Title: Genetic characterization of *Staphylococcus aureus* isolated from nasal samples of healthy ewes in Tunisia. High prevalence of CC130 and CC522 lineages

Authors: Meriam Ben Said, Mohamed Salah Abbassi, Paula Gómez, Laura Ruiz-Ripa, Senda Sghaier, Oussama El Fekih, Abdennaceur Hassen, Carmen Torres

PII: S0147-9571(17)30013-9
DOI: http://dx.doi.org/doi:10.1016/j.cimid.2017.03.002
Reference: CIMID 1127

To appear in:

Received date: 15-6-2016
Revised date: 23-2-2017
Accepted date: 9-3-2017

Please cite this article as: {http://dx.doi.org/

Genetic characterization of *Staphylococcus aureus* isolated from nasal samples of healthy ewes in Tunisia. High prevalence of CC130 and CC522 lineages.

Running title: *S. aureus* in healthy ewes, Tunisia

Meriam Ben Said [a,b], Mohamed Salah Abbassi [a,c], Paula Gómez [d], Laura Ruiz-Ripa [d], Senda Sghaier [a,b], Oussama El Fekih [a], Abdennaceur Hassen [b], Carmen Torres [d*]

[a] Université de Tunis El Manar, Institut de la Recherche Vétérinaire de Tunisie, 20 rue Jebel Lakhdhar, Bab Saadoun, Tunis 1006, Tunisie.

[b] Laboratoire de Traitement des Eaux Usées, Centre des Recherches et des Technologies des Eaux (CERTE), Technopole Borj-Cédria, BP 273, 8020, Soliman, Tunisie.

[c] Université de Tunis El Manar, Faculté de Médecine de Tunis, Laboratoire de résistance aux antibiotiques LR99ES09, Tunisie.

[d] Área de Bioquímica y Biología Molecular, Universidad de La Rioja, Logroño, Spain.

***Corresponding author**

Carmen Torres, PhD

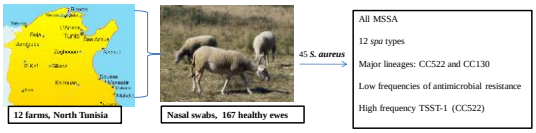
Departamento de Agricultura y Alimentación

Universidad de La Rioja

Madre de Dios, 53; 26006 Logroño, Spain

FAX: 34-941299721: Phone: 34-941299750; e-mail: carmen.torres@unirioja.es

Graphical abstract



Highlights:

- Forty-five *S. aureus* isolated from nasal swabs of 167 healthy ewes of seven farms.
- Twelve different *spa* types detected with two new ones (t15098, t15099).
- *S. aureus* assigned to six clonal complexes (CC522 and CC130 represented >80%).
- No MRSA was recovered and few resistances were observed among isolates.
- High rates of isolates with *tst* genes encoding TSST-1.

ABSTRACT

Staphylococcus aureus is a versatile bacterium, which can infect or colonize a variety of host species. The objective of this study was to characterize *S. aureus* isolates recovered from nasal swabs of 167 healthy ewes sampled from 12 farms in different areas of Tunisia during the period of 2014-2015. Genetic lineages, virulence factors and antibiotic resistance mechanisms were determined for recovered isolates. *S. aureus* was detected in 45 out of 167 tested samples (26.9 %). All isolates were methicillin-susceptible (MSSA) and the majority of them were susceptible to tested antibiotics with few exceptions (% of resistance): penicillin (8.8), ciprofloxacin (4.4), and tobramycin or tetracycline (2.2, each). Twelve different *spa* types were detected (t15098, t15099, t1773, t3576, t1534, t5428, t3750, t5970 t254, t2883,

t127 and t933), two of them were new (t15098 and t15099). *S. aureus* isolates were ascribed to *agr*I (n= 23), *agr*II (n=1) and *agr*III (n= 20), and one was non-typeable. According to the sequence-type (ST) determined and/or the *spa*-type detected, the 45 *S. aureus* isolates were assigned to six clonal complexes, with CC522 (44.4%) and CC130 (37.7%) being the most common lineages. Twenty-one (46.6%) and two (4.2%) isolates harbored the *tst* and *eta* genes encoding TSST-1 and ETA, respectively. In conclusion, nares of healthy ewes could be a reservoir of MSSA CC522 and CC130, lineages associated with TSST-1 and ETA that might represent a risk to human health.

Keywords: *S. aureus,* Healthy ewes, Nasal samples, CC130, CC522, Tunisia

1. Introduction

Staphylococcus aureus is an opportunistic pathogen and a frequent colonizer of humans and various animals [1]. However, it can also cause a wide range of infections ranging from superficial skin and soft tissue infections to deep infections such as pneumonia, septicemia and osteomyelitis in humans, as well mastitis in bovine [2]. *S. aureus* pathogenicity is mainly due to different putative virulence factors such as protein A, clumping factor, coagulase, fibrinogen, fibronectin, haemolysins, nucleases, exfoliatives toxins and enterotoxins [3,4]. In addition, antibiotic-resistant *S. aureus* isolates have been increasingly reported worldwide, especially methicillin resistance, a phenomenon considered globally as a serious public health problem. In fact, methicillin-resistant *S. aureus* (MRSA) strains have long been a problem in hospital and community setting worldwide. Moreover, livestock-associated (LA) MRSA have also been increasingly reported in the last years from a wide range of animal species including cows, pigs and chickens [5]. The potential of LA-MRSA clones, especially pig-associated strains (ST398), to infect and to cause diseases to humans represent a public health concern

[6, 7]. In addition, several studies have reported the possible transfer of MRSA from animals to people in close contact with them, such as dairy farmers [8], horse personnel [9], dog owners [10], and veterinary staff [11, 12]. Moreover, it is worthy to note that multidrug resistance is common in MRSA strains, especially against aminoglycosides and macrolides-lincosamides-streptogramins [13]. These findings highlight the importance of livestock-associated *S. aureus* as a major zoonotic agent and as a possible reservoir in animals of virulent and resistant strains affecting human health. In Tunisia, ewe herds are distributed all over the country in small family farms or in intensive husbandries. Ovine milk is used in some area of the country for artisanal transformation to fresh cheese. According to the World Bank (http://www.worldbank.org/), approximately 33% of the Tunisian population are settled in a rural region and showed frequent contact with ewes as farmers or traders of livestock. For all these last reasons, this study aimed to characterize *S. aureus* isolates recovered from nasal samples of healthy ewes in Tunisia by determining their genetic lineages, virulence traits and antimicrobial susceptibilities.

2. Materials and Methods

2.1. Sampling and microbiological identification

Nasal swabs of 167 healthy ewes were obtained from October 2014-March 2015 of 12 farms mostly located in the north of Tunisia. Swabs were incubated in 5 mL of Brain Heart Infusion broth (BHI, Becton-Dickinson) supplemented with 6.5% NaCl for 24h at 37°C and then, 0.1 mL was streaked on Mannitol Salt Agar (MSA, Becton-Dickinson) for 18-24h. One colony from each positive sample and with typical morphology was picked up. Identification of *S. aureu*s was based on colony morphology, Gram staining, the ability to coagulate rabbit plasma (Bio-Mérieux), and DNase activity. Identification of *S. aureus* was then confirmed by

a duplex PCR that amplifies the *nuc* gene and the methicillin-resistance genetic determinant *mecA* [14].

2.2. Antimicrobial susceptibility testing protocols

Susceptibility to 8 antimicrobial agents was performed using the disk-diffusion method as recommended by the Clinical and Laboratory Standards Institute, CLSI [15]; antimicrobial agents tested were (charge in µg/disk): penicillin (10 units), cefoxitin (30) tetracycline (30), tobramycin (10), gentamicin (10), erythromycin (15), clindamycin (2) and ciprofloxacin (5).

2.3. Detection of antimicrobial resistance genes

The presence of genes conferring resistance to penicillin (*blaZ*), tetracycline [*tet*(K), *tet*(L), and *tet*(M)], clindamycin [*lnu*(A), *lnu*(B), *vga*(A) and *vga*(C)], and tobramycin [*ant*(4′)-*Ia*], was analyzed by PCR [16-18]. In addition, selected isolates that belonged to the clonal complex CC130 were tested for the presence of the *mecC* gene [19]. Positive and negative controls from the collection of the University of La Rioja (Logroño, Spain) were used in all PCRs.

2.4. Molecular typing methods of *S. aureus* isolates

spa-typing was performed in all *S. aureus* isolates, as previously described [20]. The polymorphic X region of the Staphylococcal Protein A gene was amplified by PCR and sequenced. Sequence data were analyzed using Ridom Staph-Type software (www.spaserver.ridom.de) that detects *spa* repeats and assigns a *spa*-type. The *agr* allele group (I-IV) was determined by PCR as previously described [21].

Multilocus sequence typing (MLST) was performed in selected *S. aureus* isolates (isolates with new *spa* types and isolates with *spa* types repeated more than once). Seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqiL*) of *S. aureus* were amplified as previously

described [22], and Sequence Type (ST) was assigned by the MLST database (http://pubmlst.org/). The clonal complexes (CCs) of the isolates were assigned according to the ST determined and/or the *spa*-type detected.

2.5. Virulence factors

All isolates were tested by PCR for the presence of *tst* genes encoding the Toxic Shock Syndrome Toxin 1 (TSST-1), *eta* and *etb* genes encoding exfoliative ETA and ETB toxins [23], and *lukF/lukS-PV* genes encoding the Panton-Valentine leucocidin (PVL) [24].

3. Results

3.1. *S. aureus* isolates and antimicrobial susceptibility

Amongst the 167 nasal samples of healthy ewes tested in this study, *S. aureus* isolates were recovered in 45 of them (26.9 %), from seven different farms, and one isolate per positive sample was further studied. All 45 isolates showed susceptibility to cefoxitin, lacked the *mecA* gene, and were classified as methicillin-susceptible *S. aureus* (MSSA) (Table 1).

Thirty-eight (84.4%) of the isolates showed susceptibility to all antibiotics tested. By against, few resistance was detected among our isolates for penicillin (4 isolates, 8.8%), ciprofloxacin (2 isolates, 4.4%), tobramycin, clindamycin and tetracycline (one isolate, 2.2%, each one) (Table 1).

3.2. Molecular typing of *S. aureus* isolates

All the 45 *S. aureus* isolates were submitted to *spa*-typing. Twelve different *spa* types were detected, two of them being new and were registered in the database: t15098 (two isolates) and t15099 (one isolate). The remaining *spa* types were as follows (number of isolates): t1773

(15); t3576 (10); t 1534 (5) t5428 (4); t3750 (2); t5970 (2); t254, t2883, t127 and t933 (one, each one) (Table1).

MLST was performed for nine isolates (according to the criteria mentioned above) and six different STs were identified. According to the ST determined and/or the *spa*-type/s detected, the 45 *S. aureus* were assigned to six CCs (number of isolates, %): CC522 (20, 44.4), CC130 (17, 37.7), CC133 (4, 8.9), CC1, CC15 and CC80 (one, 2.2, each one) (Table 1).

The following *agr*-types were obtained *agr*I (n=23), *agr*II (n=1) and *agr*III (n=20), one isolate was non-typeable (Table 1).

3.3. Genes encoding antimicrobial resistance and virulence factors

Few resistance genes were detected in our study: *blaZ* (in two penicillin-resistant isolates) and *tet*(K) (in one tetracycline-resistant isolate). No clindamycin and tobramycin resistance genes were found among the resistant isolates. The isolates ascribed to CC130 were tested for the presence of *mecC* gene and negative results were obtained by PCR in all of them.

Twenty-one of our isolates harbored the *tst* gene (46.6%), and the *eta* gene was detected in two isolates (4.4%). None isolates harbored the *lukF/LukS-PV* or *etb* genes (Table1).

4. Discussion

A moderate recovery rate of *S. aureus* was detected in our study (26.9%), this result being similar to a previous study conducted in France where 29% of ewes carried *S. aureus* in their nares [25]; nevertheless, higher prevalence of *S. aureus* nasal carriage (44.8%) was previously found in healthy sheep in Tunisia [26]. Various *spa* types were observed among our isolates (12 different *spa* types). The presence of two new *spa* types (t15098 and t15099) in our study suggests that the data on population structure of *S. aureus* from small ruminants are still limited despite several recent studies undertaken in this field [27, 28]. Six different clonal complexes were

identified in our study, and CC522 was the most predominant one containing 20 isolates (44.4 %). These results differed from those found by Merz et al. [29] on sheep and goats milk in which CC522 was present in only two goat strains and the majority of the strains was assigned to CC130 and CC133. The lineage CC522 has previously been reported and it appeared to be restricted to Africa and Europe [28, 30, 31]. The *agr*I detected in isolates of this CC is in agreement with other reports [32]. CC130 was the second most common genetic lineage in our study (37.7%), and was associated with two *spa* types (t1773 and t5970). This CC has previously been detected in ovine, caprine and bovine isolates [27, 30, 33], and in *mecC* MRSA isolates from animal and human origins [18, 34-37]. All CC130 isolates in our study were MSSA and did not harbor the *mecC* gene; it is important to note that nares of healthy ewes could be a reservoir of MSSA isolates of this lineage. On the other hand, these strains could acquire the *mecC* or *mecA* and other resistance genes that represent a threat to humans in contact with these animals. Indeed, in Denmark, the majority of human *mecC* positive isolates were originated from rural areas; moreover, the epidemiological study of 22 patients showed that four of them had contact with livestock animals [35]. Only four of our isolates belonged to CC133 lineage and were associated with *spa* types t3750 (ST2328) and the new one t15098 (ST701). The lineage ST2328/CC133 was previously observed in small ruminants [27]; moreover, *S. aureus* CC133 isolates were also found in healthy donkeys in Tunisia, but with different ST and *spa* types [31]. Several studies described the CC133 as one of the most predominant lineages among ruminants [30, 36, 38, 39]. Guinane et al. [40] suggested that the lineage CC133 is a result of human to ruminant host jump followed by adaptive genome diversification. The clonal complexes CC15 and CC1, that are mainly related to humans [6, 41], were detected, each, in one isolate. The lineage CC80, associated with *spa*-type t2883, has previously been detected in Tunisia among one MSSA recovered from a newborn with bacteremia and from nasal swabs of healthy humans [42, 43]. More

importantly, the CC80 is one of the predominant community acquired-MRSA clones in Tunisia [44, 45], as well as in Europeans countries [46, 47].

The majority of our isolates showed susceptibility to the tested antibiotics (84.4 %), with few exceptions. The low frequency of penicillin resistance (8.8%) noticed in our study differed from data of isolates found in buffalo, bovine, ovine and caprine milk in Brazil [48], and in nasal samples of healthy humans in Tunisia [43]. Nevertheless, a similar penicillin resistance percentage was observed among sheep isolates in Tunisia [26]. The low rate of antimicrobial resistance detected in our study could be explained by the limited use of antimicrobial agents to treat ovine infections in Tunisia, and by the type of farms where the sampling was carried out. Indeed, the majority of farms included in our study were small family farms; hence, with no highly selective pressure due to antimicrobial drugs commonly used in intensive husbandry.

A high rate of isolates carried the *tst* gene in our study (46.6 %) that is in agreement with previous data on sheep isolates in Tunisia [26]. All *tst*-positive strains, except one, belonged to the clonal complexes CC130 and CC522. Moreover, two isolates harbored the *eta* gene, in addition to the *tst* gene. According to our findings, it seems that these clonal complexes are associated with toxins that might represent a risk to humans. So, it is worthy to note that our *tst* positive isolates could also harbor the *sec* and *sel* genes encoding Sec and Sel enterotoxins since, it has previously been reported that *tst-sec-sel* genes are located in the same pathogenic island [49].

To conclude, the nares of healthy ewes could be a reservoir of *S. aureus* isolates associated with CC522 and CC130 lineages. Several isolates belonging to these clonal complexes harbored the *tst* gene, related to the toxic shock syndrome, and the *eta* gene was also detected. The high rate of CC130 found in our study is interesting, since this lineage is newly associated with *mecC* MRSA from human and animal isolates in Europe. Consequently, more

studies should be undertaken in the future to gain knowledge in the genetic lineages of *S. aureus* circulating among healthy animals, as considerably as in the possibility of these isolates to acquire *mecA* or *mecC* genes, and particularly the capacity to produce virulence factors, affecting humans, due to the risk of bacterial transfer from animal to human.

Acknowledgements

We thank the farmers for their collaboration. Ben Said Meriam has a fellowship from the Tunisian Ministry of Higher Education and Scientific Research. Paula Gómez has a pre-doctoral fellowship from the Universidad de La Rioja (Spain). This study was partly supported by project SAF2016-76571-R of the Ministerio de Economía y Competitividad of Spain (MINECO) and FEDER.

References

[1] H. Hasman, A. Moodley, L. Guardabassi, M. Stegger, R.L. Skov, F.M. Aarestrup, *Spa* type distribution in *Staphylococcus aureus* originating from pigs, cattle and poultry, Vet. Microbiol. 141 (2010) 326-331.

[2] P.J. Quinn, M.E. Carter, B.K. Markey, G.R. Cater, *Staphylococcus* species. In: Clinical Veterinary Microbiology, Mosby, Edinburgh, 2000, pp. 118-126.

[3] C. Fournier, P. Kuhnert, J. Frey, R. Miserez, M. Kirchhofer, T. Kaufmann, A. Steiner, H.U. Graber, Bovine *Staphylococcus aureus*: association of virulence genes, genotype and clinical outcomes, Res. Vet. Sci. 85 (2008) 439-448.

[4] S.J. Peacock, C.E. Moore, A. Justice, M. Kantzanou, L. Story, K. Mackie, G. O'Neill, N.P. Day, Virulent combinations of adhesin and toxin genes in natural populations of *Staphylococcus aureus*, Infect. Immun. 70 (2002) 4987-4996.

[5] C. Cuny, A. Friedrich, S. Kozytska, F. Layer, U. Nubel, K. Ohlsen, B. Strommenger, B. Walter, L. Wieler, W. Witte, Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in different animal species, Int. J. Med. Microbiol. 300 (2010) 109-117.

[6] C. Lozano, C. Aspiroz, L. Charlez, E. Gomez-Sanz, M. Toledo, M. Zarazaga, C. Torres, Skin lesion by methicillin-resistant *Staphylococcus aureus* ST398-t1451 in a Spanish pig farmer: possible transmission from animals to humans, Vector Borne Zoonotic. Dis.11 (2011) 605-607.

[7] J.P. Rasigade, F. Laurent, P. Hubert, F. Vandenesch, J. Etienne, Lethal necrotizing pneumonia caused by a ST 398 *Staphylococcus aureus* strains, Emerg. Infect. Dis. 16 (2010) 1330.

[8] L. Garcia-Alvarez, M.T.G. Holden, H. Lindsay, C. Webb, D.F.J. Brown, M.D. Curran, E. Walpole, K. Brooks, D.J. Pickard, C. Teale, J. Parkhill, S.D. Bentley, G.F. Edwards, E.K. Girvan, A.M. Kearns, B. Pichon, R.L.R. Hill, A.R. Larsen, R.L. Skov, S.J. Peacock, D.J. Maskell, M.A. Holmes, 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. 11 (2011) 595-603.

[9] J. Weese, F. Caldwell, B. Willey, B. Kreiswirth, A. McGeer, J. Rousseau, D. Low, An outbreak of methicillin-resistant *Staphylococcus aureus* skin infections resulting from horses to human transmission in a veterinary hospital, Vet. Microbiol. 114 (2006) 160-164.

[10] C. Cefai, S. Ashurst, C. Owens, Human carriage of methicillin-resistant *Staphylococcus aureus* linked with a pet dog, Lancet Infect. Dis. 344 (1994) 539-540.

[11] B. Hanselman, S. Kruth, J. Rousseau, D. Low, B. Willey, A. McGeer, J. Weese, Methicillin resistant *Staphylococcus aureus* colonization in veterinary personal, Emerg. Infect. Dis.12 (2006) 1933-1938.

[12] J.C. Seguin, R.D. Walker, J.P. Caron, W.E. Kloos, G.G. George, R.J. Hollis, R.N. Jones, M.A. Pfaller, Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-animal transmission, J. Clin. Microbiol. 37 (1999) 1459-1463.

[13] M. Monaco, F. Pimentel de Araujo, M. Cruciani, E.M. Cocccia, A. Pantosti, Worldwide epidemiology and antibiotic resistance of *Staphylococcus aureus*, Curr. Top. Microbiol. Immunol. DOI 10.1007/82-2016-3.

[14] Community Reference Laboratory for Antimicrobial Resistance, Multiplex PCR for the detection of the *mecA* Gene and the Identification of *Staphylococcus aureus*. National Food Institute, 2009. Technical University of Denmark, Copenhagen, Denmark.

[15] Clinical and Laboratory Standards Institute, M100-S25.Performance Standards for Antimicrobial Susceptibility Testing, 2015, Twenty-Fifth Information Supplement.

[16] E. Gomez-Sanz, C. Torres, C. Lozano, R. Fernandez-Perez, C. Aspiroz, F. Ruiz-Larrea, M. Zarazaga, Detection, molecular characterization, and clonal diversity of methicillin-resistant *Staphylococcus aureus* CC398 and CC97 in Spanish slaughter pigs of different age groups, Foodborne Pathog. Dis. 7 (2010) 1269-1277.

[17] C. Lozano, C. Aspiroz, A. Rezusta, E. Gómez-Sanz, C. Simon, P. Gómez, C. Ortega, M.J. Revillo, M. Zarazaga, C. Torres, Identification of novel *vga(A)*-carrying plasmids and a Tn5406-like transposon in methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* of human and animal origin, Int. J. Antimicrob. Agents. 40 (2012) 306-312.

[18] D. Benito. P. Gómez, C. Aspiroz, M. Zarazaga, C. Lozano, C. Torres, Molecular characterization of *Staphylococcus aureus* isolated from humans related to a livestock farm in

Spain, with detection of MRSA-CC130 carrying *mecC* gene: A zoonotic case, Enferm. Infec. Microbiol. Clin. 34 (2016) 280-285.

[19] C. Cuny, F. Layer, B. Strommenger, W. Witte, Rare occurrence of methicillin-resistant *Staphylococcus aureus* CC130 with a novel *mecA* homologue in humans in Germany, PLoS One . 6 (2011) e 24360.

[20] D. Harmsen, H. Claus, W. Witte, J. Rothganger, H. Claus, D. Turnwald, U. Vogel, 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. 41 (2003) 5442-5448.

[21] B. Shopsin, B. Mathema, P. Alcabes, B. Said-Salim, G. Lina, A. Matsuka, J. Martinez, B.N. Kreiswirth, Prevalence of *agr* specificity groups among *Staphylococcus aureus* strains colonizing children and their guardians, J. Clin. Microbiol. 41 (2003) 456-459.

[22] M.C. Enright, N.P. Day, C.E. Davies, S.J. Peacock, B.G. Spratt, Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*, J. Clin. Microbiol. 38 (2000) 1008-1015.

[23] S. Jarraud, C. Mougel, J. Thioulouse, G. Lina, H. Meugnier, F. Forey, X. Nesme, J. Etienne, F. Vandenesch, Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease, Infect. Immun. 70 (2002) 631-641.

[24] G. Lina, Y. Piémont, F. Godail-Gamot, M. Bes, M.O. Peter, V. Gauduchon, F. Vandenesch, J. Etienne, Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia, Clin. Infect. Dis. 29 (1999) 1128-1132.

[25] E. Vautor, V. Magnone, G. Rios, K.L. Brigand, D. Bergonier, G. Lina, H. Meugnier, P. Barbry, R. Thiery, M. Pepin, Genetic differences among *Staphylococcus aureus* isolates from

dairy ruminant species: a single-dye DNA microarray approach, Vet. Microbiol, 133 (2009) 105-114.

[26] H. Gharsa, K. Ben Slama, C. Lozano, E. Gómez-Sanz, N. Klibi, R. Ben Sallem, P. Gómez, M. Zarazaga, A. Boudabous, C. Torres, Prevalence, antibiotic resistance, virulence traits and genetic lineages of *Staphylococcus aureus* in healthy sheep in Tunisia. Vet. Microbiol, 156 (2012) 367-373.

[27] J. Eriksson, C. Espinosa-Gongora, I. Stamphøj, A.R. Larsen, L. Guardabassi, Carriage frequency, diversity and methicillin resistance of *Staphylococcus aureus* in Danish small ruminants, Vet. Microbiol, 163 (2013) 110-115.

[28] E.M. Smith, P.F. Needs, G. Manley, L.E. Green, Global distribution and diversity of ovine-associated *Staphylococcus aureus*, Infect. Genet. Evol. 22 (2014) 208-215.

[29] A. Merz, R. Stephan, S. Johler, *Staphylococcus aureus* isolates from goat and sheep milk seems to be closely related and differ from isolates detected from bovine milk, Front. Microbiol, 7 (2016) 319.

[30] M.C. Porrero, H. Hasman, A.I. Vela, J.F. Fernandez-Garayzabal, L. Dominguez, F.M. Aarestrup, Clonal diversity of *Staphylococcus aureu*s originating from the small ruminants goats and sheep, Vet. Microbiol, 156 (2012) 157-161.

[31] H. Gharsa, B. Ben Sallem, K. Ben Slama, E. Gómez-Sanz, C. Lozano, A. Jouini, N. Klibi, M. Zarazaga, A. Boudabous, C. Torres, 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. 8 (2012) 203.

[32] G.K. Bar-Gal, S.E. Blum, L. Hadas, R. Ehricht, S. Monecke, G. Leitner, 2015 Host-specificity of *Staphylococcus aureus* causing intramammary Infections in dairy animals assessed by genotyping and virulence genes, Vet. Microbiol. 176 (2015) 143-154.

[33] J.M. Sung, D.H. Lloyd, J.A. Lindsay, *Staphylococcus aureus* host specificity: Comparative genomics of human versus animal isolates by multi-strain microarray. Microbiology, 154 (2008) 1949-1959.

[34] M. Stegger, P.S. Anderson, A. Kearns, B. Pichon, M.A. Holmes, G. Edwards, F. Laurent, C. Teale, R. Skov, A.R. Larsen, Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harboring either *mecA* or the new homologue $mecA_{LG251}$. Clin. Microbial. Infect, 18 (2012) 395-400.

[35] A. Peterson, M. Stegger, O. Heltberg, J. Christensen, A. Zeuthen, L.K. Knudsen, M. Sorum, L. Schouls, J. Larsen, R. Skov, A.R. Larsen, Epidemiology of methicillin-resistant *Staphylococccus aureus* carrying the novel *mecC* gene in Denmark corroborates a zoonotic reservoir with transmission to humans. Clin. Microbiol. Infect, 19 (2013) E16-E19.

[36] P. Gómez, C. Lozano, D. González-Barrio, M. Zarazaga, F. Ruiz-Fons, C. Torres, High prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) carrying the *mecC* gene in a semi-extensive red deer (*Cervus elaphus hispanicus*) farm in Southern Spain, Vet. Microbiol. 177 (2015) 326-331.

[37] P. Gómez, C. Lozano, M.C. Camacho, J.F. Lima-Barbero, J.M. Hernández, M. Zarazaga, U. Höfle, C. Torres C. Detection of MRSA ST3061-t843-*mec*C and ST398-t011-*mec*A in white stork nestlings exposed to human residues. J. Antimicrob. Chemother. 71(2016):53-7.

[38] N.L. Ben Zakour, D.E. Sturdevant, S. Even, C.M. Guinane, C. Barbey, P.D. Alves, M.F. Cochet, M. Gautier, M. Otto, J.R. Fitzgerald, Y. Le Loir, Genome-wide analysis of ruminant *Staphylococcus aureus* reveals diversification of the core genome, J. Bacteriol. 190 (2008) 6302-6317.

[39] D.S. Smyth, E.J. Feil, W.J. Meaney, P.J. Hartigan, T. Tollersrud, J.R. Fitzgerald, M.C. Enright, C.J. Smyth, Molecular genetic typing reveals further insights into the diversity of animal-associated *Staphylococcus aureus*, J. Med. Microbiol. 58 (2009) 1343-1353.

[40] C.M. Guinane, N.L. Ben Zakour, M.A. Tormo-Mas, L.A. Weinert, B.V. Lowder, R.A. Cartwrigh, D.S. Smyth, C.J. Smyth, J.A. Lindsay, K.A. Gould, A. Witney, J. Hinds, J.P. Bollback, A. Rambaut, J.R. Penadés, J.R. Fitzgerald, Evolutionary genomics of *Staphylococcus aureus* reveals insights into the origin and molecular basis of ruminant host adaptation, Genome. Biol. Evol. 2 (2010) 254-266.

[41] S. Monecke, C. Luedicke, P. Slickers, R. Ehrich, Molecular epidemiology of *Staphylococcus aureus* in asymptomatic carriers, Eur. J. Clin. Microbiol. Dis. 28 (2009) 1159-1165.

[42] A. Kechrid, M. Pérez-Vázquez, H. Smaoui, D. Hariga, M. Rodríguez-Baños, A. Vindel, F. Baquero, R. Cantón, R. Del Campo, Molecular analysis of community-acquired methicillin-susceptible and resistant *Staphylococcus aureus* isolates recovered from bacteraemic and osteomyelitis infections in children from Tunisia. Clin. Microbiol. Infect, 17 (2010) 1020-1026.

[43] K. Ben Slama, H. Gharsa, N. Klibi, A. Jouini, C. Lozano, E. Gómez-Sanz, M. Zarazaga, A. Boudabous, C. Torres, Nasal carriage of *Staphylococcus aureus* in healthy humans with different levels of contact with animal in Tunisia: genetic lineage, methicillin resistance, and virulence factors, Eur. J. Clin. Microbiol. Infect. Dis. 30 (2011) 499-508.

[44] M. Ben Nejma, M. Mastouri, B. Bel Hadj Jrad, M. Nour, Characterization of ST80 Panton-Valentine leukocidin-positive community-acquired methicillin-resistant *staphylococcus aureu*s clone in Tunisia, Diagn. Microbiol. Infect. Dis. 77 (2008) 20-24.

[45] M. Ben Jomàa-Jemili, I. Teruyo, Z. Meng, J. Jingxun, L. Shanshuang, I. Boutiba-Ben Boubaker, A. Hammami, H. Xiao, H. Keiichi, Molecular Characterization of methicillin-resistant Panton-Valentine leukocidin-positive *Staphylococcus aureus* clones disseminating in Tunisian hospitals and in the community, BMC. Microbiol. 13 (2013) 2.

[46] F. Vandenesch, T. Naim, M.C. Enright, G. Lina, G.R. Nimmo, H. Heffernan, N. Liassine, M. Bes, T. Greenland, M.E. Reverdy, J. Etienne, Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine Leucocidin genes: worldwide emergence, Emerg. Infect. Dis. 9 (2003) 978–984.

[47] O. Denis, A. Deplano, H. De Beenhouwer, M. Hallin, G. Huysmans, M.G. Garrino, Y. Glupczynski, X. Malaviolle, A. Vergirson, M.J. Struelens, Polyclonal emergence and importation of Community-acquired methicillin-resistant *Staphylococcus aureus* strains harboring Panton-Valentine leucocidin genes in Belgium, J. Antimicrob. Chemother. 56 (2005) 1103-1106.

[48] M. Aires-de-Sousa, C.E.S.R. Parente, O. Vieira-da-Motta, I.C.F. Bonna, D.A. Sliva, H. de Lancaster, Characterization of *Staphylococcus aureus* isolates from buffalo, bovine, ovine and caprine milk samples collected in Rio de Janeiro State, Brazil, Appl. Environ. Microbiol. 73 (2007) 3845-3849.

[49] J.A. Orden, J. Goyache, J. Hernandez, A. Domenech, G. Suarez, E. Gomez-Lucia, Detection of enterotoxins and TSST-1 secreted by *Staphylococcus aureus* isolated from ruminant mastitis. Comparison of ELISA and immunoblot. J. Appl. Bacteriol. 72 (1992) 486-489.

List of Tables

Table 1. Characteristics of 45 *S. aureus* isolates recovered from healthy ewes in Tunisia.

Number of isolates	Area Farm ID[a]	*spa*-type[b]	ST /CC[c]	*agr*-type	Virulence genes[d]	Phenotype of resistance[d,e]	Resistance genes
15	MB3, MB4, MB5	t1773	ST130/ST2011 / CC130	III	*tst* (11), *eta* (1)	PEN(1), CLI (1)	
10	MO, MB3, MB5	t3576	ST2057 / CC522	I	*tst* (5), *eta* (1)	CIP(1)	
5	MB2, MB5	t1534	ST2057 /CC522	I	*tst* (4)	Susceptible	
4	MB3, MB4	t5428	ST2079 / CC522	I	-	TOB(1)	
2	MA	**t15098**	ST701/CC133	I	*tst* (1)	Susceptible	
2	B	t3750	ST2328/CC133	III	-	Susceptible	
2	B	t5970	ST2011 / CC130	III	-	CIP(1)	
1	MB3	t254	(CC15)	II	-	PEN, TET	*blaZ, tet(K)*
1	MB3	t2883	(CC80)	I	-	PEN	*blaZ*
1	MB4	t127	(CC1)	III	-	PEN	
1	MB4	**t15099**	ST2079/CC522	I	-	Susceptible	
1	MB5	t933	ND[e]	NT[e]	-	Susceptible	

[a] Geographical areas of the farms: B: Beja, MA: Mater, MB: Menzel Bouzelfa , MO: Morneg. The number indicates the farm identity in each geographical area.

[b] New *spa*-types are shown in bold.

[c] In some cases the clonal complex (CC) was assumed according to the *spa*-type (in this case it is shown in parenthesis).

[d] In some cases, not all the isolates of the group presented the characteristic indicated, the number of isolates with this characteristic is indicated in superscript.

[e] PEN: penicillin, CLI: clindamycin , CIP: ciprofloxacin, TOB: tobramycin, TET: tetracycline.; ND: non determined; NT: non typeable.