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Prevalence, antibiotic resistance, virulence traits and genetic lineages of *Staphylococcus aureus* in healthy sheep in Tunisia☆

Haythem Gharsa [a], Karim Ben Slama [a], Carmen Lozano [b], Elena Gómez-Sanz [b], Naouel Klibi [a], Rym Ben Sallem [a], Paula Gómez [b], Myriam Zarazaga [b], Abdellatif Boudabous [a], Carmen Torres [b,*]

[a] *Laboratoire des Microorganismes et Biomolécules Actives, Faculté des Sciences de Tunis, Université Tunis-El Manar, 2092 Tunis, Tunisia*
[b] *Área de Bioquímica y Biología Molecular, Universidad de La Rioja, Madre de Dios 51, 26006 Logroño, Spain*

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ABSTRACT

Nasal swabs of 163 healthy sheep were obtained from two farms and one abattoir in Tunisia during 2010. Samples were inoculated in Baird Parker agar and ORSAB medium for *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) recovery, respectively. MRSA was detected in 5 of these 163 samples (3%) in ORSAB medium, and one isolate per sample was further studied. MRSA isolates were *mecA*-positive, typed as ST153-CC80-t044-*agr*III, and contained *bla*Z, *ant*(6)-Ia, *aph*(3′)-IIIa, *erm*(C), *tet*(K), and *fus*B genes encoding penicillin, streptomycin, kanamycin, erythromycin, tetracycline and fusidic acid resistance, respectively. These MRSA isolates showed indistinguishable or closely related PFGE-patterns and harboured the *luk*F/*luk*S gene encoding the Panton-Valentine leukocidin and the *luk*-ED, *hla*, *hld*, and hlg_v genes. Methicillin-susceptible *S. aureus* (MSSA) were recovered in 68 of the 163 samples (41.7%) and one isolate per sample was characterized. Most of MSSA (82.4%) showed susceptibility to the tested antibiotics with exceptions: penicillin (6%, carrying *blaZ* gene), tetracycline (19%, carrying *tet*(K) gene) and fusidic acid (9%). The following toxin-genes were identified among MSSA: *tst* (53 isolates), *luk*-M (52), *luk*-ED, *hla*, *hlb*, *hld* and hlg_v (67), *hlg* (1), *sec* (49), *sel* (52), and the *egc*-cluster-like *sen-sem-sei-seo-seg* (1). Ten *spa*-types (two of them new ones) and nine sequence types (six new ones) were detected among the 73 *S. aureus* isolates, and they were ascribed to *agr* types I and III. All MRSA and MSSA isolates were able to coagulate bovine plasma and MRSA harboured the immune-evasion-gene-cluster type E. *Conclusions*. Nares of healthy sheep could be a reservoir of PVL-positive community-associated-MRSA and also of TSST-positive *S. aureus* isolates, with potential implications in public health.

1. Introduction

Staphylococcus aureus is an important pathogen that can colonize the nares of different animals, including farm animals (Weese and Duijkeren, 2009). This microorganism is able to produce a wide range of extracellular toxins and virulence factors. Strains producing the Panton-Valentine leukocidin (PVL), the toxic shock syndrome toxin (TSST), and staphylococcal enterotoxins (SE) pose a risk for humans and animals being associated with skin and soft tissue infections and even with cases of severe pneumonia (Jarraud et al., 2002; Francis et al., 2005). It is also relevant to consider that the transmission of *S. aureus* is possible either by direct contact with animals or through contaminated food as milk or cheese (Vautor et al., 2005).

Of particular concern is methicillin-resistant *S. aureus* (MRSA) which is resistant to most of beta-lactams. MRSA

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* Corresponding author. Tel.: +34 941299750; fax: +34 941299721.
E-mail addresses: carmen.torres@unirioja.es, carmen.torres@daa.unirioja.es (C. Torres).

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strains usually harbour multiple antimicrobial resistance genes what constitutes an important clinical problem due to the limited therapeutic options left (Fluit et al., 2001). The first cases of MRSA in animals were reported in 1972 from bovine mammary infections. Thereafter, more MRSA strains were detected in cattle and the number of reported cases is recently increasing. Moreover, MRSA has been described in other animal species such as horses, dogs and pigs, among others (Weese and Duijkeren, 2009; Madec and Haenni, 2010) but data about MRSA in sheep remain scarce. In the last years, a livestock-associated genetic lineage of MRSA (LA-MRSA) of the sequence type ST398 has emerged in farm animals, especially in pigs, and human infections owing to this clonal lineage have also been reported (Kock et al., 2009; Lozano et al., 2011). Recent cases of infections related to strains of porcine origin (clone ST398) have highlighted the possibility of transference of MRSA from animals to humans (Madec and Haenni, 2010; Lozano et al., 2011). In addition, data on MRSA (and mainly non-ST398 MRSA) in other animal species than pigs are still scarce.

So far, few data do exist about the genetic lineages of *S. aureus* or the prevalence of MRSA that colonize nares of healthy sheep (Vautor et al., 2005; Alzohairy, 2011). Additionally, as far as we know, no previous studies of this type have been performed in animals in Tunisia and very few in other African countries (Elbir et al., 2010). There are only data about the prevalence and lineages of *S. aureus* in healthy humans with different level of contact with farms or domestic animals in that country (Ben Slama et al., 2011). Sheep is an important agriculture animal species in Tunisia as well as in other Northern African countries. Therefore, the present study was undertaken to determine the carriage rate, resistance mechanisms, virulence traits, and genetic lineages of nasal *S. aureus* of healthy sheep in Tunisia.

2. Materials and methods

2.1. Sampling and microbiological isolation

Nasal swabs of 163 healthy sheep were obtained in two farms (59 animals) and in one big abattoir (104 animals) that receive sheep from farms of all Tunisia. Samples were obtained during March–May 2010.

Swabs were incubated in Tryptone Soy Broth (TSB) for 48 h and then, they were subcultured on Baird Parker agar (BD) and ORSAB medium (Oxacillin Resistance Screening Agar Base, Oxoid) for 24–48 h, for *S. aureus* and MRSA recovery, respectively. *S. aureus* suggestive colonies were initially identified by conventional methods [Gram-staining, catalase test, oxidase test, DNase production, and ability to coagulate rabbit plasma (BioRad)]. *S. aureus* identification was then confirmed by amplification of the species-specific *nuc* gene (Gómez-Sanz et al., 2010).

All isolates were tested for their capacity to coagulate bovine plasma (Sigma–Aldrich) following standard methodology (Guinane et al., 2010).

2.2. Antimicrobial susceptibility testing

Susceptibility to 18 antimicrobial agents was performed using the disk-diffusion method in accordance with the Clinical and Laboratory Standards Institute recommendations (CLSI, 2010). Antimicrobial agents tested were (charge in μg/disk): 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). Double-disk diffusion test (*D*-test) with erythromycin and clindamycin disks was implemented in all isolates to detect inducible clindamycin resistance.

2.3. Detection of mecA gene and SCCmec-typing

The presence of the *mecA* gene was studied by PCR in all oxacillin and/or cefoxitin resistant isolates (Zhang et al., 2004). The SCC*mec*-typing was performed for *mecA* positive isolates by a multiplex PCR strategy as previously described (Kondo et al., 2007).

2.4. Detection of antimicrobial resistance genes

The ribosomal methylases encoded by *erm*(A), *erm*(B) and *erm*(C) genes, which confer resistance to erythromycin and clindamycin, and the efflux pump encoded by *msr*(A) gene, conferring resistance to erythromycin, were studied by PCR in erythromycin-resistant isolates (Gómez-Sanz et al., 2010). In addition, *tet*(K), *tet*(M) and *tet*(L) genes conferring resistance to tetracycline, *bla*Z gene to penicillin, *fus*B and *fus*C genes to fusidic acid, and *aph*(3′)-IIIa and *ant*(6)-Ia genes to kanamycin and streptomycin, were studied by PCR in all antimicrobial-resistant *S. aureus* isolates (Gómez-Sanz et al., 2010). Mutations in elongation factor G were determined by sequence analysis of *fus*A in all fusidic acid-resistant isolates (O'Neill et al., 2002).

2.5. Pulsed-field-gel-electrophoresis (PFGE)

All *S. aureus* isolates were characterized by PFGE with *Sma*I restriction enzyme digestion as previously described (Bouzaiane et al., 2008). Samples were run on 1% agarose gel in 0.5% TBE buffer at 14 °C on a CHEF DR-II PFGE system by using an initial switching time of 5 s which was increased to 40 s during 20 h at 6 V/cm. The DNA fingerprints generated by PFGE were analyzed with visual method and by the digitalized method by *Gel-Pro* version 3.1 software. The obtained results were submitted to MVSP software generating dendrogram according to simple matching coefficient and UPGMA algorithm.

2.6. Other molecular typing methods of S. aureus isolates

Spa-typing was performed in all *S. aureus* isolates as elsewhere described (Harmsen et al., 2003). The polymorphic X region of *spa* gene was amplified by PCR, and sequences were analyzed using Ridom Staph-Type software version 1.5.21 (Ridom GmbH), which automatically detects *spa* repeats and assigns a *spa*-type according to http://spaserver.ridom.de/.

Identification of *agr* allele group (I–IV) was determined by multiplex PCR as earlier described (Shopsin et al., 2003).

Multilocus-sequence-typing (MLST) was performed in selected *S. aureus* isolates (in all 5 MRSA and in one isolate of each detected *spa*-type). The allelic profile of each strain was obtained by sequencing internal fragments of 7 unlinked housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*). Alleles of the 7 genes defined the allelic profile, which corresponded to a sequence type (ST) assigned by the MLST database (http://www.mlst.net).

2.7. 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*), *tst* gene encoding the TSST (Hwang et al., 2007), *lukS-lukF* genes encoding PVL leukocidin, *luk*E-*luk*D genes encoding the bicomponent leukotoxin LukE-LukD, *luk*M gene encoding leukocidin M, as well as *eta* and *etb* genes encoding exfoliative ETA and ETB toxins, respectively, and *hla*, *hlb*, *hld*, *hlg* and hlg_v genes encoding haemolysin toxins (Jarraud et al., 2002).

2.8. Detection of the immune-evasion-gene-cluster (IEC)

All isolates were tested by PCR for the presence of six genes (*chp*, *sak*, *sea*, *sep*, *scn* and *hlb*) encoding the IEC, that allows its classification into seven IEC types (from A to G), as previously reported (Van Wamel et al., 2006).

3. Results

3.1. Field survey results for S. aureus isolates from sheep

Among the 163 nasal samples of sheep tested in this study, 73 of them were positive for *S. aureus* recovery (44.8%), and one isolate per sample was further studied. Five of these *S. aureus* isolates were MRSA (3% of tested animals), and the remaining 68 isolates were MSSA (41.7% of tested animals).

3.2. Characteristics of MRSA detected in this study

All 5 MRSA isolates showed indistinguishable or closely related patterns and they were ascribed to *spa*-type t044, sequence type ST153 (included in clonal complex CC80) and *agr*III (Fig. 1 and Table 1). The SCC*mec* cassette of these 5 MRSA was not typeable by the employed methods in this study. These strains presented resistance, in addition to beta-lactams, to streptomycin, kanamycin, erythromycin, and clindamycin (inducible type) and four of them to tetracycline and fusidic acid, and harboured the genes *bla*Z, *ant*(6)-Ia, *aph*(3′)-IIIa, *erm*(C), *tet*(K), and *fus*B, respectively. The *fus*A gene identified in the 4 fusidic-acid-resistant MRSA isolates did not show mutations in its sequence that could justify this resistance phenotype. Additionally, they also contained the *lukS-luk*F genes encoding the Panton-Valentine leukocidin, as well as the *luk*-ED, *hla*, *hld* and hlg_v virulence genes. These MRSA strains also presented the ability to coagulate bovine plasma. In addition, they carried the *sak*, *hlb* and *scn* genes and were classified into the immune-evasion-gene-cluster IEC type E.

3.3. Characteristics of MSSA detected in this study

The characteristics of the 68 MSSA isolates recovered in this study are shown in Table 1. All 68 MSSA were submitted to *spa*-typing and 9 different *spa*-types were detected (number of isolates): t1534 (18), t1773 (19), t3576 (17), t5428 (6), t967 (4), t267 (1), and t223 (1), with two new ones registered as t7579 (1) and t7630 (1).

Amplification of the *agr* locus showed that *agr* group I was predominant (detected in 47 of 68 MSSA isolates, 69.1%), being the remaining isolates ascribed to *agr* group III.

Analysis of *Sma*I macrorestriction profiles of the 68 MSSA isolates revealed 16 different PFGE patterns (Fig. 1). These patterns were classified from S2 to S17, each defining a clone. The pulsotype S9 was the most frequently detected one (26% of isolates, 19 isolates), followed by the pulsotype S4b (12.3% of isolates, 9 isolates) and pulsotype S2b (11% of isolates, 8 isolates). PFGE showed a high clonality among strains with the same *spa*-type, except in the strains belonging to *spa*-type t3576 which were ascribed to 5 different pulsotypes.

Eight different sequence-types were identified by MLST among MSSA, six of them being new and registered as ST2056, ST2057, ST2076, ST2078, ST2079, and ST2083 (Table 1).

All MSSA strains presented the ability to coagulate bovine plasma. Only one of the 68 MSSA isolates carried the genes of the IEC system and the positive strain contained the genes *sak*, *chp*, *hlb* and *scn*, and consequently was classified into the IEC type B, according to previously referred criteria (Van Wamel et al., 2006). This IEC-positive strain belonged to sequence type ST2076.

3.4. Characterization of antimicrobial resistance mechanisms and virulence genes among MSSA

Most of MSSA (82.4%) showed susceptibility to the tested antimicrobial agents, with the following exceptions: penicillin (6% of isolates, carrying *bla*Z gene), tetracycline (19%, carrying *tet*K gene), and fusidic acid (9%). All 5 MRSA and 68 MSSA isolates showed susceptibility to vancomycin, teicoplanin, gentamicin, tobramycin, mupirocin, chloramphenicol, amikacin, ciprofloxacin and trimethoprim-sulfamethoxazole.

Fifty-three of 68 MSSA carried the *tst* gene (78%), encoding TSST, and none of them were PVL positive. Other virulence genes carried by MSSA isolates were: *luk*-M (61 isolates, 89,7%), *luk*-ED, *hla*, *hlb*, *hld*, and hlg_v (67 isolates, 98,5%), *hlg* (1 isolate, 1,5%), *sec* (48 isolates, 70,6%), *sel* (51 isolates, 75%), and the *ecg* cluster-like: *sen-sem-sei-seo-seg* (1 isolate, 1,5%). In contrast, 13 virulence genes were absent among our staphylococci: *sea*, *seb*, *sek*, *sep*, *seq*, *see*, *seu*, *seh*, *sed*, *sej*, *ser*, *eta* and *etb*.

No relation was found between the presence of virulence genes and antimicrobial resistance.

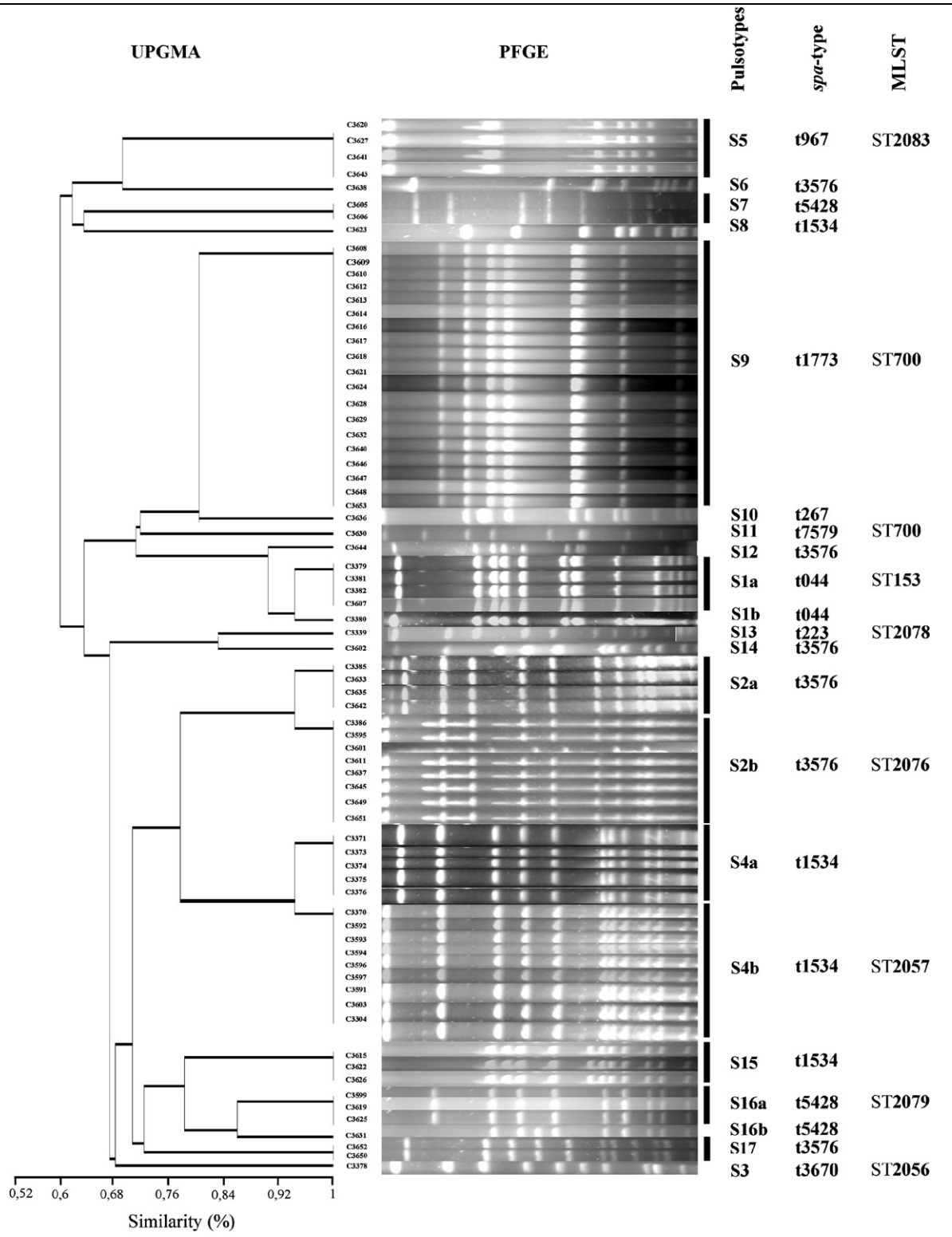

Fig. 1. Dendrogram showing the grouping of 73 *S. aureus* isolates from sheep using PFGE. Similarities are based on simple matching coefficient and UPGMA algorithm of *Sma*I macrorestriction profiles obtained by PFGE.

Table 1
Phenotypic and genotypic characteristics of the 5 MRSA and 68 MSSA isolates recovered from healthy sheep in Tunisia.[a]

No. isolates	*spa*-type	MLST[b]	PFGE pattern	*agr* type	Toxin genes detected	IEC type[c]	Phenotype of antibiotic resistance[d]	Resistance genes detected[e]
5	t044	ST153	S1a, S1b	III	*luk-ED, lukS-lukF, hla, hld, hlg$_\nu$*	E	P, FX, OX, TE[4], S, KA, E, CC, FA[4]	*mecA, blaZ, aph(3')*-IIIa, *ant*(6)-Ia, *tet*(K)[4], *erm*(C), *fusB*[4]
15	t1534	ST2057[e]	S4a, S4b, S8, S15	I	*tst-sec*^{13}*-sel*^{14}*, luk-ED, luk*-M, *hla, hlb, hld, hlg$_\nu$*	–	FA[3]	
2	t1534		S4a	I	*luk*-ED, *luk*-M, *hla, hlb, hld, hlg$_\nu$*	–	NR	
1	t1534		S4b	III	*tst-sec-sel, luk*-ED, *luk*-M, *hla, hlb, hld, hlg$_\nu$*	–	FA	
1	t7579[e]	ST700	S11	III	*tst-sec-sel, luk*-ED, *luk*-M, *hla, hlb, hld, hlg$_\nu$*	–	TE	*tet*(K)
1	t7630[e]	ST2056[e]	S3	I	*luk*-ED, *luk*-M, *hla, hlb, hld, hlg$_\nu$*	–	NR	
19	t1773	ST700	S9	III	*tst-sec*^{18}*-sel, luk*-ED, *luk*-M, *hla, hlb, hld, hlg$_\nu$*	–	P[1], TE[10]	*blaZ*[1], *tet*(K)[10]
16	t3576	ST2076[e]	S2a, S2b, S12, S14, S17	I	*tst-sec*^{15}*-sel*^{15}*, luk*-ED, *luk*-M, *hla, hlb, hld, hlg$_\nu$*	–	FA[1]	
1	t3576		S6	I	*tst, seg-sei-sem-sen-seo*	B	P, TE	*blaZ, tet*(K)
6	t5428	ST2079[e]	S7, S16a, S16b	I	*luk*-ED, *Luk*-M, *hla, hlb, hld, hlg$_\nu$*	–	NR	
4	t967	ST2083[e]	S5	I	*luk*-ED, *hla, hlb, hld, hlg$_\nu$*	–	P[1]	
1	t267	ST1476	S10	I	*luk*-ED, *hla, hlb, hld, hlg$_\nu$*	–	P, TE	*blaZ, tet*(K)
1	t223	ST2078[e]	S13	I	*tst-sec-sel, luk*-ED, *luk*-M, *hla, hlb, hld, hlg$_\nu$*	–	NR	

[a] In some cases, not all the isolates of the group presented the characteristic indicated, but only some of them and the number of isolates with this characteristic is indicated in superscript.
[b] The MLST of one isolate of each *spa* type was determined, with the exception of the 5 MRSA for which MLST was performed for all of them.
[c] The IEC type was determined according to Van Wamel et al. (2006). IEC –, all six tested genes of the system were negative for this strain.
[d] P: penicillin; OX: oxacillin, FX: cefoxitin, TE: tetracycline, S: streptomycin, KA: kanamycin, E: erythromycin, CC: clindamycin and .FA: fusidic acid. NR: no resistance detected.
[e] New *spa*-types or sequence types were detected and registered as indicated.

4. Discussion

The high recovery rate of *S. aureus* detected among nasal samples of healthy sheep in this study (44.8%), including PVL-positive MRSA isolates, is of relevance and represents, to our knowledge, the first study of this type performed in Tunisia. Previous works carried out in France and in Saudi Arabia in healthy sheep showed a lower nasal carriage of *S. aureus* (29% and 37.5%, respectively) (Vautor et al., 2005; Alzohairy, 2011).

The 5 MRSA strains recovered in our study were typed as ST153-t044 (single-locus variant of ST80), and *agr* type III, and harboured the genes encoding PVL. ST153 belongs to the clonal complex CC80, one of the major PVL-positive community acquired-MRSA lineages disseminated worldwide. It is of interest to remark that the clone CC80-t044 detected in our animal samples were frequently detected among community associated-MRSA in Tunisia in a previous study (Ben Nejma et al., 2008), as well as in other countries (Deurenberg and Stobberingh, 2008). In addition, the unique MRSA detected in healthy humans in Tunisia in a previous study (a veterinarian working with farm animals) was of the same clonal complex CC80 (although of different sequence type and *spa*-type) (Ben Slama et al., 2011). Some authors refer that *S. aureus* of the clone CC80 frequently shows resistance to tetracycline, streptomycin, kanamycin and fusidic acid (with the presence of the gene *fus*B), but not to gentamicin (McLaws et al., 2011), as four of the 5 MRSA isolates detected in our study.

Eight STs were identified among the MSSA strains, with 6 new STs detected. There are few data about the two remaining already-known STs but there seems to be an association between these clonal lineages and farm animals. ST700 detected in 20 of our MSSA isolates was previously identified in one strain recovered from one goat and belongs to CC130, which animal origin has been suggested (Shore et al., 2011). ST1476 lineage detected in one of our MSSA isolates belongs to CC97, which is mainly related to ruminants. This ST and CC have been also identified in Italian pig holdings and in Spanish pigs (Battisti et al., 2010; Gómez-Sanz et al., 2010).

Additionally, no MRSA ST398 was detected among our strains, clonal lineage related to farm animals (Gómez-Sanz et al., 2010; Lozano et al., 2011). This genetic lineage is mostly detected among pigs and cattle, and as far as we know, it has not been previously identified in sheep samples.

There is association between specific lineages of *S. aureus* and specific animal species. For Islamic countries, as it is the case of Tunisia, that do not consume pork, sheep is a very important agriculture animal species and the meat of sheep represents half of the total red meat consumed in Tunisia, according to recent published data (Brahmi et al., 2010). Important differences in the type of agricultural animals and type of consumed red meat do exist in European countries comparing to those of the Islamic culture.

The capacity of coagulate bovine plasma detected in our MRSA and MSSA isolates is a characteristic previously reported for small ruminants and bovine *S. aureus* ecovars

(Guinane et al., 2010); the referred authors associated this capacity with the presence of a novel staphylococcal pathogenicity island (SaPlov2), which was detected in their study in *S. aureus* strains of the clonal complex CC133, a well adapted clone to the bovine ecosystem. According to our results, it seems that other clonal complexes recovered from sheep might harbour this SaPlov2 element or similar ones, due to the fact that all our strains (of clonal complexes different from CC133) showed also the capacity to coagulate bovine plasma.

Beside the presence of *luk*S-*luk*F genes, coding for PVL toxin in the 5 MRSA isolates, several other virulence genes were detected in our MSSA isolates. The high percentage of *tst*-positive strains detected in our study (78% of MSSA) is in agreement with the results found by other authors in sheep (Vautor et al., 2009), but it is higher than the results obtained in MSSA strains from human nasal samples (Ben Slama et al., 2011).

Remarkably, most of the strains which harboured the *tst* gene also contained the *luk*-M, *luk*-ED, *sec* and *sel* genes. These genes might be either located in the same or associated genetic element or be somehow related to the identified clonal lineages. A relation among *tst*, *sec* and *sel* genes has been observed in many studies and it is known that there is a pathogenic island which encodes them (SaPI2) (Orden et al., 1992). On the other hand, the operon *egc* (enterotoxin gene cluster) which contains the *seg-sei-sem-sen-seo* genes (Hwang et al., 2007; Vautor et al., 2009) was only detected in one of our strains. This cluster is considered as a putative source of superantigen in *S. aureus*, being a possible source of new SE genes which are responsible for the horizontal transfer of virulence and/or antimicrobial resistance genes among *S. aureus* strains (Jarraud et al., 2001; Úbeda et al., 2005). Studies on MSSA human strains have detected a higher prevalence of this operon and it seems to be linked to the clonal background (Holtfreter et al., 2007). All strains except for the one which harboured the *egc* cluster presented *hla*, *hld* and hlg_{ν} genes, and 93% of them the *hlb* gene. These toxins are produced by most of *S. aureus* strains (Dinges et al., 2000). No positive strains were obtained for the remaining toxin genes tested (*sea*, *seb*, *sek*, *sep*, *seq*, *see*, *seu*, *seh*, *sed*, *sej*, *ser*, *eta* and *etb*).

All our MRSA strains (typed as ST153) and one of the 68 MSSA strains (typed as ST2076) carried the IEC system (type E or B). In previous studies it has been documented that IEC has an extremely high incidence among clinical human *S. aureus* strains (Van Wamel et al., 2006). According with our results, it seems that this IEC system is very scarcely distributed in MSSA sheep isolates but in those MRSA ones. This system is mobilized by bacteriophages that integrate the genes of IEC cluster in the gene *hlb* of *S. aureus* (Van Wamel et al., 2006).

Most of our MSSA showed susceptibility to the antimicrobials tested with few exceptions. The low level of penicillin resistance detected among our isolates contrast with the high frequency of this type of resistance reported for human isolates, even in commensal ones (Ben Slama et al., 2011; Lozano et al., 2011). The finding of tetracycline resistance among 23% of our *S. aureus* isolates could be explained by the high use of this antibiotic in animal industry. In regards to fusidic acid resistance, the rate found in different countries is very variable, with percentages ranging from 0.3% to 52.5% (Chen et al., 2010). No mutations in elongation factor G have been detected in the *fus*A sequences of the analyzed fusidic acid resistant *S. aureus* isolates.

In conclusion, the nares of healthy sheep could be a reservoir of PVL-positive MRSA isolates and also of *S. aureus* isolates carrying *tst*, *sec* and *sel* genes. More studies should be performed in the future to gain knowledge in the genetic lineages of *S. aureus* circulating among healthy animals, as well as in the capacity of these strains to produce virulence factors, due to the risk of animal-to-human bacterial transference.

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