Original article

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

Carmen Lozano a, Alba Mari b, Carmen Aspiroz c, Elena Gómez-Sanz a, Sara Ceballos a, Blanca Fortuño a, Fernando Barcenilla d, Alfredo Jover-Sáenz d, Carmen Torres a,∗

a Área Bioquímica y Biología Molecular, Universidad de La Rioja, Logroño, Spain
b Ambulatorio Área Básica de Salud Balafia-Pardinyes-Secà, Lérida, Spain
c Unidad de Microbiología, Hospital Rayo Villanova, Zaragoza, Spain
d Unidad Funcional de Infección Nosocomial, Hospital Universitario Arnau de Vilanova, Lérida, Spain

A R T I C L E  I N F O

Article history:
Received 2 June 2014
Accepted 11 September 2014
Available online 4 November 2014

Keywords:
Staphylococcus aureus
CC398
MRSA
MSSA
Staphylococcus pseudintermedius
PVL
TSS-T1
Nasal carriage

A B S T R A C T

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 coagulate 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 virulance 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 were 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 XCC398. 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 lincomycines (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, sit, 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.

© 2014 Elsevier España, S.L.U. and Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica. All rights reserved.

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

R E S U M E N

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 primaría.

Palabras clave:
Staphylococcus aureus
CC398
SARM
SASM

∗ Corresponding author.
E-mail address: carmen.torres@unirioja.es (C. Torres).

http://dx.doi.org/10.1016/j.eimc.2014.09.007
0213-005X/© 2014 Elsevier España, S.L.U. and Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica. All rights reserved.
**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* (CoP) 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. It has been demonstrated that *S. aureus* nasal carriers have a higher risk of developing an infection with this microorganism. 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. Initially, sporadic cases caused by CA-MRSA clones were described in our country. These community associated cases were detected in immigrant patients especially in those coming from South American countries. 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. Moreover, in 2005, a new clonal lineage named MRSA ST398 was reported. Since then, its relationship with farm animals, especially pigs, has been observed. The risk of colonization and infection of farmers has been identified, this clone being also identified in healthy humans without animal contact.

*S. pseudintermedius* is the main colonizing agent of healthy dogs and cats and also is a common pathogen of these animals, usually causing skin and soft tissue infections (STTs). The transmission of *S. pseudintermedius* isolates between pets and their owners has been suggested in some studies. Moreover, cases of infections in humans caused by this microorganism have been described. 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 linkages 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. PCR-RFLP of *pta* gene with Mbol endonuclease was performed to differentiate between *S. intermedius* and *S. pseudintermedius* isolates.

**Molecular typing of isolates**

Single-locus DNA sequencing of the gene spa encoding *S. aureus* protein A 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). SCC mec-typing was performed by multiplex PCR strategy in MRSA. 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 Panton-Valentine leucocidin, [PVL], or presented methicillin resistance) (www.saureus.mlst.net) and in one *S. pseudintermedius* isolate. 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. 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. 

**Virulence factors**

The presence of genes encoding PVL ( lukF/PV/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. In S. pseudintermedius the virulence genes tested by PCR were: lukS/P-1, siet, se-int, expB, sec_animal, and expA.

**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 sample of each nationality): 36 isolates from patients of Lérida, 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 r002-ST146-CC5-SCmec-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 ST98-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, ST342-CC942, ST2498-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 SCmec 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 157 which consisted of a new repeat combination (r01 r09 r21 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 pto29). This isolate was susceptible to all tested antibiotics and presented the toxin genes lukS/P-1, 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%. Nevertheless, some recent studies have reported lower percentages (about 20%). In this sense, Bode et al. reported a prevalence of 18.8% using real-time PCR. Den Heijer et al. 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, was of 18.8%. In another study carried out in Spain 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 varied between 0.2 and 2%. However, in a very recent study in our country, a higher value was identified (6%).

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%). However, these uneven results mainly depend on the body-site in which the samples are taken.

In humans, the prevalence of this microorganism is associated with individuals having regular contact with pets such as veterinarians and owners. 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.
A high diversity of spa-types was identified which is in accordance with other studies about MSSA strains.\textsuperscript{12,13} Remarkably, the strains C2739, C2899, 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

### Table 1

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>spa-type\textsuperscript{a}</th>
<th>MLST (CC)\textsuperscript{b}</th>
<th>Toxin genes</th>
<th>Antimicrobial resistance phenotype\textsuperscript{c}</th>
<th>Antimicrobial resistance genes</th>
<th>Nationality of patient (Continent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2823</td>
<td>t1197</td>
<td>ST398 (CC398)</td>
<td>tST</td>
<td>PEN-TET-ERY-CLI-CIP-LEV-SXT</td>
<td>blaZ, tet(M), erm(C), dfrS1, dfrK</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2741</td>
<td>t002</td>
<td>ST146 (CC5)</td>
<td>tST</td>
<td>PEN-GXA-FOX-ERY-CLI-TOB-CIP-LEV</td>
<td>mecr, erm(C), ant(4) 6-Lu, aph(3')-Ila</td>
<td>Spanish (Europe) unknown</td>
</tr>
<tr>
<td>C2743</td>
<td>t002</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2992</td>
<td>t008</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Romanian (Europe)</td>
</tr>
<tr>
<td>C2993</td>
<td>t008</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Nigerian (Africa)</td>
</tr>
<tr>
<td>C2998</td>
<td>t008</td>
<td></td>
<td></td>
<td>TET</td>
<td>tet(M)</td>
<td>Unknown (Europe)</td>
</tr>
<tr>
<td>C2770</td>
<td>t010</td>
<td></td>
<td></td>
<td>ERY-CIP-LEV</td>
<td>msr(A)/msr(B)</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2754</td>
<td>t078</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2789</td>
<td>t084</td>
<td></td>
<td></td>
<td>TET-SXT</td>
<td>tet(K), dfrS1</td>
<td>Malian (Africa)</td>
</tr>
<tr>
<td>C2790</td>
<td>t084</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Angolan (Africa)</td>
</tr>
<tr>
<td>C2778</td>
<td>t084</td>
<td></td>
<td></td>
<td>PEN-SXT</td>
<td>blaZ, dfrS1</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2771</td>
<td>t094</td>
<td></td>
<td></td>
<td>PEN-TET</td>
<td>blaZ, tet(K)</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2794</td>
<td>t189</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Mauritian (Africa)</td>
</tr>
<tr>
<td>C2753</td>
<td>t346</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Nigerian (Africa)</td>
</tr>
<tr>
<td>C2751</td>
<td>6387</td>
<td>ST15 (CC5)</td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2755</td>
<td>6387</td>
<td>ST15 (CC5)</td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2739</td>
<td>6391</td>
<td>ST8 (CC5)</td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2769</td>
<td>6392</td>
<td>ST534 (CC5)</td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2824</td>
<td>t164</td>
<td></td>
<td></td>
<td>SXK</td>
<td>dfrS1</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2888</td>
<td>t164</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Indian (Asia)</td>
</tr>
<tr>
<td>C2772</td>
<td>t012</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2756</td>
<td>t012</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2742</td>
<td>t012</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2759</td>
<td>t021</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Colombian (South America)</td>
</tr>
<tr>
<td>C2780</td>
<td>t018</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2776</td>
<td>t253</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2988</td>
<td>t198</td>
<td>ST30 (CC30)</td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Moroccan (Africa)</td>
</tr>
<tr>
<td>C2991</td>
<td>t198</td>
<td>ST30 (CC30)</td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Moroccan (Africa)</td>
</tr>
<tr>
<td>C2750</td>
<td>t1076</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2757</td>
<td>t1333</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2777</td>
<td>6388</td>
<td>ST34 (CC30)</td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2760</td>
<td>6390</td>
<td>ST34 (CC30)</td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2740</td>
<td>t015</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2744</td>
<td>t031</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2784</td>
<td>t065</td>
<td>PEN-ERY-CLI</td>
<td></td>
<td>erm(A), blaZ</td>
<td>Spanish (Europe)</td>
<td></td>
</tr>
<tr>
<td>C2990</td>
<td>t230</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Moroccan (Africa)</td>
</tr>
<tr>
<td>C2995</td>
<td>t230</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Morocco (Africa)</td>
</tr>
<tr>
<td>C2788</td>
<td>t1330</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2781</td>
<td>t1337</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2821</td>
<td>t269</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2786</td>
<td>t272</td>
<td>erb</td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2782</td>
<td>t272</td>
<td>erb</td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2787</td>
<td>6889</td>
<td>ST121 (CC121)</td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2747</td>
<td>1445</td>
<td>ST942 (CC942)</td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2822</td>
<td>1445</td>
<td>ST2500 (CC042)</td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2746</td>
<td>t360</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2777</td>
<td>t779</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2745</td>
<td>t869</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Ecuadorian (South America)</td>
</tr>
<tr>
<td>C2775</td>
<td>t1326</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2783</td>
<td>t1900</td>
<td></td>
<td></td>
<td>PEN-CIP-LEV</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2889</td>
<td>t2353</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Spanish (Europe)</td>
</tr>
<tr>
<td>C2785</td>
<td>t2646</td>
<td></td>
<td></td>
<td>PEN</td>
<td>blaZ</td>
<td>Brazilian (South America)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The new spa-types are marked in bold.

\textsuperscript{b} The new STs are marked in bold.

\textsuperscript{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.\textsuperscript{29} Recently, a second epidemiological event consisting in the emergence of MSSA CC398 human infections has been detected.\textsuperscript{30} Indeed, it is thought that the origin of livestock-associated MRSA CC398 could be MSSA of humans.\textsuperscript{31,32} So, MSSA CC398 strains have previously been detected as colonizer agents in healthy humans in Spain\textsuperscript{12,13} and in other countries.\textsuperscript{32}
clones is known as USA300 which is typed as ST8-t008 and contains the PVL genes.\textsuperscript{3,4} 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.\textsuperscript{10}

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 identified\textsuperscript{11} and the same applies with the tst gene.\textsuperscript{12,13} 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.\textsuperscript{7,8} Moreover, in a very recent study the spa type t002 was the most commonly detected among nasal MRSA from European patients.\textsuperscript{24}

High percentage of penicillin resistance was detected among our S. aureus isolates (76%), in agreement with other studies.\textsuperscript{7,24} The values of resistance to methicillin, macrolides and tetracycline were also in accordance with the ranges observed by den Heijer et al.,\textsuperscript{25} 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.\textsuperscript{14,15} Moreover, this isolate harbored the lukS/F-I, sieti, se-int, an expB toxin genes. The expB gene was recently described,\textsuperscript{21} and it seems to be related to cases of superficial dermatitis in dogs.\textsuperscript{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 lukS/lukF-PV and tst should be noted and considered in the development of control measures.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

Part of this work has been financed by Project SAF2012-35474 of the Ministry of Economy and Competitiveness and the European Regional Development Fund (ERDF). C. Lozano has a contract associated with Project SAF2012-35474.

References

36. Tenover FC, Goering RV. Methicillin-resistant Staphylococcus aureus strain USA300: origin and epidemiology.