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Letter to Editor

The First Draft Genome Sequence of Methicillin-Resistant- ST80 *Staphylococcus aureus* Isolated from Sheep's Nasal Cavities in Tunisia

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Abstract

We report the draft genome sequence of methicillin-resistant *Staphylococcus aureus* strain S56. The S56 was isolated from a nasal sample obtained from a healthy sheep in Tunisia and was typed as t044-ST80. Its genome is composed of 2.839.420 bp and contained several genes implicated in the virulence, biofilm production and antibiotic resistance.

Keywords: *S. aureus; Ovine; Whole Genome Sequencing; ST80; t044*

Staphylococcus aureus, especially Methicillin-resistant *Staphylococcus aureus* (MRSA) is a critically important human pathogen that is also an emerging concern in veterinary medicine. Resistance to methicillin is mediated by the presence of *mecA* gene, which encodes an additional penicillin-binding protein (PBP2a or PBP2') with low affinity for β-lactam antibiotics. Recently, a novel methicillin-resistance determinant, $mecA_{LGA251}$ or *mecC* has been described with 70% homology with *mecA* [1].

Although there are some continent variations, the clonal complexes (CC) CC5, CC8 and CC22 are currently the most prevalent CCs worldwide among hospital-acquired-MRSA (HA-MRSA) isolates. However, the community-acquired/associated MRSA (CA-MRSA) mainly belong to clonal complexes CC8 (ST8), CC30 (ST30), CC59, CC80 and CC93 [1].

In Tunisia, little is known about the epidemiology of MRSA from animal origins. Therefore, we studied a collection of 76 *S. aureus* isolates collected from nasal swabs of sheep (42 isolates), goats (22 isolates), and cows (12 isolates) in different regions from Tunisia. Swabs were incubated in Tryptone Soy Broth (TSB) for 48h and then, they were subcultured on Baird Parker agar and Oxoid Brilliance MRSA agar. *S. aureus* were initially detected by conventional methods (Gram-staining, catalase test, oxidase test, DNase production, and ability to coagulate rabbit plasma (bioMérieux, Marcy l'Etoile, France)). Identification of *S. aureus* isolates was then confirmed by real time PCR (RT-PCR, ABI 7500, Taqman-Roche mastermix). Screening for the presence of *mecA* and *mecC* was performed by multiplex PCR [2].

The positive and negative control strains from the collection of the laboratory of Microbiology, Instituto Tecnológico Agrario de Castilla y León, Valladolid, Spain; were included in each PCR experiment. Only one isolate, from ovine nasal swab, named *S. aureus* S56 was *mec*A-positive. This strain was further characterized by whole genome sequencing to investigate its genomic properties. DNA was extracted by genomic DNA isolation kit (Sigma, Spain) and the genome was sequenced using Ilumina Miseq 125-bp paired-end sequence technology as described previously. The raw reads were paired in CLC genomics workbench 9.0, resulting in 712,116 reads with a mean size of 202 bp. The reads were assembled in CLC genomics workbench 9.0 using standard settings, including scaffolding. The assembly was corrected by NCBI PGAP. The final version contains 69 contigs with sizes between 0.251 and 340.826 Kb (the average contig length was 41.151 Kb). The total assembly size of MRSA isolate was 2,839,420 bp, with an N50 value of 157673 bp, 2,858 coding sequences and a GC content of 32.3%.

Sequencing showed that the *S. aureus* S56 had the *spa* type t044 (07-23-12-34-34-33-34) and the *agr*1 and belonged to the Sequence type (ST) 80 (allelic profile 1-3-1-14-11-51-10).The *ant*(6)-Ia, *aph*(3')-III, *bla*Z, *fus*B, *mec*A, *nor*A, and *tet*(K) genes encoding streptomycin, kanamycin, penicillin, fusidic acid, methicillin, quinolones, and tetracycline-resistance, respectively, were detected (Table 1). Indeed, this strain was resistant to all the aforementioned antibiotics by antibiogram. Sequence data revealed that S56 carried the genes encoding Panton-Valentine leucocidin toxin as well as the *edinB*, *lukE* and *lukD* toxin genes. In addition, the haemolysin genes (*hla*, *hlb*, *hld*, *hlg A*, *hlgB* and *hlgC*) and genes encoding cell surface proteins, including those implicated in adhesion to components of the extracellular matrix (*atl*, *eap*, *ebpS*, *efb*, *sdrH icaA*, *icaB*, and *icaC*) were also present. The IEC genes *scn* and *sak* encoding the human-specific immune evasion proteins SCIN (staphylococcal complement inhibitor), and staphylokinase were detected in this strain as well as the genes encoding aureolysin (*aur*) and serine protease A and B (*splA*/*splB*). The *femA*, *femB*, *femX* genes were also present in our strain, those genes are responsible of regulation of the expression of methicillin resistance and affects glycine content of peptidoglycan in methicillin-resistant.

To the best of our knowledge this is the first report from Tunisia of the whole genome sequencing of a t044-ST80-SARM strain from ovine nasal cavities. This strain is similar to the European clone t044-ST80-CA-MRSA [3]. The ST80 clone is predominant in Europe and also in Tunisia [3,4]. The importance of this clone arise for its virulence factors such as PVL and leucocidine D/E toxins, the human-specific immune evasion cluster, hemolysins, the epidermal cell differentiation inhibitor B (EdinB), as well as its ability to produce biofilm. In addition this strain was multidrug resistant being resistant not only to methicillin and penicillin but also to tetracycline, streptomycin, kanamycin and fusidic acid (TSKF pattern) [5,6].

	Phenotypic and genotypic characteristics
Genome size	2.839.420 bp
GC%	32.3%
Sequence type	ST 80
spa type	t044
Resistance profil	Methicillin, penicillin, streptomycine, kanamycin, fusidic acid, quinolone, and tetracycline
Genes encoding antibiotic resistance	*mecA*, *blaZ*, *ant(6)-Ia*, *aph(3')-III*, *fusB*, *norA*, *tet(K)*
Expression regulators of methicillin resistance	*femA*, *femB*, *femX*
Hemolysin genes	*hla*, *hlb*, *hld*
Leucocidin genes	*lukS-PV,lukF-PV,lukE,lukD, edinB*
immune evasion cluster (IEC) genes	*scn*, *sak*
Gene encoding surface proteins	*atl*, *eap*, *ebpS*, *efb*, *sdrH, icaA*, *icaB*, *icaC*
Exoenzyme genes	*aur*, *spIB*, *spIA*

Table 1: *Phenotypic and genotypic characteristics of the ovine methicillin-resistant S. aureus S56.*

Ethics Statement

Not needed.

Acknowledgments

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