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Molecular Characterization of *Staphylococcus aureus* from Nasal Samples of Healthy Farm Animals and Pets in Tunisia

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Abstract

A total of 261 healthy farm and pet animals (75 cattle, 52 goats, 100 dogs, and 34 cats) from different regions of Tunisia were screened for *Staphylococcus aureus* nasal carriage. Molecular typing of isolates (by *spa*- and multilocus sequence-typing) was performed, and their antimicrobial resistance and virulence genotypes were determined by PCR and sequencing. *S. aureus* isolates were detected in 17 of 261 tested samples (6.5%). All *S. aureus* isolates recovered were methicillin-susceptible (MSSA), and one isolate/sample was further studied. Eight different *spa* types were detected (t189, t279, t582, t701, t1166, t1268, t1534, and t1773), and eight different sequence types were identified (ST6, ST15, ST45, ST133, ST188, ST700 [clonal complex CC130], ST2057, and a new ST2121). MSSA from pets (six isolates) showed resistance to (number of isolates, resistance gene): penicillin (six, *blaZ*), tetracycline (one, *tet*[M]), erythromycin one, *erm*[A]), streptomycin (one, *ant*[6]-*Ia*), and ciprofloxacin (one). All isolates from farm animals showed susceptibility to the tested antimicrobials, except for two penicillin-resistant isolates. Five *S. aureus* isolates from goats and cats harbored the *lukF/lukS*-PV genes, encoding the Panton–Valentine leukocidin, and six isolates from goats harbored the *tst* virulence gene. In addition, diverse combinations of enterotoxin genes were detected, including two variants of the *egc* cluster. Goats and cats could represent a reservoir of important toxin genes, with potential implications in animal and human health.

Key Words: Animals—Nasal carriage— Panton–Valentine leukocidin (PVL)—*Staphylococcus aureus*—toxic shock syndrome toxin (TSST-1)—Tunisia.

Introduction

STAPHYLOCOCCUS AUREUS IS A COMMON NASAL and skin colonizer of healthy humans and of different animal species. However, it is also one of the most important opportunistic pathogens responsible for a variety of infections in both humans and animals. It is frequently associated with mastitis in cattle, sheep, and goats, causing chronic infections in cows and clinical and subclinical infections in goats and sheep. Most infections in pets usually involve skin and other soft tissues, with postoperative infections of surgical wounds being very common (Peton et al. 2013).

S. aureus is able to produce numerous adhesins and virulence factors that facilitate cell invasion and bacterial growth, and they are associated with the severity of *S. aureus* infections. One of these virulence factors is the Panton–Valentine leukocidin (PVL), a cytotoxin that causes leukocyte destruction and tissue necrosis related to staphylococcal skin and pulmonary infections. Other important virulence factors are the toxic shock syndrome toxin (TSST-1) and staphylococcal enterotoxins (SEs) responsible for food poisoning (Argudin et al. 2010, Zecconi and Scali 2013). It is also important to consider the capacity of *S. aureus* to acquire antibiotic resistance; therefore, methicillin-resistant *S. aureus*

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(MRSA) represents a major therapeutic problem with an increasing frequency in severe infections.

The epidemiology and ecology of this bacterial species in animals has gained interest in the last years, not only for its importance in veterinary medicine but also for increasing evidence of its zoonotic potential. The presence of *S. aureus* in animals plays an important role in its epidemiology, and contact with positive animals, especially MRSA carriers, may pose a risk to human health (Weese 2010, Graveland et al. 2011, Fitzgerald 2012). Animal-associated clonal lineages of *S. aureus* are emerging, as it is the case of MRSA ST398 among farm animals, mainly pigs, or CC97 among bovines (Fitzgerald 2012). People exposed to livestock have a greater risk of colonization and subsequently of infection. Molecular typing techniques have demonstrated that isolates from pets usually show similar characteristics to those of the human isolates of the same geographic region (Haenni et al. 2012). Cases of *S. aureus* transmission between humans and animals have increasingly been reported (Van Duijkeren et al. 2005, Vitale et al. 2006, Fitzgerald 2012, Walther et al. 2012, Gómez-Sanz et al. 2013).

During last decade, most studies on *S. aureus* in humans and animals have focused on the molecular epidemiology of MRSA. Recently, there have been an increasing number of studies focused on clonal lineages of methicillin-susceptible *S. aureus* (MSSA) in different countries. It is thought that MRSA emerged by the acquisition of *mecA* or *mecC* genes in MSSA and, for that, it is highly interesting to study the clones and the characteristics of MSSA strains in diverse ecosystems. Thus, we will be able to know more about the evolution of this microorganism.

The objective of this study was to determine the nasal carriage of *S. aureus* in healthy farm and pet animals in Tunisia, to carry out the molecular typing of recovered isolates, and to determine their content in antimicrobial resistance and virulence genes.

Materials and Methods

Sampling and microbiological isolation

Nasal swabs of 127 healthy farm animals (75 cattle and 52 goats) were obtained during 2010–2011 from two farms of the north and central region of Tunisia and one abattoir of Tunis (Tunisia) that received cattle and goats from the entire country. In addition, nasal samples of 134 healthy pets (100 dogs and 34 cats) were obtained in the National School of Veterinary Medicine of Sidi Thabet and in two veterinary clinics of Tunis. These animals came from Tunis and several regions around Tunis and were brought in by their owners for vaccination. The ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines were followed in this study. The origin of the 261 samples is shown in Table 1. All nasal swabs were incubated in tryptone soy broth (TSB) and later subcultured on Baird–Parker (BP) agar and on oxacillin-resistance screening agar base (ORSAB) supplemented with oxacillin (2 mg/L) for *S. aureus* and MRSA recovery, respectively. Plates were incubated at 37°C for 24–48 h. One *S. aureus*–suspected colony per positive plate was selected and identified by conventional methods (Gram-staining, catalase test, oxidase test, DNase production, and ability to coagulate rabbit plasma [BioRad]). *S. aureus* identification and methicillin resistance/susceptibility were then confirmed by amplification of the species-specific *nuc* gene and the *mec*A gene by multiplex PCR (Gharsa et al. 2012a). No clinical studies or patient data were included in this study.

Antimicrobial susceptibility testing

Susceptibility to 18 antimicrobial agents was performed using the disk-diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) recommendations (Clinical and Laboratory Standards Institute 2012). Antimicrobial agents tested were (charge in μg/disk, except unit/disk for penicillin): Penicillin (10), oxacillin (1), cefoxitin (30), kanamycin (30), gentamicin (10), tobramycin (10), tetracycline (30), chloramphenicol (30), trimethoprim–sulfamethoxazole (1.25/23.75), erythromycin (15), clindamycin (2), amikacin (30), ciprofloxacin (5), mupirocin (5), vancomycin (30), teicoplanin (30), fusidic acid (10), and streptomycin (10).

Detection of antimicrobial resistance genes

The presence of several genes that confer resistance to penicillin (*bla*Z), tetracycline (*tet*[K], *tet*[L], *tet*[M], and *tet*[O]), macrolides/lincosamides (*erm*[A], *erm*[B], *erm*[C]), and streptomycin (*ant*[6]-*Ia*) was analyzed by PCR (Gharsa et al. 2012a). In addition, isolates that belonged to the lineage CC130 were tested for the presence of the novel *mec*C gene (formerly named $mecA_{LGA251}$) (Cuny et al. 2011, García-Alvarez et al. 2011).

Pulsed-field-gel-electrophoresis

All *S. aureus* isolates were characterized by pulsed-field-gel-electrophoresis (PFGE) with *SmaI* restriction enzyme digestion as previously described (Bouzaiane et al. 2008). Samples were run on a 1% agarose gel in 0.5 × Tris-borate-EDTA (TBE) buffer at 14°C on a CHEF DR-II PFGE system using switching times ranging from 5 to 40 s during 20 h at 6 V/cm. The DNA fingerprints generated by PFGE were analyzed visually and digitally according to Tenover criteria

Table 1. Origin and Number of Animals Tested in This Study for *S. aureus* Recovery

Animal	*Farm 1*	*Farm 2*	*Abattoir*	*NSVM*[a]	*Clinic 1*	*Clinic 2*	*Total*
Cow	22	—	53	—	—	—	75
Goat	—	52	—	—	—	—	52
Dog	—	—	—	80	11	9	100
Cat	—	—	—	16	14	4	34

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(Tenover et al. 1995) and by Gel-Pro v. 3.1 software, respectively. The obtained results were submitted to MVSP software generating a dendrogram according to simple matching coefficient and the unweighted pair group method with arithmetic mean (UPGMA) algorithm.

Other molecular typing methods of S. aureus *isolates*

S. aureus protein A (*spa*) typing was performed in all *S. aureus* isolates as described elsewhere (Harmsen et al. 2003). The polymorphic X region of the *spa* gene was amplified by PCR, and sequences were analyzed using Ridom Staph-Type software v.1.5.21 (Ridom GmbH), which automatically detects *spa* repeats and assigns a *spa* type according to http://spaserver.ridom.de/. The identification of the allele group of the accessory gene regulator locus (*agr*) was determined by two duplex PCRs, as previously described (Shopsin et al. 2003).

Multilocus sequence typing (MLST) was performed in all *S. aureus* isolates detected. The allelic profile of each isolate was obtained by sequencing internal fragments of seven unlinked housekeeping genes (*arc*C, *aro*E, *glp*F, *gmk*, *pta*, *tpi*, and *yqi*L) to determine the sequence type (ST) and clonal complex (CC) assigned by MLST and BURST (Based Upon Related Sequence Types) analyses (www.mlst.net).

Detection of staphylococcal toxin genes

All isolates were tested by PCR for the presence of 18 genes coding for staphylococcal enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq*, *ser*, and *seu*). The presence of the enterotoxin gene cluster *egc* was considered when five SE genes (*seg*, *sei*, *sem*, *sen*, and *seo)* were detected; the *egc*-like cluster included six SE genes (*seg*, *sei*, *sem*, *sen*, *seo,* and *seu*) (Jarraud et al. 2001, Hwang et al. 2007).

Isolates were also screened for the presence of *tst* gene encoding the TSST-1 (Jarraud et al. 2002), *lukF/lukS*-PV genes encoding PVL, *lukE-lukD* genes encoding the bicomponent leukotoxin LukE-LukD, *lukM* gene encoding leukocidin M, as well as *eta* and *etb* genes encoding exfoliative toxins A and B, respectively, and genes encoding hemolysins A, B, D, G, and G_v (Jarraud et al. 2001, Lozano et al. 2011).

Results

Field survey results for S. aureus *isolates from healthy animals*

To isolate *S. aureus* from nasal samples, each enrichment culture of nasal swabs was subcultured on BP medium. At 48 h postinoculation, *S. aureus*-like colonies were identified in 17 of the 261 nasal samples (6.5%), including 10 goats (19.2%), four dogs (4%), two cats (5.9%), and one cow (1.3%). From each putative *S. aureus*–positive sample, one *S. aureus*–like colony was selected and further tested for identification of the species using both conventional methods and a species-specific PCR method, as described in Materials and Methods. All 17 isolates were confirmed as *S. aureus* and all of them were MSSA.

The enrichment cultures of nasal swabs were also tested for MRSA recovery by inoculating to ORSAB plates. No staphylococcal colonies were detected on ORSAB plates for all samples tested.

Characteristics of S. aureus *isolates detected in healthy farm animals*

S. aureus isolates were recovered from 11 of the 127 tested samples (8.7%) of farm animals. These isolates were recovered from 10 goat samples and one cow sample. The characteristics of the 11 MSSA recovered isolates are shown in Table 2. Four different *spa* types were detected among these *S. aureus* isolates (t1534, t1268, t1773, and t1166), and four different STs were identified by MLST (ST2057, ST45, ST700, and ST133). The STs detected among these MSSA isolates of farm animals were classified into four clonal complexes: CC522 (four isolates), CC45 (three isolates), CC130 (three isolates), and CC133 (one isolate). Typing of the *agr* locus showed that *S. aureus* isolates were ascribed to *agr* type I (eight isolates), to *agr* type II (two isolates), and to a nontypeable *agr* (one isolate).

Analysis of SmaI macrorestriction profiles of the 11 MSSA isolates revealed five different PFGE patterns (P1–P5) (Fig. 1 and Table 2).

Two isolates from goat samples showed resistance to penicillin (with the *bla*[Z] gene), and the remaining isolates from farm animal samples were susceptible to all tested antimicrobial agents. The three MSSA isolates that belonged to the clonal complex CC130 were tested by PCR for the *mec*C gene, obtaining a negative result.

Three *S. aureus* isolates from goats harbored the *lukF/lukS*-PV genes and six isolates presented the *tst* gene. Other virulence genes carried by MSSA isolates were (number of isolates): *hla* (eleven), *hlb* (eight), *hld* (eleven), *hlg* (three), hlg_v (three), *lukED* (eight), *lukM* (six), *sec* (six), *sed* (two), *seg* (one), *sei* (one), *sej* (three), *sel* (nine), *ser* (four), and *egc*-like cluster: *seg-sei-sem-sen-seo-seu* (three) (Table 2). The remaining PCR virulence genes tested were negative among these isolates.

Characteristics of S. aureus *isolates detected in healthy pets*

S. aureus isolates were recovered from six of the 134 tested samples of healthy pets (4.5%), obtained from four dogs and two cats, all of them of the National School of Veterinary Medicine. The characteristics of the six MSSA isolates from pet animals are shown in Table 2. Four different *spa* types were detected among these isolates (t189, t279, t582, and t701), and four STs were also identified by MLST (ST6, ST15, ST188, and a new ST registered as ST2121). ST2121 showed the allelic profile numbered as follows: *arcc*(2)-*aroe*(2)-*glpf*(2)-*gmk*(2)-*pta*(7)-*tpi*(3)-*yqil*(2). The sequence types detected among these *S. aureus* isolates of pet animals were classified into three clonal complexes: CC5 (three isolates), CC15 (two isolates), and CC30 (which enclosed the new ST2121). Typing of the *agr* locus showed that *S. aureus* isolates were ascribed to *agr*I (four isolates), *agr*II (one isolate), and *agr*III (one isolate).

Analysis of SmaI macrorestriction profiles of the six MSSA isolates revealed four different PFGE patterns (P6–P9), and three subtypes in pulsotype P7 (P7a–P7c) (Fig. 1 and Table 2). The three isolates with closely related PFGE patterns corresponded to those typed as ST6, *spa* type t701, and *agr*I, and they were recovered from two unrelated dogs and one cat.

All MSSA isolates showed penicillin resistance (harboring the *bla*Z gene); one isolate also exhibited tetracycline and erythromycin resistance (with the *tet*[M] and *erm*[A] genes),

TABLE 2. CHARACTERISTICS OF THE 17 *S. AUREUS* RECOVERED FROM NASAL SAMPLES OF FARM AND PET ANIMALS IN THIS STUDY

Number of isolates	*Origin*[a]	spa-*type*	*MLST ST/CC*	agr	*PFGE*	*PVL*	tst	*Other toxin genes detected*[b]	*Antimicrobial resistance*[b] *Phenotype*	*Resistance genes*
4	Goat/F2	t1534	ST2057/CC522	I	P1	–	+	*hla, hlb, hld, lukED, lukM, sec, sed*[1]*,seg*[1]*, sei*[1]*, sej*[3]*,sel*		
3	Goat/F2	t1268	ST45/CC45	I	P2	+	–	*hla, hld, hlg, hlg$_v$, sel,ser*[1]*, egc*-like[c]	PEN[2]	*bla*(Z)[2]
2	Goat/F2	t1773	ST700/CC130	III	P3	–	+	*hla, hlb, hld, lukM, lukED, sec, sed*[1]*, sel, ser*[1]		
1	Cow/A	t1773	ST700/CC130	NT	P4	–	–	*hla, hlb, hld, lukED*		
1	Goat/F2	t1166	ST133/CC133	I	P5	–	–	*hla, hlb, hld, lukED, ser*		
1	Cat/NSVM	t279	ST15/CC15	II	P6	+	–	*hla, hld, lukED, sei*	PEN-CIP	*bla*(Z)
1	Cat/NSVM	t701	ST6/CC5	I	P7$_a$	+	–	*hla, hlb, hld, lukED, sea, ser*	PEN	*bla*(Z)
2	Dog/NSVM	t701	ST6/CC5	I	P7$_b$, P7$_c$	–	–	*hla, hlb, hld, lukED, sea, see, ser*	PEN	*bla*(Z)
1	Dog/NSVM	t582	ST2121[d]/CC30	III	P8	–	–	*hla, hld, hlg, hlg$_v$,egc$_v$*[c]	PEN-TET-ERY	*bla*(Z), *tet*(M), *erm*(A)
1	Dog/NSVM	t189	ST188/CC15	I	P9	–	–	*hla, hlb, hld, lukED, ser*	PEN-STR	*bla*(Z), *ant*(6)-*Ia*

[a]Animal type and location indicated.
[b]When only some of the isolates of the group presented the gene, the number is indicated as a superscript.
[c]*egc*-like include *seg, sei, sem, sen, seo* and *seu; egc$_v$* includes *seg, sei, sem, sen*, and *seu.*
[f] New ST detected.
MLST, multilocus sequence typing; ST, sequence type; CC, clonal complex; PFGE, pulsed-field gel electrophoresis; PVL, Panton–Valentine leukocidin; F2, farm 2; PEN, penicillin; A, abattoir; NT, nontypeablel; NSVM, National School of Veterinary Medicine; CIP, ciprofloxacin; TET, tetracycline; ERY, erythromycin; STR, streptomycin.

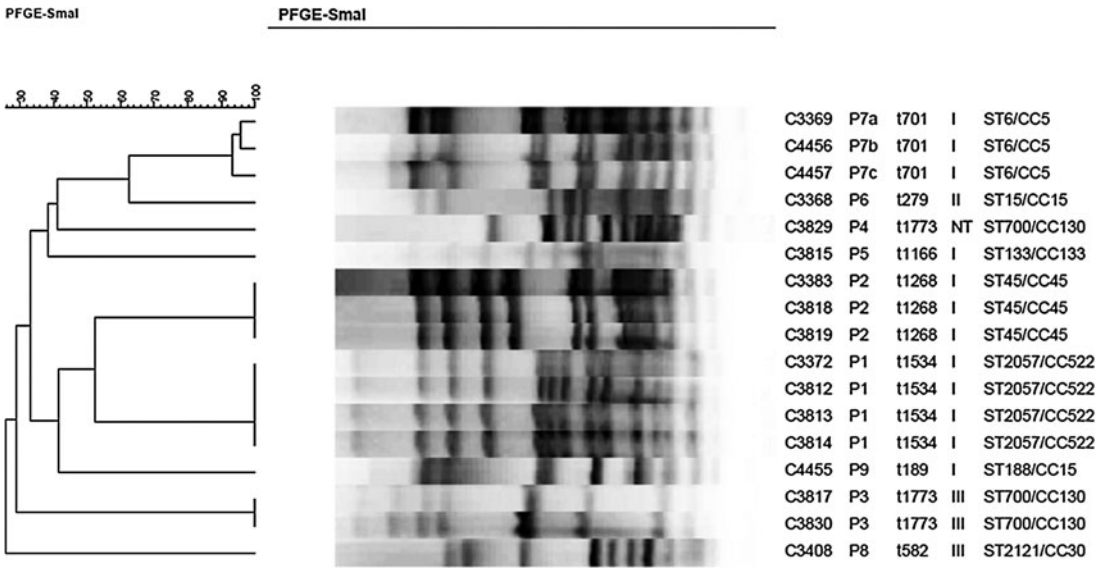

FIG. 1. Dendrogram of pulsed-field gel electrophoresis SmaI patterns among 17 *Staphylococcus aureus* isolates recovered in this study, generated by GelCompar II software using the unweighted pair group method with arithmetic mean (UPGMA) algorithm and the Dice similarity coefficients.

and one additional isolate presented streptomycin resistance (containing the *ant*[6]-*Ia* gene) (Table 2).

The two *S. aureus* isolates from cats harbored the *lukF/lukS*-PV genes encoding the PVL. Other virulence genes carried by MSSA from pets were (number of isolates): *hla* (six), *hlb* (four), *hld* (six), *hlg* (one), hlg_v (one), *lukED* (five), *sea* (three), *see* (two), *sei* (two), *ser* (four), and an *egc* cluster variant that included the *seg-sei-sem-sen-seu* genes (one) (Table 2). The remaining virulence genes tested were negative among these isolates from pets.

Discussion

A relatively low recovery rate of *S. aureus* isolates was detected among nasal samples of healthy animals in this study (6.5%), with different rates depending on the animal species. However, MRSA was not detected in the analyzed samples. To our knowledge, this is the first study of this type performed in Tunisia. Higher *S. aureus* nasal carriage rates in goats (>64%) and cows (7.5%) were identified by others in Norway, Canada, or Denmark (Barbour et al. 1997, Mork et al. 2010, Eriksson et al. 2013). Regarding pets, a study performed in Canada showed a rate of 14% in dogs and 4.3% in cats, MRSA being detected in 1.5% of analyzed dogs (Hanselman et al. 2009). Other studies carried out in healthy pets detected a rate of 8.8% in China (four) and 12% in Spain (Gómez-Sanz et al. 2013). However, lower values have been also detected in other studies (Wan et al. 2011). These different carriage rates might be due to the different animal population studied or to the employed methodologies, among other factors.

The high diversity of genetic lineages of *S. aureus* detected among farm animals and pets is noteworthy. In these terms, the results of molecular typing by MLST, PFGE, and *spa* typing (Table 2 and Fig. 1) exhibited elevated variability. Higher diversity in the different molecular typing approaches has been previously shown in MSSA isolates in comparison with MRSA (Grundmann et al. 2010).

Four STs distributed in four CCs were identified among our *S. aureus* isolates from goat and bovine samples. Lineage ST700 detected in three MSSA isolates (from two goats and one cow) belonging to CC130, which was mainly related to bovine animals. However, this ST has been also identified in goats (Smyth et al. 2009, Porrero et al. 2012, Eriksson et al. 2013). It is important to remark that our CC130 isolates did not harbor the recently described *mec*C gene. This gene was first described in MRSA (*mec*A negative) of this lineage in cattle in 2011 (García-Alvarez et al. 2011), and since then several studies have reported its presence in MRSA of a wide range of mammal species in Europe, the majority of them belonging to the same lineage (Cuny et al. 2011, Paterson et al. 2012). Alternatively, the clonal complex CC522, also detected in isolates from goats in our study, had previously been identified in samples of this animal species in Spain (Porrero et al. 2012). CC133, detected in one MSSA from goats, is considered as one of the major small-ruminant lineages (Guinane et al. 2010, Eriksson et al. 2013). The three PVL-positive MSSA from goats belonged to the clonal complex CC45 and to the *agr* type I, characteristics previously detected among human MRSA strains (Seidl et al. 2011). A possible human origin of this clonal complex has been previously suggested (Sobral et al. 2012).

The four STs identified among MSSA from pets were distributed in three different CCs, as is the case of CC5, CC15, and CC30. The presence of *S. aureus* that belongs to CC5 among pets is becoming unexceptional (Haenni et al. 2012, Walther et al. 2012), and its presence in other animals as poultry is commonly observed (Hasman et al. 2010). The clonal complex CC15, identified in two MSSA isolates in our study (one dog and one cat), has been recently described in MSSA of pets in France and in a recent study on *S. aureus* of donkeys in Tunisia (Gharsa et al. 2012a, Sobral et al. 2012). In addition, a new ST belonging to CC30 was identified (ST2121). This CC is one of the predominant hospital- and community-associated MRSA clones, and MSSA isolates of this lineage have also been described (Monecke et al. 2011), its presence in swine isolates being also unexceptional (Hasman et al. 2010). These data and our results illustrate the spread of *S. aureus* human lineages among different animal species.

The detection of PVL-positive MSSA isolates in farm animals (6% of tested goats) and pets (6% of tested cats) is of relevance due to the important role that this toxin seems to have in serious infections (Melles et al. 2006). A high prevalence of the PVL genes in MRSA isolates recovered from dogs has been detected in other studies (Rankin et al. 2005, Van Duijkeren et al. 2005). On the other hand, none of the canine *S. aureus* isolates harbored the PVL genes in other reports (Boost et al. 2008, Hanselman et al. 2009, Walther et al. 2012, Gómez-Sanz et al. 2013). Regarding its detection in cats, there are several studies that have identified these toxin genes in MRSA of these animals, but not in MSSA isolates (Rankin et al. 2005, Vitale et al. 2006). The existence of the PVL genes in three *S. aureus* isolates from goats in our study population is also relevant. A recent study revealed the presence of *lukF/lukS*-PV genes in ewe isolates (Van Duijkeren et al. 2005), but not in goats.

Our results showed that *S. aureus* isolated from goats had a high enterotoxigenic potential. Similar results were found in *S. aureus* isolated from goat and bovine mastitis in Turkey (Unal et al. 2012). *S. aureus* isolates from our goat and sheep population contained *sec* and *sel* genes, whereas those from cows carried mainly *sea*, *sej*, and *sed* genes. Moreover, six *S. aureus* isolates of goats that harbored the *sec* gene were simultaneously positive for the *tst* gene. These combinations of toxin *tst*/*sec* genes have been described before in *S. aureus* isolated from goats (Scherrer et al. 2004). The frequency of other enterotoxins (*sed*, *seg*, *sei*, *sej*, *sel*, *sem*, *sen*, *seo*, *ser*, *seu*) in our goat isolates is in agreement with previous studies (Valle et al. 1990), which described healthy goats as an important reservoir of enterotoxigenic staphylococci. An *egc*-like cluster was detected in three of our 10 isolates from goats. Similarly, the detection of this *egc* cluster was reported in goats, and it has been suggested that its presence may confer a survival advantage of *S. aureus* in animals by modulating the immune response (Smyth et al. 2005).

With regard to the enterotoxin genes detected in isolates from pets, a previous study performed in Egypt revealed that 10% and 2.1% of dogs and cats, respectively, carried enterotoxigenic *S. aureus* isolates (Abdel-Moein et al. 2011). Additionally, a recent report from Spain revealed that 75% of pet animals (dogs, cats) investigated presented at least one enterotoxin gene (Gómez-Sanz et al. 2013). These data highlight the potential role of enterotoxigenic *S. aureus*–positive pets in the epidemiology of household food poisonings. An *egc* cluster variant carrying *seg-sei-sem-sen-seu* was detected in one *S. aureus* isolate of a dog. The *egc* cluster

and its variants are considered as a putative source of superantigens in *S. aureus* (Jarraud et al. 2001).

All of our MSSA isolates showed susceptibility to the tested antimicrobials, with few exceptions. Penicillin resistance was detected in a high proportion of tested isolates of pets. However, this result contrasts with the low rate detected in farm animals in the current study and in a recent report on sheep isolates in Tunisia (Gharsa et al. 2012b). The low penicillin resistance among these animals is in agreement with other authors (Unal et al. 2012). However, elevated rates of penicillin resistance are very common among the general *S. aureus* population.

It should be taken in consideration that in this study the data were obtained from two farms and one abattoir. However, more extensive studies with farms and abattoirs in the entire country are necessary to determine the global situation in this country.

Conclusions

The data reported here demonstrate that the nares of healthy pets, goats, and cows could be reservoirs of PVL-positive and TSST-1–positive *S. aureus* isolates and other virulence genes, such as those encoding enterotoxins. It is noteworthy that not only MRSA but also MSSA isolates from these animals should be studied. More studies should be performed in the future to expand the knowledge on the genetic lineages of *S. aureus* circulating among healthy farm animals and pets, as well as on the capacity of these isolates to produce virulence factors, due to the risk of animal-to-human bacterial transference and therefore its potential animal and human health implications.

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Author Disclosure Statement

No competing finantial interests exist.

References

Abdel-Moein KA, Samir A. Isolation of enterotoxigenic *Staphylococcus aureus* from pet dogs and cats: A public health implication. Vector Borne Zoonotic Dis 2011; 11:627–629.

Argudin MA, Mendoza MC, Rodicio MR. Food poisoning and *Staphylococcus aureus* enterotoxins. Toxins 2010; 2:1751–1773.

Barbour EK, Nabbut NH, Hamadeh SK, Al-Nakhli HM. Bacterial identity and characteristics in healthy and unhealthy and respiratory tracts of sheep and calves. Vet Res Commun 1997; 21:421–430.

Boost MV, O'donoghue MM, James A. Prevalence of *Staphylococcus aureus* carriage among dogs and their owners. Epidemiol Infect 2008; 136:953–964.

Bouzaiane O, Abbassi M, Gtari M, Belhaj O, et al. Molecular typing of staphylococcal communities isolated during municipal solid waste composting process. Ann Microbiol 2008; 58:387–394.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement. CLSI document M100-S22. Wayne, Pennsylvania: CLSI, 2012.

Cuny C, Layer F, Strommenger B, Witte W. Rare occurrence of methicillin-resistant *Staphylococcus aureus* CC130 with a novel *mecA* homologue in humans in Germany. PLoS One 2011; 6:e24360.

Eriksson J, Espinosa-Gongora C, Stamphoj I, Larsen AR, et al. Carriage frequency, diversity and methicillin resistance of *Staphylococcus aureus* in Danish small ruminants. Vet Microbiol 2013; 12:110–115.

Fitzgerald JR. Livestock-associated *Staphylococcus aureus*: origin, evolution and public health threat. Trends in Microbiology 2012; 20:192–198.

Garcia-Alvarez L, Holden MTG, Lindsay H, Webb CR, et al. Methicillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: A descriptive study. Lancet Infect Dis 2011; 1:595–603.

Gharsa H, Ben Sallem R, Ben Slama K, Gómez-Sanz E, et al. High diversity of genetic lineages and virulence genes in nasal *Staphylococcus aureus* isolates from donkeys destined to food consumption in Tunisia with predominance of the ruminant associated CC133 lineage. BMC Vet Res 2012a; 8:203.

Gharsa H, Slama KB, Lozano C, Gómez-Sanz E, et al. Prevalence, antibiotic resistance, virulence traits and genetic lineages of *Staphylococcus aureus* in healthy sheep in Tunisia. Vet Microbiol 2012b; 156:367–373.

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.

Graveland H, Wagenaar J, Bergs K, Heesterbeek H, et al. Persistence of livestock associated MRSA CC398 in humans is dependent on intensity of animal contact. PLoS ONE 2011; 6–e16830.

Grundmann H, Aanensen DM, Van Den Wijngaard CC, Spratt BG, et al. European Staphylococcal Reference Laboratory Working Group. Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: A molecular-epidemiological analysis. PLoS One 2010; 7:e1000215.

Guinane CM, Ben Zakour NL, Tormo-Mas MA, Weinert LA, et al. Evolutionary genomics of *Staphylococcus aureus* reveals insights into the origin and molecular basis of ruminant host adaptation. Genome Biol Evol 2010; 12:454–466.

Haenni M, Saras E, Châtre P, Médaille C, et al. USA300 variant and other human-related methicillin-resistant *Staphylococcus aureus* strains infecting cats and dogs in France. J Antimicrob Chemoth 2012; 67:326–329.

Hanselman BA, Kruth SA, Rousseau J, Weese SJ. Coagulase positive staphylococcal colonization of humans and their household pets. Can Vet J 2009; 50:954–958.

Harmsen D, Claus H, Witte W, Rothganger J, et al. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. J Clin Microbiol 2003; 41:5442–5448.

Hasman H, Moodley A, Guardabassi L, Stegger M, et al. *Spa* type distribution in *Staphylococcus aureus* originating from pigs, cattle and poultry. Vet Microbiol 2010; 141:326–331.

Hwang SY, Kim SH, Jang EJ, Kwon NH, et al. Novel multiplex PCR for the detection of the *Staphylococcus aureus* superantigen and its application to raw meat isolates in Korea. Int J Food Microbiol 2007; 117:99–105.

Jarraud S, Peyrat MA, Lim A, Tristan A, et al. *egc*, a highly prevalent operon of enteroxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus*. J Immunol 2001; 166:669–677.

Jarraud S, Mougel C, Thioulouse J, Lina G, et al. Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease. Infect Immun 2002; 70:631–641.

Lozano C, Aspiroz C, Charlez L, Gómez-Sanz E, et al. Skin lesion by methicillin-resistant *Staphylococcus aureus* ST398-t1451 in a Spanish pig farmer: Possible transmission from animals to humans. Vector Borne Zoonotic Dis 2011; 11:605–607.

Melles DC, van Leeuwen WB, Boelens HA, Peeters JK, et al. Panton-Valentine leukocidin genes in *Staphylococcus aureus*. Emerg Infect Dis 2006; 12:1174–1175.

Monecke S, Coombs G, Shore AC, David C, et al. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. PLoS One 2011; 6:e17936.

Mork T, Kvitle B, Mathisen T, Jorgensen HJ. Bacteriological and molecular investigations of *Staphylococcus aureus* in dairy goats. Vet Microbiol 2010; 141:134–141.

Paterson GK, Larsen AR, Robb A, Edwards GE, et al. The newly described *mecA* homologue, *mecA*LGA251, is present in methicillin-resistant *Staphylococcus aureus* isolates from a diverse range of host species. J Antimicrob Chemoth 2012; 67:2809–2813.

Peton V, Le Loir Y. *Staphylococcus aureus* in veterinary medicine. Infect Genet Evol 2013; 21:1567–1581.

Porrero MC, Hasman H, Vela AI, Fernandez-Garayzabal JF, et al. Clonal diversity of *Staphylococcus aureus* originating from the small ruminants goats and sheep. Vet Microbiol 2012; 156:157–161.

Rankin S, Roberts S, O'Shea K, Maloney D, et al. Panton Valentine leukocidin (PVL) toxin positive MRSA strains isolated from companion animals. Vet Microbiol 2005; 108:145–148.

Scherrer D, Corti S, Muehlherr JE, Zweifel C, et al. Phenotypic and genotypic characteristics of *Staphylococcus aureus* isolates from raw bulk-tank milk samples of goats and sheep. Vet Microbiol 2004; 101:101–107.

Seidl K, Chen L, Bayer AS, Abdel Hady W, et al. Relationship of *agr* expression and function with virulence and vancomycin treatment outcomes in experimental endocarditis due to methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2011; 55:5631–5639.

Shopsin B, Mathema B, Alcabes P, Said-Salim B, et al. Prevalence of *agr* specifity groups among *Staphylococcus aureus* strains colonizing children and their guardians. J Clin Microbiol 2003; 41:456–459.

Smyth DS, Feil EJ, Meaney WJ, Hartigan PJ, et al. Molecular genetic typing reveals further insights into the diversity of animal-associated *Staphylococcus aureus*. J Med Microbiol 2009; 58:1343–1353.

Smyth DS, Hartigan PJ, Meaney WJ, Fitzgerald JR, et al. Superantigen genes encoded by the *egc* cluster and SaPIbov are predominant among *Staphylococcus aureus* isolates from cows, goats, sheep, rabbits and poultry. J Med Microbiol 2005; 54:401–411.

Sobral D, Schwarz S, Bergonier D, Brisabois A, et al. High throughput multiple locus variable number of tandem repeat analysis (MLVA) of *Staphylococcus aureus* from human, animal and food sources. Plos One 2012; 7:e33967.

Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. J Clin Microbiol 1995; 33:2233–2239.

Unal N, Askar S, Macun HC, Sakarya F, et al. Panton–Valentine leukocidin and some exotoxins of *Staphylococcus aureus* and antimicrobial susceptibility profiles of staphylococci isolated from milks of small ruminants. Trop Anim Health Prod 2012; 44:573–579.

Valle J, Gomez-Lucia E, Piriz S, Goyache J, et al. Enterotoxin production by staphylococci isolated from healthy goats. Appl Env Microbiol 1990; 56:1323–1326.

Van Duijkeren EV, Wolfhagen MJHM, Heck MEOC, Wannet WJB. Transmission of a Panton-Valentine leucocidin-positive, methicillin-resistant *Staphylococcus aureus* strain between humans and a dog. J Clin Microbiol 2005; 43:6209–6211.

Vitale CB, Gross TL, Weese JS. Methicillin resistant *Staphylococcus aureus* in cat and owner. Emerg Infect Dis 2006; 12:1998–2000.

Walther B, Hermes J, Cuny C, Wieler LH, 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.

Wan MT, Fu SY, Lo YP, Huang TM, et al. Heterogeneity and phylogenetic relationships of community-associated methicillin-sensitive/resistant *Staphylococcus aureus* isolates in healthy dogs, cats and their owners. J Appl Microbiol 2011; 112:205–221.

Weese JS. Methicillin-resistant *Staphylococcus aureus* in animals. ILAR Journal 2010; 51:233–244.

Zecconi A, Scali F. *Staphylococcus aureus* virulence factors in evasion from innate immune defenses in human and animal diseases. Immunol Lett 2013; 150:12–22.

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