUMEN

Palabras clave:

Staphylococcus aureus

CC398

SARM

SASM

Introducción: *Staphylococcus aureus* y *Staphylococcus pseudintermedius* son 2 especies de gran importancia que pueden producir enfermedades tanto en personas como en animales. El objetivo del trabajo fue estudiar el estado de portador nasal de aislados de *Staphylococcus* coagulasa positiva (SCoP) en pacientes de un centro de atención primaria.

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Staphylococcus pseudintermedius
LPV
TSST-1
Portador nasal

Métodos: Se analizaron muestras nasales de 281 pacientes sin patología infecciosa. Se tiparon los aislados SCoP y se estudiaron sus fenotipos y genotipos de resistencia y sus perfiles de virulencia.

Resultados: Se aislaron SCoP en 56/281 pacientes (19,9%): 55 de los aislados fueron *S. aureus* (19,6%), 54 sensibles a la meticilina (SASM) y uno resistente a la meticilina (SARM). El aislado restante correspondió a *S. pseudintermedius* (0,4%). Se detectó una alta diversidad de tipos de *spa* ($n=40$), identificándose 6 nuevos tipos. Se realizó el multi-locus-sequence-typing de 13 cepas SASM y una cepa SARM seleccionadas y se detectaron los siguientes STs: ST8, ST15, ST30, ST34, ST121, ST146, ST398, ST554, ST942, ST2499 y ST2500 (los 2 últimos nuevos). Una de las cepas SASM se tipó como t1197-ST398-(Clonal Complex)CC398. La cepa SARM se tipó como t002-ST146-CC5-SCCmec-IVc y mostró un fenotipo de multirresistencia. Se detectó resistencia a: penicilina (76%), macrólidos (7%), tetraciclina (7%), trimetoprim-sulfametoxazol (7%), quinolonas (7%) y lincosamidas (5%). Se identificaron los genes (número de cepas): *lukF/lukS*-PV (5), *tst* (17), *eta* (1) y *etb* (2). La cepa de *S. pseudintermedius* presentó un *spa* nuevo (t057), una secuencia tipo nueva (ST180), y contenía los genes *lukS/F-I*, *siet*, *se-int* y *expB*.

Conclusiones: Se detectó una alta diversidad genética entre los aislados de SASM. Destaca la identificación de una cepa SASM CC398 (en un veterinario) y otra de *S. pseudintermedius*, y la frecuente detección de los genes *lukF/lukS*-PV, *tst*, *eta* o *etb* entre las cepas SASM.

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Introduction

Staphylococcus spp. are known to be colonizer agents of humans and animals. Some of the species included in this genus are also important pathogens, which are able to produce numerous virulence factors. Moreover, these microorganisms can acquire different resistance genes, highlighting the increase of methicillin resistance mediated by the gene *mecA*. There are two main coagulase positive *Staphylococcus* (CoPS) species, *Staphylococcus aureus* and *Staphylococcus pseudintermedius*, which are highly important due to their capacity of producing important diseases in humans and animals, respectively.

S. aureus is the most known and virulent species in humans. This pathogen can produce slight skin infections, food poisoning or life threatening diseases such as pneumonia, meningitis, or septicemia, among others. Up to one-third of the healthy human population is intermittently colonized and another third is *S. aureus* persistent nasal carrier.¹ It has been demonstrated that *S. aureus* nasal carriers have a higher risk of developing an infection with this microorganism.² There has been an important increase of the rate of methicillin resistant *S. aureus* (MRSA) in hospital environments (HA-MRSA) and in the community (CA-MRSA), with some clones being predominant in determined geographic areas.^{3,4} Initially, sporadic cases caused by CA-MRSA clones were described in our country.^{5,6} These community associated cases were detected in immigrant patients especially in those coming from South American countries.⁶ However, nowadays, the differences between HA-MRSA and CA-MRSA have diminished and infections apparently caused by CA-MRSA have been increasingly detected in our hospitals.^{7,8} Moreover, in 2005, a new clonal lineage named MRSA ST398 was reported.^{9,10} Since then, its relationship with farm animals, especially pigs, has been observed. The risk of colonization and infection of farmers has been identified,¹¹ this clone being also identified in healthy humans without animal contact.¹²

S. pseudintermedius is the main colonizing agent of healthy dogs and cats¹³ and also is a common pathogen of these animals, usually causing skin and soft tissue infections (SSTIs).¹⁴ The transmission of *S. pseudintermedius* isolates between pets and their owners has been suggested in some studies.^{15,16} Moreover, cases of infections in humans caused by this microorganism have been described.¹⁷ Therefore, the presence of this microorganism as a colonizer or infecting agent in humans cannot be discarded.

There are few data about the real prevalence of *S. aureus* and *S. pseudintermedius* carriage in patients of the community in our country. The aim of this study was to investigate the CoPS carriage (CoPSC) in a population attending to a Primary Healthcare Center

(for non-infectious diseases), located in a region of Spain characterized by a high percentage of immigration and high density of pig farms. In addition, it was intended to know the genetic lineages of recovered isolates and to study their antibiotic resistance mechanisms and virulence factors.

Materials and methods

Bacterial isolates

Nasal swabs of 281 non-infectious patients were obtained from October 2009 to March 2011 in a Health Primary Center of Lérida (Catalonia, Spain). Informed consent was obtained from all the patients. Individuals tested were from different geographic locations (number of patients): Lérida (Catalonia, Spain) (154), Center-Europe (18), Asia (15), Africa (69), South America (23) and unknown (2). Samples were seeded on blood agar (Oxoid®), CNA agar (bioMérieux®), and ORSA (Oxoid®) plates for the recovery of CoPS, and plates were incubated at 35 °C for 36 h. Identification of *S. aureus* and *S. (pseud)intermedius* was performed by conventional methods, coagulase and API STAPH (bioMérieux®), and by specific PCR.¹⁵ PCR-RFLP of *pta* gene with *MboI* endonuclease was performed to differentiate between *S. intermedius* and *S. pseudintermedius* isolates.¹⁵

Molecular typing of isolates

Single-locus DNA sequencing of the gene *spa* encoding *S. aureus* protein A^{7,15} was carried out in all *S. aureus* and *S. pseudintermedius* isolates and *S. aureus* sequences obtained were analyzed using Ridom Staph-Type software version 1.5.21 (Ridom GmbH). SCCmec-typing was performed by multiplex PCR strategy in MRSA.⁷ Multilocus Sequence Typing (MLST) was implemented in 14 selected *S. aureus* isolates (*S. aureus* isolates which presented new *spa*-types, or *spa*-types related to ST398, or isolates which contained the genes *lukF/lukS*-PV encoding the Pantone-Valentine leucocidin, [PVL], or presented methicillin resistance) (www.saureus.mlst.net) and in one *S. pseudintermedius* isolate.¹⁵ In the 14 *S. aureus* isolates, their Clonal Complexes (CCs) were achieved using BURST analyses.

Susceptibility testing and detection of antimicrobial resistance genes

Susceptibility testing was carried out by VITEK 2 system (bioMérieux®) and disk-diffusion agar method following

the CLSI guidelines.¹⁸ Antibiotics tested were as follows: penicillin, oxacillin, cefoxitin, erythromycin, telithromycin, clindamycin, quinupristin-dalfopristin, gentamicin, streptomycin, kanamycin, tobramycin, tetracycline, ciprofloxacin, levofloxacin, chloramphenicol, trimethoprim-sulfamethoxazole, vancomycin, teicoplanin, mupirocin, fusidic acid, linezolid, fosfomycin, nitrofurantoin, and rifampin.

The presence of *blaZ*, *mecA*, *erm(A)*, *erm(B)*, *erm(C)*, *erm(F)*, *erm(T)*, *msr(A)*/*msr(B)*, *tet(K)*, *tet(L)*, *tet(M)*, *tet(O)*, *dfrS1*, *dfrD*, *dfrG*, *dfrK*, and *vanA* resistance genes was investigated by PCR.^{7,15,19}

Virulence factors

The presence of genes encoding PVL (*lukF/lukS-PV*), TSST-1 (*tst*), Exfoliative Toxin A (ETA) (*eta*), B (ETB) (*etb*), and D (ETD) (*etd*) was studied by PCR in all *S. aureus* isolates.⁷ In *S. pseudintermedius* the virulence genes tested by PCR were: *lukS/F-I*, *siet*, *se-int*, *expB*, *sec_{canine}*, and *expA*.^{15,20,21}

Results

Bacteria isolates detected

CoPS isolates were recovered from 56 of the 281 tested samples (19.9%) and one isolate per positive sample was further studied. Among the 56 CoPS isolates, 55 of them were *S. aureus* (19.6%): 54 methicillin susceptible *S. aureus* (MSSA) and one methicillin resistant *S. aureus* (MRSA). The remaining isolate was *S. pseudintermedius* (0.4%).

Characteristics of *S. aureus* isolates

Fifty-five *S. aureus* isolates were obtained from nasal samples of patients of different nationalities (% of recovery respect to the studied samples of each nationality): 36 isolates from patients of L rida, Spain (23.4%), one from Center Europe (5.5%), 2 from Asia (13%), 10 from Africa (14%), 4 from South America (17%), and 2 of unknown nationality. A high diversity of *spa*-types was detected among these isolates obtained (Table 1). Thus, 40 different *spa*-types were identified, 6 of them being new ones. These new *spa*-types were registered in Ridom database with the accession numbers t6387, t6388, t6389, t6390, t6391, and t6392. Fourteen selected isolates were studied by MLST (one MRSA and 13 MSSA). The MRSA isolate was typed as t002-ST146-CC5-SCCmec-IVc. The seven MSSA isolates with new *spa*-types belonged to ST8-CC5, ST15-CC5, ST34-CC30, ST121-CC121, and ST554-CC5. One MSSA isolate presented the *spa* type t1197 and belonged to ST398-CC398. This isolate was obtained from a veterinarian who worked with cattle and pigs. Other 5 MSSA isolates were selected because they contained the genes *lukF/lukS-PV* and belonged to ST30-CC30, ST942-CC942, ST2499-CC22, and ST2500-CC942, the last two STs being new.

Forty-two *S. aureus* isolates showed penicillin resistance (76%) and all of them presented the *blaZ* gene. Four isolates were macrolide resistant (7%), three of them being lincosamide resistant (5%). In these isolates, the *msr(A)*/*msr(B)*, *erm(A)* or *erm(C)* genes were identified. Other detected resistances were: methicillin resistance (2%) encoded by *mecA* gene, tetracycline resistance (7%) mediated by *tet(K)* and *tet(M)* genes, trimethoprim-sulfamethoxazole resistance (7%) encoded by *dfrS1* and *dfrK* genes, and quinolone resistance (7%). One isolate showed diminished susceptibility to vancomycin (MIC = 3 mg/L) (Table 1).

Moreover, five isolates contained the *lukF/lukS-PV* genes (9%), 17 isolates the *tst* gene (31%), one isolate the *eta* gene (2%), and two isolates the *etb* gene (4%). Two of the *lukF/lukS-PV*-positive isolates also harbored the gene *tst* (Table 1). The PVL positive isolates

were obtained from five patients of different nationalities: Europe (1 isolate), South America (1 isolate), India (1 isolate), and Africa (2 isolates) (Table 1).

Characteristics of the MRSA isolate

Only one sample contained a MRSA isolate (0.4%). This isolate (C2741) presented the *spa* type t002 and was typed as ST146-CC5, and its SCCmec was type IVc and presented resistance, in addition to beta-lactams, to macrolides, lincosamides, tobramycin, and quinolones. The patient was an 80-years-old Spanish man who presented multiple pathologies (liver cirrhosis, chronic arterial hypertension, atrial fibrillation, etc.), and had a high contact with the Primary Healthcare Center. However, he did not have hospital admissions in the previous year.

Characteristics of the *S. pseudintermedius* isolate

One methicillin susceptible *S. pseudintermedius* isolate was obtained (0.4%) from an Indian patient that referred no animal contact. This isolate (C2536) presented a new *spa*-type t57 which consisted of a new repeat combination (r01 r09 r21 r02 r02 r03 r03 r06 r05). Moreover, the isolate C2536 also belonged to a new ST named ST180 which presented two new alleles (*cnp60.41* and *pta_29*). This isolate was susceptible to all tested antibiotics and presented the toxin genes *lukS/F-I*, *siet*, *se-int*, and *expB*.

Discussion

A rate of *S. aureus* of 19.6%, a low percentage of MRSA (0.4%) and a low percentage of *S. pseudintermedius* (0.4%) were found as colonizer agents in the studied patients. Some authors refer that the *S. aureus* nasal colonization rate in the general population is about 30%.²² Nevertheless, some recent studies have reported lower percentages (about 20%). In this sense, Bode et al.²³ reported a prevalence of 18.8% using real-time PCR. Den Heijer et al.²⁴ had similar results (21.6%) in a cross-sectional study which included 9 European countries. The differences detected among the different countries were from 12.7% (Hungary) to 29.4% (Sweden); the percentage detected in Spain, in that study,²⁴ was of 18.8%. In another study carried out in Spain¹² the percentage detected (19.1%) was very similar to the one found in our studied population. In our work, the highest rate of colonization was found in European patients (20%) and the lowest value in Asian patients (13%). However, it should be taken into account that the majority of samples studied were from patients of our country (European patients).

The prevalence of MRSA in our study was 0.4%. The percentages detected in other studies performed among healthy people^{12,24} varied between 0.2 and 2%. However, in a very recent study in our country, a higher value was identified (6%).²⁵

Regarding *S. pseudintermedius* rate, this microorganism is more commonly found in dogs than in humans. Different studies have reported *S. pseudintermedius* colonization values on healthy dogs highly variable (23 and 92%).²⁶ However, these uneven results mainly depend on the body-site in which the samples are taken.²⁷ In humans, the presence of this microorganism is associated with individuals having regular contact with pets such as veterinarians and owners.^{15,28} Nevertheless, our *S. pseudintermedius* isolate was obtained from an Indian patient that referred no contact with animals.

Interestingly, one MSSA isolate belonging to CC398 was obtained from a patient who worked as a veterinarian in a farm with pigs and cattle. In the case of methicillin resistant strains, this CC is related to livestock animals. It has been observed that people in contact with farm animals are more frequently carriers of

Table 1
Characteristics of the 55 *S. aureus* isolates recovered from nasal samples of ambulatory patients.

Strain number	<i>spa</i> -type ^a	MLST (CC) ^b	Toxin genes	Antimicrobial resistance phenotype ^c	Antimicrobial resistance genes	Nationality of patient (Continent)
C2823	t1197	ST398 (CC398)		PEN-TET-ERY-CLI-CIP-LEV-SXT	<i>blaZ</i> , <i>tet</i> (M), <i>erm</i> (C), <i>dfrS1</i> , <i>dfrK</i>	Spanish (Europe)
C2741	t002	ST146 (CC5)		PEN-OXA-FOX-ERY-CLI-TOB-CIP-LEV	<i>mecA</i> , <i>erm</i> (C), <i>ant</i> (4')-Ia, <i>aph</i> (3')-IIIa	Spanish (Europe)
C2758	t002		<i>tst</i>			unknown
C2743	t002					Spanish (Europe)
C2992	t008			PEN	<i>blaZ</i>	Romanian (Europe)
C2993	t008			PEN	<i>blaZ</i>	Nigerian (Africa)
C2989	t008			TET	<i>tet</i> (M)	Unknown
C2770	t010			ERY-CIP-LEV	<i>msr</i> (A)/ <i>msr</i> (B)	Spanish (Europe)
C2754	t078			PEN	<i>blaZ</i>	Spanish (Europe)
C2789	t084			TET-SXT	<i>tet</i> (K), <i>dfrS1</i>	Malian (Africa)
C2790	t084			PEN	<i>blaZ</i>	Angolan (Africa)
C2778	t084		<i>eta</i>	PEN-SXT	<i>blaZ</i> , <i>dfrS1</i>	Spanish (Europe)
C2771	t094		<i>tst</i>	PEN-TET	<i>blaZ</i> , <i>tet</i> (K)	Spanish (Europe)
C2994	t189			PEN	<i>blaZ</i>	Mauritanian (Africa)
C2753	t346			PEN	<i>blaZ</i>	Nigerian (Africa)
C2751	t6387	ST15 (CC5)				Spanish (Europe)
C2755	t6387	ST15 (CC5)				Spanish (Europe)
C2739	t6391	ST8 (CC5)		PEN		Spanish (Europe)
C2769	t6392	ST554 (CC5)				Spanish (Europe)
C2824	t164			SXT	<i>dfrS1</i>	Indian (Asia)
C2888	t164			PEN	<i>blaZ</i>	Moroccan (Africa)
C2773	t005			PEN	<i>blaZ</i>	Spanish (Europe)
C2772	t005	ST2499 (CC22)	<i>lukF/lukS</i> -PV	PEN		Indian (Asia)
C2748	t012		<i>tst</i>	PEN	<i>blaZ</i>	Spanish (Europe)
C2756	t012		<i>tst</i>	PEN	<i>blaZ</i>	Spanish (Europe)
C2742	t012		<i>tst</i>	PEN	<i>blaZ</i>	Spanish (Europe)
C2759	t021		<i>tst</i>	PEN	<i>blaZ</i>	Colombian (South America)
C2780	t018		<i>tst</i>	PEN	<i>blaZ</i>	Spanish (Europe)
C2776	t253		<i>tst</i>	PEN	<i>blaZ</i>	Spanish (Europe)
C2988	t789	ST30 (CC30)	<i>lukF/lukS</i> -PV, <i>tst</i>	PEN	<i>blaZ</i>	Moroccan (Africa)
C2991	t789	ST30 (CC30)	<i>lukF/lukS</i> -PV, <i>tst</i>	PEN	<i>blaZ</i>	Moroccan (Africa)
C2750	t1076			PEN	<i>blaZ</i>	Spanish (Europe)
C2757	t1333			PEN	<i>blaZ</i>	Spanish (Europe)
C2777	t6388	ST34 (CC30)	<i>tst</i>	PEN	<i>blaZ</i>	Spanish (Europe)
C2760	t6390	ST34 (CC30)		PEN	<i>blaZ</i>	Spanish (Europe)
C2740	t015			PEN	<i>blaZ</i>	Spanish (Europe)
C2744	t031			PEN	<i>blaZ</i>	Spanish (Europe)
C2784	t065			PEN-ERY-CLI	<i>erm</i> (A), <i>blaZ</i>	Spanish (Europe)
C2990	t230			PEN	<i>blaZ</i>	Moroccan (Africa)
C2995	t230			PEN	<i>blaZ</i>	Moroccan (Africa)
C2788	t330					Spanish (Europe)
C2781	t3537			PEN	<i>blaZ</i>	Spanish (Europe)
C2821	t269			PEN	<i>blaZ</i>	Spanish (Europe)
C2786	t272		<i>etb</i>			Spanish (Europe)
C2782	t272		<i>etb</i>	PEN	<i>blaZ</i>	Spanish (Europe)
C2787	t6389	ST121 (CC121)	<i>tst</i>	PEN	<i>blaZ</i>	Spanish (Europe)
C2747	t1445	ST942 (CC942)	<i>lukF/lukS</i> -PV, <i>tst</i>	VAN [†]		Colombian (South America)
C2822	t1445	ST2500 (CC942)	<i>lukF/lukS</i> -PV, <i>tst</i>	PEN		Spanish (Europe)
C2746	t360		<i>tst</i>	PEN	<i>blaZ</i>	Spanish (Europe)
C2779	t779			PEN	<i>blaZ</i>	Spanish (Europe)
C2745	t869			PEN	<i>blaZ</i>	Ecuadorian (South America)
C2775	t1326		<i>tst</i>	PEN	<i>blaZ</i>	Spanish (Europe)
C2783	t1900			PEN-CIP-LEV	<i>blaZ</i>	Spanish (Europe)
C2889	t2353					Spanish (Europe)
C2785	t2646		<i>tst</i>			Brazilian (South America)

^a The new *spa*-types are marked in bold.

^b The new STs are marked in bold.

^c PEN, penicillin; TET, tetracycline; ERY, erythromycin; CLI, clindamycin; TOB, tobramycin; CIP, ciprofloxacin; LEV, levofloxacin; SXT, trimethoprim-sulfamethoxazole

MRSA CC398 than people who do not have that contact.²⁹ Recently, a second epidemiological event consisting in the emergence of MSSA CC398 human infections has been detected.³⁰ Indeed, it is thought that the origin of livestock-associated MRSA CC398 could be MSSA of humans.^{31,32} So, MSSA CC398 strains have previously been detected as colonizer agents in healthy humans in Spain¹² and in other countries.³²

A high diversity of *spa*-types was identified which is in accordance with other studies about MSSA strains.^{12,33} Remarkably, the strains C2739, C2989, C2992, and C2993 showed characteristics typical to community strains. These isolates were susceptible to all antimicrobial tested except penicillin or tetracycline, were obtained from non-infectious patients and three of them presented the *spa*-type t008. One of the most important community MRSA

clones is known as USA300 which is typed as ST8-t008 and contains the PVL genes.^{34,35} In our case, isolates were methicillin susceptible. It has been hypothesized that MSSA of CC8, sequence type 8, is the presumptive ancestor of the first MRSA USA500 and USA300 strains.³⁶

Interestingly, the PVL toxin genes were not found in these isolates but in strains belonging to CC30. The presence of this toxin in CC30 clones has frequently been identified³⁷ and the same applies with the *tst* gene.^{12,38} The CC30 is one of the major *S. aureus* lineages and it is related to hospital-acquired and community-acquired infections worldwide.

Only one strain was methicillin resistant. This isolate was typed as ST146-CC5, *spa*-type t002 and SCCmec IVc and presented a multiresistance phenotype. Strains with similar characteristics are considered hospital acquired (HA) MRSA. Although strains with the *spa*-type t002 are very common in the hospitals of our country, the ST146 is found less frequently.^{7,39} Moreover, in a very recent study the *spa* type t002 was the most commonly detected among nasal MRSA from European patients.²⁴

High percentage of penicillin resistance was detected among our *S. aureus* isolates (76%), in agreement with other studies.^{7,24} The values of resistance to methicillin, macrolides and tetracycline were also in accordance with the ranges observed by den Heijer et al.,²⁴ highlighting the trimethoprim-sulfamethoxazole resistance rate which was very elevated (7%).

With respect to the *S. pseudintermedius* strain isolated in this study the detection of a new *spa*-type (t57) and a new ST (ST180) is remarkable. However, this result was expected as this isolate was methicillin susceptible (MS) and a high clonal diversity has been previously observed among MS *S. pseudintermedius* isolates.^{15,40} Moreover, this isolate harbored the *lukS/F-I*, *siet*, *se-int*, an *expB* toxin genes. The *expB* gene was recently described,²¹ and it seems to be related to cases of superficial dermatitis in dogs.⁴⁰

Conclusions

In conclusion, a high genetic diversity of *S. aureus* was detected in nasal samples of ambulatory patients. The identification of MSSA CC398 and *S. pseudintermedius* isolates in two patients, one of them veterinarian, is highly remarkable. The detection of virulent strains in general population which contained the genes *lukF/lukS-PV* and *tst* should be noted and considered in the development of control measures.

Conflict of interest

The authors declare no conflict of interest.

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References

- Gordon RJ, Lowy FD. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clin Infect Dis. 2008;46:350–9.
- Wertheim HF, Melle DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis. 2005;5:751–62.
- Deurenberg RH, Stobbering EE. The evolution of *Staphylococcus aureus*. Infect Genet Evol. 2008;8:747–63.
- Grundmann H, Aanensen DM, van den Wijngaard CC, Spratt BG, Harmsen D, Friedrich AW. Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. PLoS ONE. 2010;7:e1000215.
- Aspiroz C, Martín I, Lozano C, Torres C. First case of community-acquired Pantone-Valentine leukocidin-positive (ST88) methicillin-resistant *Staphylococcus aureus* bacteremia in Spain in a patient with meningitis. Enferm Infecc Microbiol Clin. 2010;28:70–1.
- Cercenado E, Cuevas O, Marín M, Bouza E, Trincado P, Boquete T, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in Madrid, Spain: transcontinental importation and polyclonal emergence of Pantone-Valentine leukocidin-positive isolates. Diagn Microbiol Infect Dis. 2008;61:143–9.
- Lozano C, Porres-Osante N, Crettaz J, Rojo-Bezares B, Benito D, Olarte I, et al. Changes in genetic lineages, resistance, and virulence in clinical methicillin-resistant *Staphylococcus aureus* in a Spanish hospital. J Infect Chemother. 2013;19:233–42.
- Menegotto F, González-Cabrero S, Lorenzo B, Cubero A, Cuervo W, Gutiérrez MP, et al. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a Spanish hospital over a 4-year period: clonal replacement, decreased antimicrobial resistance, and identification of community-acquired and livestock-associated clones. Diagn Microbiol Infect Dis. 2012;74:332–7.
- Armand-Lefevre L, Ruimy R, Andrement A. Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. Emerg Infect Dis. 2005;11:711–4.
- Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg Infect Dis. 2005;11:1965–6.
- Lozano C, Aspiroz C, Ezpeleta AI, Gómez-Sanz E, Zarazaga M, Torres C. Empyema caused by MRSA ST398 with atypical resistance profile, Spain. Emerg Infect Dis. 2011;17:138–40.
- Lozano C, Gómez-Sanz E, Benito D, Aspiroz C, Zarazaga M, Torres C. *Staphylococcus aureus* nasal carriage, virulence traits, antibiotic resistance mechanisms, and genetic lineages in healthy humans in Spain, with detection of CC398 and CC97 strains. Int J Med Microbiol. 2011;301:500–5.
- van Duijkeren E, Catry B, Greko C, Moreno MA, Pomba MC, Pyörälä S, et al. Review on methicillin-resistant *Staphylococcus pseudintermedius*. J Antimicrob Chemother. 2011;66:2705–14.
- Perreten V, Kadlec K, Schwarz S, Grönlund Andersson U, Finn M, Greko C, et al. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. J Antimicrob Chemother. 2010;65:1145–54.
- Gómez-Sanz E, Torres C, Lozano C, Zarazaga M. High diversity of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* lineages and toxigenic traits in healthy pet-owning household members. Underestimating normal household contact? Comp Immunol Microbiol Infect Dis. 2013;36:83–94.
- Laarhoven LM, de Heus P, van Luijn J, Duim B, Wagenaar JA, van Duijkeren E. Longitudinal study on methicillin-resistant *Staphylococcus pseudintermedius* in households. PLoS ONE. 2011;6:e27788.
- Riegel P, Jesel-Morel L, Laventie B, Boisset S, Vandenesch F, Prévost G. Coagulase-positive *Staphylococcus pseudintermedius* from animals causing human endocarditis. Int J Med Microbiol. 2011;301:237–9.
- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: twentieth informational supplement. M100-S21 Wayne, PA, USA: CLSI; 2011.
- Woodford N, Morrison D, Johnson AP, Briant V, George RC, Cookson B. Application of DNA probes for rRNA and *vanA* genes to investigation of a nosocomial cluster of vancomycin-resistant enterococci. J Clin Microbiol. 1993;31:653–8.
- Iyori K, Futagawa-Saito K, Hisatsune J, Yamamoto M, Sekiguchi M, Ide K, et al. *Staphylococcus pseudintermedius* exfoliative toxin EXI selectively digests canine desmoglein 1 and causes subcorneal clefts in canine epidermis. Vet Dermatol. 2011;22:319–26.
- Iyori K, Hisatsune J, Kawakami T, Shibata S, Murayama N, Ide K, et al. Identification of a novel *Staphylococcus pseudintermedius* exfoliative toxin gene and its prevalence in isolates from canines with pyoderma and healthy dogs. FEMS Microbiol Lett. 2010;312:169–75.
- van Belkum A, Melle DC, Nouwen J, van Leeuwen WB, van Wamel W, Vos MC, et al. Coevolutionary aspects of human colonisation and infection by *Staphylococcus aureus*. Infect Genet Evol. 2009;9:32–47.
- Bode LG, Kluytmans JA, Wertheim HF, Bogaers D, Vandenbroucke-Grauls CM, Roosendaal R, et al. Preventing surgical-site infections in nasal carriers of *Staphylococcus aureus*. N Engl J Med. 2010;362:9–17.
- den Heijer CD, van Bijnen EM, Paget WJ, Pringle M, Goossens H, Bruggeman CA, et al. Prevalence and resistance of commensal *Staphylococcus aureus*, including methicillin-resistant *S. aureus*, in nine European countries: a cross-sectional study. Lancet Infect Dis. 2013;13:409–15.
- Teira R, Teira A, Campo AB, de Benito I. Prevalence of nasopharyngeal colonization by methicillin-resistant *Staphylococcus aureus* in a population of high school students in Torrelavega (Spain). Enferm Infecc Microbiol Clin. 2013;31:349.
- Paul NC, Bärghman SC, Moodley A, Nielsen SS, Guardabassi L. *Staphylococcus pseudintermedius* colonization patterns and strain diversity in healthy dogs: a cross-sectional and longitudinal study. Vet Microbiol. 2012;160:420–7.
- Bannoehr J, Guardabassi L. *Staphylococcus pseudintermedius* in the dog: taxonomy, diagnostics, ecology, epidemiology and pathogenicity. Vet Dermatol. 2012;23:253–66.
- Paul NC, Moodley A, Ghibaud G, Guardabassi L. Carriage of methicillin-resistant *Staphylococcus pseudintermedius* in small animal veterinarians: indirect evidence of zoonotic transmission. Zoonoses Public Health. 2011;58:533–9.

29. Verkade E, Kluytmans J. Livestock-associated *Staphylococcus aureus* CC398: animal reservoirs and human infections. *Infect Genet Evol.* 2013; pii:S1567-1348(13)00062-2.
30. Valentin-Domelier AS, Girard M, Bertrand X, Violette J, François P, Donnio PY, et al. Methicillin-susceptible ST398 *Staphylococcus aureus* responsible for bloodstream infections: an emerging human-adapted subclone? *PLoS ONE.* 2011;6:e28369.
31. Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, et al. *Staphylococcus aureus* ST398: host adaptation and emergence of methicillin resistance in livestock. *MBio.* 2012;3:e00305–311.
32. Uhlemann AC, Porcella SF, Trivedi S, Sullivan SB, Hafer C, Kennedy AD, et al. Identification of a highly transmissible animal-independent *Staphylococcus aureus* ST398 clone with distinct genomic and cell adhesion properties. *MBio.* 2012;3, e00027–12.
33. Rolo J, Miragaia M, Turlej-Rogacka A, Empel J, Bouchami O, Faria NA, et al. High genetic diversity among community-associated *Staphylococcus aureus* in Europe: results from amulticenter study. *PLoS ONE.* 2012;7:e34768.
34. Suzuki M, Yamada K, Nagao M, Aoki E, Matsumoto M, Hirayama T, et al. Antimicrobial ointments and methicillin-resistant *Staphylococcus aureus* USA300. *Emerg Infect Dis.* 2011;17:1917–20.
35. Uhlemann AC, Kennedy AD, Martens C, Porcella SF, Deleo FR, Lowy FD. Toward an understanding of the evolution of *Staphylococcus aureus* strain USA300 during colonization in community households. *Genome Biol Evol.* 2012;4: 1275–85.
36. Tenover FC, Goering RV. Methicillin-resistant *Staphylococcus aureus* strain USA300: origin and epidemiology.
37. Chen L, Chavda KD, Solanki M, Mediavilla JR, Mathema B, Schlievert PM, et al. Genetic variation among Pantone-Valentine leukocidin-encoding bacteriophages in *Staphylococcus aureus* clonal complex 30 strains. *J Clin Microbiol.* 2013;51:914–9.
38. Nienaber JJ, Sharma Kuinkel BK, Clarke-Pearson M, Lamlertthong S, Park L, Rude TH, et al. Methicillin-susceptible *Staphylococcus aureus* endocarditis isolates are associated with clonal complex 30 genotype and a distinct repertoire of enterotoxins and adhesins. *J Infect Dis.* 2011;204:704–13.
39. Torres-Sangiao E, Pérez-Castro S, Fernández-Natal MI, Cisterna-Cáncer R, Zapico-González M, Fernández-Pérez B, et al. Identification of international circulating lineages of methicillin-resistant *Staphylococcus aureus* in the north of Spain and their glycopeptide and linezolid susceptibility. *J Med Microbiol.* 2012;61:305–7.
40. Walther B, Hermes J, Cuny C, Wieler LH, Vincze S, Abou Elnaga Y, et al. Sharing more than friendship – nasal colonization with coagulase-positive staphylococci (CPS) and co-habitation aspects of dogs and their owners. *PLoS ONE.* 2012;7:e35197.