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Original Article

Characterization of *Staphylococcus aureus* from Raw Meat Samples in Tunisia: Detection of Clonal Lineage ST398 from the African Continent

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Abstract

Livestock-associated *Staphylococcus aureus* isolates, and especially those belonging to ST398, have been increasingly described in colonized and infected animals and humans, and also in food samples in several countries. The purpose of this study was to determine the frequency of *S. aureus* and methicillin-resistant *S. aureus* (MRSA) isolates in raw meat samples destined for food consumption in Tunisia, and to characterize the recovered isolates. One hundred sixty-nine food samples of animal origin were collected. Samples were inoculated onto selective mediums for *S. aureus* and MRSA recovery. Different molecular typing methods were implemented (pulsed-field gel electrophoresis [PFGE], multilocus sequence typing, *spa*-, *agr*-, and SCC*mec* typing). MRSA was detected in 2 of these 169 samples (1.2%), both of which were of chicken origin. The two MRSA isolates (one/sample) were typed as ST30-CC30-t012-*agr*III-*SCCmec*V and ST398-CC398-t4358-*agr*I-*SCCmec*IVa. The MRSA ST398 strain presented resistance, in addition to β-lactams, to tetracycline (*tet*[M]) and erythromycin (*erm*[C]) and harbored the *sen*, *hla*, *hlg*, and *hlgv* virulence genes. Methicillin-susceptible *S. aureus* (MSSA) isolates were recovered from 42 of the 169 tested samples (24.8%). A high diversity of *spa* types ($n=21$) and *Sma*I-PFGE patterns (27 different profiles; 4 nontypeable) were detected among MSSA isolates. Four MSSA isolates were typed as ST398/CC398. The percentage of antimicrobial resistance and detected genes in MSSA isolates were as follows: tetracycline (28.6% *tet*[K] and *tet*[L]), kanamycin (9.5%, *aph*[3′]-IIIa), tobramycin (2.4%, *ant*[4′]-Ia), erythromycin (14.3%, *erm*[A], *erm*[C], *msr*[*A*]), and penicillin (95%). The genes *lukS-lukF* were detected in two MSSA isolates (4.5%), the gene *tst* in one isolate, and the gene *eta* in five isolates. To our knowledge, this is the first detection of MRSA and MSSA ST398 in food in an African country. The risk of transmission of *S. aureus* and MRSA carrying different antimicrobial resistance and virulence genes through the food chain cannot be ignored.

Introduction

Staphylococcus aureus is part of the normal microbiota of humans and animals, although it is also considered an opportunistic pathogen. *S. aureus* infections are facilitated by the expression of several virulence factors, which include several toxin groups such as the Panton-Valentine leukocidin (PVL), the toxic shock syndrome toxin, adhesins, hemolysins, exfoliative toxins (ETA and ETB), and staphylococcal enterotoxins (SEs). The ingestion of one or more of SEs in high doses via food products contaminated with enterotoxin-positive *S. aureus* isolates can produce staphylococcal food poisoning. For that, the presence of *S. aureus* strains carrying enterotoxin genes in food constitutes a health risk for consumers.

Moreover, *S. aureus* is able to acquire multiple resistance genes, which limit the therapeutic options. Methicillin-resistant *S. aureus* (MRSA) isolates appeared in 1961, and in

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1972, MRSA was isolated for the first time in animals (Devriese *et al*., 1972). Since then, they have been detected in different animal species (Madec and Haenni, 2010). In 2005, a new sequence type of MRSA (ST398) appeared in Europe associated with livestock animals (Armand-Lefevre *et al*., 2005). MRSA CC398 has been previously found in animals (especially in pigs), in people in contact with these animals (veterinarians and farmers) and their relatives (Khanna *et al*., 2008), and in food of animal origin (Lozano *et al*., 2009; Fessler *et al*., 2011). Until now, MRSA CC398 has been detected in European countries (Armand-Lefevre *et al*., 2005), Canada (Khanna *et al*., 2008), United States (Mediavilla *et al*., 2012), Asia (Lim *et al*., 2012), South America (Arriola *et al*., 2011), and Australia (Groves *et al*., 2013).

The studies carried out in Africa have been mainly focused on human isolates. Different CCs (CC1, CC5, CC15, CC30, CC88, CC152, CC121, or CC672) have been found among hospital and community MRSA and MSSA isolates, depending on the region (Breurec *et al*., 2011; Basset *et al*., 2015). Concerning data of Tunisia, one study carried out in healthy people only detected one MRSA belonging to CC80 (Ben Slama *et al*., 2011), and this clonal lineage was also observed in hospitalized children in this country (Ben Nejma *et al*., 2014). In some studies performed in healthy animals, the CCs identified among MRSA and MSSA isolates have also been diverse (CC1, CC6, CC22, CC72, CC130, CC80 or CC522 among others) (Gharsa *et al*., 2012a, 2012b), highlighting the predominance of CC133 among donkeys destined for food consumption (Gharsa *et al*., 2012a). Remarkably, only one study has identified the clonal lineage CC398 in one skin sample from a dog in Zambia, and this isolate was susceptible to methicillin (Youn *et al*., 2014).

The purpose of this study was to determine the frequency of *S. aureus* and MRSA isolates in raw meat samples destined for food consumption in Tunisia, and to investigate the content in antimicrobial resistance and virulence genes and the genetic lineages of recovered isolates.

Materials and Methods

Sampling and microbiological isolation

A total of 164 raw food samples of animal origin (84 poultry, 42 lambs, 30 calves, 4 horses, 3 rabbits, and 1 pig) were collected from 8 poultry markets, 6 butcheries, 3 farms, and 2 supermarkets in 10 different regions of Tunisia during October 2010 to March 2011. Samples were processed for recovery of MRSA and *S. aureus* as previously described (Lozano *et al*., 2012; Benito *et al*., 2014). 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) (Biomerieux, Marcy l'Etoile, France). *S. aureus* identification was then confirmed by amplification of the species-specific *nuc* gene (Gómez-Sanz *et al*., 2010) (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/fpd).

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, 2012). Antimicrobial agents tested were (charge in μg/disk): penicillin (10 units), 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.

DNA extraction method

DNA of all *S. aureus* isolates recovered was extracted. For that, bacteria were harvested from brain–heart infusion agar plates, suspended in 45 μL of sterile water with 5 μL of lysostaphin (3000 U/mL) and incubated at 37°C for 10 min. After that, 45 μL of sterile water, 5 μL of proteinase K solution (2 mg/mL), and 150 μL of Tris HCl (0.1 M pH 8) were added. Cell suspensions were then incubated at 60°C for 10 min and later they were placed at 100°C for 5 min.

*Detection of methicillin resistance genes and SCC*mec*-typing*

The presence of the *mecA* gene was studied by polymerase chain reaction (PCR) in all oxacillin- and/or cefoxitin-resistant isolates using primers and conditions as previously reported (Gómez-Sanz *et al*., 2010). The *mecC* gene was also tested by PCR in all isolates (García-Álvarez *et al*., 2011). The SCC*mec*-typing was performed for *mecA* positive isolates by a multiplex PCR strategy described by Kondo *et al*. (2007) (Supplementary Table S1).

Detection of other antimicrobial resistance genes

The ribosomal methylases encoded by *erm*(A), *erm*(B), *erm*(C), and *erm*(T) 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. In addition, *tet*(K), *tet*(M), and *tet*(L) genes conferring resistance to tetracycline, and *aph*(3′)-IIIa and *ant*(4′)-Ia genes to kanamycin and tobramycin, were studied by PCR in all antimicrobial-resistant *S. aureus* isolates. We used the primers and conditions as mentioned by Lozano *et al*. (2009, 2012) (Supplementary Table S1).

Pulsed-field gel electrophoresis

All *S. aureus* isolates were characterized by pulsed-field gel electrophoresis (PFGE) with *Sma*I restriction enzyme digestion as previously described (Gómez-Sanz *et al*., 2010). The DNA fingerprints generated by PFGE were analyzed with the visual method and by the digitalized method by GelCompar II version 6.5 software (Applied Maths, Kortrijk, Belgium) generating a dendrogram according to Dice coefficient and unweighted-pair group method with arithmetic mean algorithm. In the strains in which no PFGE pattern was obtained with *Sma*I enzyme, the enzyme *Apa*I was used.

Other molecular typing methods of S. aureus *isolates*

Spa typing was performed in all *S. aureus* isolates as described elsewhere (Lozano *et al*., 2011). Identification of *agr*

allele group (I–IV) was determined by multiplex PCR as described earlier (Shopsin *et al*., 2003). Multilocus sequence typing was performed in selected *S. aureus* isolates (isolates with *spa* types associated with ST398 and isolates with the two most common *spa*-types found in this study) (http://saureus.mlst.net/) (Supplementary Table S1).

Detection of staphylococcal toxin genes

All isolates were tested by PCR for the presence of *sea, seb, sec, sed, see, seg, seh, sei, sej, sek, sel, sem, sen, seo, sep, seq, ser, seu*, *tst*, *lukS-lukF*, *lukE-lukD*, *lukM*, *eta*, *etb*, *hla, hlb, hld, hlg,* and *hlgv* genes (Lozano *et al*., 2011) (Supplementary Table S1).

Analysis of the possible origin of selected strains

The presence of several mobile genetic elements (MGEs) recently associated with an avian origin and the detection of the genes comprising the immune evasion cluster (IEC) (*scn*, *chp*, *sak*, *sea*, and/or *sep*) related to human origin were performed by PCR in ST398 strains (Van Wamel *et al*., 2006; Lowder *et al*., 2009) (Supplementary Table S1).

Results

Field survey results for S. aureus *isolates*

Forty-three food samples of the 164 studied (26.2%) contained *S. aureus* and 1 isolate per sample was further studied. Two of the *S. aureus* isolates were MRSA, and both were recovered from chicken samples and contained the *mecA* gene; the remaining 41 *S. aureus* isolates were methicillin susceptible (MSSA). The *mecC* gene was not identified in the isolates of this study.

Characteristics of MRSA isolates detected in this study

The two MRSA isolates showed different characteristics (Table 1). The *spa*-type of the isolate C4995 was t012, ST30, and *agr*III. The *mecA* gene was carried by the *SCCmec*V. This isolate only presented resistance for β-lactams. It also contained the *seg*, *sei*, *sem*, *seu*, *hla*, and *hld* virulence genes. The second isolate, MRSA C5019, was ascribed to *spa* t4358, ST398, *agr*I, and *SCCmec*IVa. This strain showed a multiresistance phenotype that also included, in addition to β-lactams, tetracycline, erythromycin, and clindamycin (inducible type) and contained the resistance genes *mecA, erm*(C), and *tet*(M). Moreover, this strain harbored the genes *sen*, *hla*, *hlg*, and *hlgv*.

Characteristics of MSSA isolates detected in this study

The 41 MSSA isolated in our study showed 20 different *spa* types (Table 1). Four of the *spa* types were detected in 20 of the MSSA isolates (t005 in 10 isolates; t008 in 5 isolates; t189 in 3 isolates; and t024 in 2 isolates). Four of the MSSA isolates were ascribed to ST398 and to CC398, and they belonged to *spa* types t899 (two chicken isolates), t034 (one veal isolate), and a new *spa* type t13938, which presented a new repeat sequence (r652) (chicken isolate) (Table 1).

Characterization of the *agr* system showed a variable frequency with a predominance of *agr* group I (28 isolates, 68.3%), *agr* group II (10 isolates, 24.3%), and only 4 isolates belonged to *agr* group III (9.8%). The *agr* group IV has not been detected.

Characterization of antimicrobial resistance mechanisms and virulence genes among MSSA

All but 2 of the 41 MSSA isolates showed penicillin resistance (95%). Resistance to tetracycline was detected in 12 isolates (6 isolates containing *tet*[K]; 3 isolates of the combination *tet*[K] and *tet*[L]; 1 isolate *tet*[M]; and in 1 isolate, none of these genes was identified). The four isolates resistant to kanamycin harbored the *aph*(3′)-IIIa gene. One isolate was tobramycin resistant and contained the *ant*(4′)-Ia gene. The *msrA, erm*(A), *erm*(C), or *erm*(T) genes responsible for resistance to erythromycin were detected in four isolates.

All isolates carried at least two virulence genes. The genes *lukS-lukF* were detected in two isolates (4.9%), the gene *tst* was detected in one isolate, and the gene *eta* in four isolates.

PFGE patterns of S. aureus *isolates*

Analysis of *Sma*I macrorestriction profiles of the non-ST398 *S. aureus* isolates (37 MSSA and 1 MRSA) revealed 28 (S1–S27) different PFGE patterns. In pattern S2, two different subtypes were detected (S2a and S2b). Regarding the results of *ApaI* PFGE experiment performed in the five ST398 isolates (4 MSSA and 1 MRSA), two patterns were found (A1 and A2) and different subtypes of A1 were identified (A1a-A1c). The two MSSA ST398-t899 recovered from chicken samples showed the sample PFGE pattern (A1b) (Table 1).

Analysis of the possible origin of ST398 strains

None of the five ST398 strains studied harbored the MGEs associated with avian origin. Moreover, the genes of the IEC systems were not detected in two of these ST398 strains, but the remaining three ST398 strains (C5013, C5019, and C4993) presented the IEC type B (*scn-chp-sak*).

Discussion

The prevalence of *S. aureus* detected among the food samples analyzed in this study was 26.2%. The presence of this microorganism in meat has been studied in several countries (Schraft *et al*., 1992; Lozano *et al*., 2009; Huong *et al*., 2010; Benito *et al*., 2014). The percentage of food samples with *S. aureus* detected in our study was similar to that in some studies (Schraft *et al*., 1992; Huong *et al*., 2010). However, other studies detected higher prevalence rates (50–65%) (Kitai *et al*., 2004; Young *et al*., 2014), while others reported lower percentages (Normanno *et al*., 2007; Benito *et al*., 2014). Only 1.2% of our samples carried MRSA isolates. As occurred with *S. aureus*, the MRSA percentages detected in other studies have been variable (from 0 to 37%) (Normanno *et al*., 2007; Lozano *et al*., 2009; Fessler *et al*., 2011). These variations may be due in part to differences in the methodologies applied and especially to the origin of the samples. Regarding African studies, MRSA isolates have been previously detected in milk and meat samples in Nigeria, South Africa, and Kenya (Shitandi *et al*., 2004; Ateba *et al*., 2010; Ndahi *et al*., 2014). However, in those studies the *mecA* gene was not found or was not studied.

Although our MRSA prevalence was low, the risk of MRSA transmission through the food chain cannot be disregarded, and foodborne disease outbreaks caused by MRSA have been reported (Jones *et al*., 2002). One of our MRSA strains (C5019) was typed as ST398. To our knowledge, this

TABLE 1. PHENOTYPIC AND GENOTYPIC CHARACTERISTICS OF THE 43 *STAPHYLOCOCCUS AUREUS* ISOLATES RECOVERED FROM MEAT IN TUNISIA[a]

Isolates	*Origin*	*Type of establishment (region)*[b]	spa-*type (ST)*[c,d]	*PFGE pattern*[e]	agr *type*	*Enterotoxin genes*	*Other virulence genes detected*	*Phenotype of resistance*	*Resistance genes*[c]
C4995	Chicken	Farm (7)	t012 (ST30)	S1	III	*seg, sei, sem, seu*	*hla, hld*	P, OXA, FOX	***mec*A**
C5019	Chicken	Poultry market (1)	t4358 (**ST398**)	A1a	I	*sen*	*hla, hlg, hlgv*	P, OXA, FOX, TE, E	***mec*A**, *erm*(C), *tet*(M)
C5021	Chicken	Poultry market (1)	t899 (**ST398**)	A1b	I	*sen*	*hla, hlg, hlgv*	P, TE, E	*erm*(A), *erm*(C), *tet*(M)
C5013	Chicken	Poultry market (1)	t899 (**ST398**)	A1b	I		*hla, hlg, hlgv*	P	
C5011	Veal	Butchery (1)	t034 (**ST398**)	A2	I	*sen*	*hla, hlb, hld, hlgv*	P, TE, E	*tet*(K), *erm*(C)
C4993	Chicken	Supermarket (1)	**t13938**[f] **(ST398)**	A1c	I	*sen*	*hla, hlg, hlgv*	E	*erm*(A), *erm*(C), *erm*(T)
C5018	Chicken	Poultry market (1)	t005 (ST22)	S2a	I	*seb, seg, sei, sem, seo, seu*	*hla, hlg*	P	
C5003	Chicken	Poultry market (1)	t005	S2a	I	*sei, sem, seo, seu*	*hla, hld, hlg*	P	
C5006	Chicken	Poultry market (1)	t005	S2a	I	*seg, sei, sem, seu, seo*	*hla, hlg*	P	
C5026	Chicken	Supermarket (1)	t005	S2a	I	*seg, sei, sem, seu, seo*	*hla, hlg*	P	
C4983	Sheep	Butchery (1)	t005	S2a	I	*sep*	*hla, hlgv*	P	
C4989	Sheep	Butchery (9)	t005	S2a	I	*seg, sei, sem, seu, seo*	***eta***, *hla, hld*	P	
C5017	Sheep	Butchery (1)	t005	S2b	II	*seg, sei, sem, seu, seo*	*hla, hld, hlgv*	P	
C5000	Veal	Butchery (3)	t005	S3	I	*sen, sep*	*hla, hlgv*	P	
C4997	Veal	Butchery (1)	t005	S2a	I	*seg, sei, sem, seu, seo*	*hla, hld, hlg*		
C4996	Sheep	Butchery (1)	t008 (ST8)	S4	I		*hla, hld, hlgv, luckE*	P, TE, K	*aph*(3′)-IIIa, *tet*(K)
C5005	Sheep	Supermarket (1)	t008	S4	I		*hla, hld, hlgv, luckE*	P, TE	*tet*(K)
C5004	Sheep	Butchery (2)	t008	S4	I	*sen*	*hla, hld, hlg, hlgv*	P	
C4984	Sheep	Butchery (2)	t008	S5	I	*sep*	*hla, hld, hlgv*	P	
C4994	Chicken	Poultry market (1)	t008	S6	I	*sen*	*hla, hlg, hlgv*	P, TE, E	*tet*(L), *msrA, erm*(C)
C5015	Veal	Butchery (9)	t024	S7	I	*sed, sej, ser*	*hla, hld, hlgv*	P	
C5023	Veal	Butchery (4)	t024	S8	I	*sed, sej, ser*	*hla, hld, hlgv*	P	
C4985	Sheep	Butchery (2)	t189	S9	I	*seb, sep*	*eta, hla, hld, hlgv, luckE*	P, TE	*tet*(K)
C5014	Sheep	Butchery (4)	t189	S10	I	*sep*	*hla, hld, hlgv*	P	
C4999	Sheep	Butchery (8)	t189	S11	I	*sep*	*hla, hlgv*	P	
C5002	Veal	Butchery (3)	t084	S12	II		*hla, hld, hlgv*	P	
C4998	Sheep	Butchery (10)	t084	S12	II	*sen*	*hla, hld, hlgv*	P, TE, TOB	*tet*(K), *ant*(4′)-Ia
C5025	Chicken	Poultry market (1)	t786	S13	III	*sen, sep*	***lukS-lukF***, *hla, hlgv*	P, TE	
C4991	Chicken	Supermarket (1)	t786	S14	III	*sen, sep*	*hla, hld, hlgv*	P, TE	*tet*(K), *tet*(L)
C5009	Veal	Butchery (2)	t1313	S15	II	*sei, sem, seo, seu*	*hla, hld, hlgv*	P, K, E	*aph*(3′)-IIIa
C4992	Sheep	Butchery (8)	t1313	S16	II	*seg, sei, sem, seu, seo*	*hla, hlgv*	P, K, E	*aph*(3′)-IIIa
C4987	Veal	Butchery (4)	t2413	S17	II		*hla, hlgv*	P	
C4988	Veal	Butchery (1)	t2413	S17	II	*sen*	*hla, hld, hlgv*	P	
C5010	Chicken	Farm (3)	t045	S18	II	*sei, sem, seo, sep, seu*	*hla, hld, hlgv*	P	
C5008	Chicken	Poultry market (1)	t091	S19	I	*sen, sep*	*hla, hld, hlgv*	P	
C5016	Sheep	Butchery (2)	t181	S20	II	*sen*	***eta***, *hla, hld, hlgv*	P, TE	*tet*(K), *tet*(L)
C4990	Chicken	Poultry market (10)	t223	S21	I	*seg, seo, seu*	***tst***, *hla, hld*	P	
C5020	Chicken	Poultry market (1)	t267	S22	III	*sen, sep*	***lukS-lukF***, *hla, hld, hlgv*	P, TE	*tet*(K)
C5001	Chicken	Poultry market (2)	t304	S23	I	*sen*	***eta***, *hla, hld, hlgv*	P, CIP	
C4986	Sheep	Butchery (4)	t502	S24	II	*sed, sei, sej,sem, ser,seu*	*hla, hld, hlgv*	P	
C4982	Chicken	Supermarket (1)	t701	S25	I	*sen*	*hla, hlgv*	P, TE	*tet*(K), *tet*(L)
C5024	horse	Supermarket (1)	t1166	S26	I	*sen, seo*	***eta***, *hla, hlb, hld, hlgv*	P	
C5022	Chicken	Farm (2)	t3380	S27	I	*seg*	*hla, hlgv*	P, K	*aph*(3′)-IIIa

[a]The two methicillin-resistant strains appear shaded.
[b]The samples were obtained from 10 different regions of Tunisia: 1, Grand Tunis; 2, Menzel Bourguiba; 3, Bizerte; 4, Beja; 5, Mahdia; 6, Nabeul; 7, Mjez Beb; 8, Kef; 9, Sidi Bouzid; 10, Ben Guerden.
[c]The Sequence Type (ST), after being determined by multilocus sequence typing, is indicated in parentheses.
[d]The new *spa* type and the sequence type ST398 appear in bold.
[e]PFGE patterns are indicated by A or S depending on the enzyme used (*ApaI* or *SmaI*, respectively).
[f]New *spa*-type with a new repeat sequence (r652).
P, penicillin; OXA, oxacillin, FOX, cefoxitin; E, erythromycin; CC, clindamycin; TE, tetracycline; S, streptomycin; CIP, ciprofloxacin; K, kanamycin; TOB, tobramycin.

is the first time that this clonal lineage has been reported in food samples in Africa. MRSA CC398 strains have been previously identified in food samples in other countries (Lozano *et al*., 2009), highlighting the study of Fessler *et al*. (2011) with a high prevalence of MRSA (37%) in poultry products (most of them of lineage CC398). Remarkably in our study, the MRSA ST398 isolate as well as three of the four MSSA ST398 isolates were obtained from chicken samples. We must take into consideration that in our study, only one sample from pig was included due to the fact that in this country, as in other Muslim countries, the consuming of pork is very low.

Our MRSA ST398 strain was typed as *agr*I-t4358-*SCCmec*IV, and exhibited a multiresistance phenotype. In addition to the MRSA ST398 strain (C5019), the other four MSSA strains belonged to the lineage ST398. Interestingly, two of them showed an identical PFGE pattern (A1b) and both were recovered from samples obtained in supermarkets of the same region. There are several recent publications in which it has been suggested that MRSA CC398 strains could have their origin in human MSSA CC398 strains (Price *et al*., 2012). Interestingly, the IEC type B was identified in two of the four MSSA ST398 strains as well as in the MRSA ST398 strain. The IEC system is a set of genes that allows evasion of the human defenses that are associated with strains of human origin. Its presence or absence in *S. aureus* strains isolated from food is very important, since it gives us information about the possible origin of the isolates (human or animal origin) (Benito *et al*., 2014). The presence of these genes in MSSA ST398 has been previously reported (Uhlemann *et al*., 2012). However, these genes are rarely found among MRSA ST398 strains (Haenni *et al*., 2011).

Our ST398 strains presented *agr*I and the *spa* types t034 (1 strain), t899 (two strains), t4358 (1 strain), and a new *spa* type t13938 (1 strain) with a new repeat sequence (r652) (Table 1). There are several *spa* types associated with lineage CC398, some of them being more commonly found in MRSA strains (t011, t108, t1197, or t1255 among others) and others in MSSA (t034 or t571) (Price *et al*., 2012). The *spa* type t4358 detected in the MRSA ST398 strain has been identified earlier in strains belonging to ST9, another clonal lineage also related to animals (Yan *et al*., 2014). The same occurs with *spa* type t899, which has been found in strains of both sequence types ST398 and ST9 by others (Larsen *et al*., 2012; Monaco *et al*., 2013). In fact, these two *spa* types (t4358 and t899) are the most common ones in pigs from China and Malaysia (Larsen *et al*., 2012).

The remaining MRSA strain (C4995) was typed as ST30-t012-*agr*III-*SCCmec*V and did not carry the *lukS-lukF* genes encoding for PVL. However, PVL genes have been previously detected in MRSA strains belonging to ST30 (Hsu *et al*., 2006). Moreover, this toxin has been also found in MSSA CC30 isolates obtained from five major African towns (Breurec *et al*., 2011). MRSA CC30 is one of the most frequently found lineages in both colonization and causing infections in humans (van Belkum *et al*., 2009). Moreover, this clone has been also detected in samples from pigs (Agersø *et al*., 2012).

A high diversity of *spa* types was identified in the strains of this study. Twenty-three different *spa* types were identified, t005 and t008 being the most predominant ones. MSSA t005-CC22 strains have been previously detected in samples from different origins (Vincze *et al*., 2013; Aiken *et al*., 2014), and the *spa* type t008 is related to community strains and is one of the most common *spa* types identified among MSSA in human isolates (Tavares *et al*., 2014). Other *spa* types found in this study (t223, t267, t701, and t1166) were previously detected in healthy donkeys and sheep in Tunisia (Gharsa *et al*., 2012a, b). It is important to mention that the *spa* type t1166 is related to the ruminant-associated CC133.

Some relevant virulence factors were identified among our strains. The genes *lukS-lukF* were detected in two MSSA isolates (4.5%), the gene *tst* in one isolate, and the gene *eta* in five isolates. Some genes encoding enterotoxins, which are responsible for food poisoning, were also identified. Interestingly, many of our strains harbored genes that are part of the operon *egc* (*seg, sei, sem*, *sen*, and *seo*), but no strain showed the complete operon. The absence of one or more genes in the *egc* cluster has been previously reported (Lozano *et al*., 2011). In relation to the phenotype of resistance detected, most of the strains were penicillin resistant (95%), in accordance with other studies about MSSA strains (Lozano *et al*., 2011). It is important to note that 29.3% of the strains were tetracycline resistant. Tetracycline is widely used in the animal industry, and our strains were obtained from meat samples.

In conclusion, a relatively high (25%) and low (1.2%) percentage of MSSA and MRSA, respectively, were obtained among raw meat samples in our study. Among MSSA strains, a high diversity of *spa* types and PFGE patterns was obtained. Interestingly, to our knowledge, this is the first report of detection of MSSA and MRSA of the clonal lineage ST398 in food samples (meat of poultry and veal origins) in a country of the African continent. More information about the spread of *S. aureus* ST398 strains and other lineages in this continent is necessary. The risk of transmission of *S. aureus* and MRSA carrying different antimicrobial resistance and virulence genes through the food chain cannot be ignored, especially in raw meat.

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Disclosure Statement

No competing financial interests exist.

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