



Methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in dairy sheep and in-contact humans: An intra-farm study

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ABSTRACT

Staphylococcus aureus is involved in a wide variety of diseases in humans and animals, and it is considered one of the most significant etiological agents of intramammary infection in dairy ruminants, causing both clinical and subclinical infections. In this study, the intra-farm prevalence and circulation of methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) were investigated on an Italian dairy sheep farm previously identified as MRSA-positive by testing bulk tank milk (first isolation in 2012). Human samples (nasal swabs, hand skin samples, and oropharyngeal swabs) from 3 persons working in close contact with the animals were also collected, and the genetic characteristics and relatedness of the MRSA isolates from human and animal sources within the farm were investigated. After 2 yr from the first isolation, we confirmed the presence of the same multidrug-resistant strain of MRSA sequence type (ST)1, clonal complex (CC)1, *spa* type t127, staphylococcal cassette chromosome *mec* (SCC*mec*) type IVa, showing identical pulsed field gel electrophoresis (PFGE) and resistance profiles at the farm level in bulk tank milk. Methicillin-resistant *S. aureus* isolates were detected in 2 out of 556 (0.34%) individual milk samples, whereas MSSA isolates were detected in 10 samples (1.8%). The MRSA were further isolated from udder skin samples from the 2 animals that were MRSA-positive in milk and in 2 of the 3 examined farm personnel. All MRSA isolates from both ovine and human samples belonged to ST(CC)1, *spa* type t127, SCC*mec* type IVa, with some isolates from animals harboring genes considered markers of human adaptation. In contrast, all MSSA isolates belonged

to ruminant-associated CC130, ST700, *spa* type t528. Analysis by PFGE performed on selected MRSA isolates of human and animal origin identified 2 closely related (96.3% similarity) pulsotypes, displaying only minimal differences in gene profiles (e.g., presence of the immune evasion cluster genes). Although we observed low MRSA intra-farm prevalence, our findings highlight the importance of considering the possible zoonotic potential of CC1 livestock-associated MRSA, in view of the ability to persist over years at the farm level. Biosecurity measures and good hygiene practices could be useful to prevent MRSA spread at the farm level and to minimize exposure in the community and in categories related to farm animal industry (e.g., veterinarians, farmers, and farm workers).

Key words: livestock-associated methicillin-resistant *Staphylococcus aureus*, dairy sheep, clonal complex 1, zoonosis

INTRODUCTION

Staphylococcus aureus is involved in a wide variety of diseases in humans and animals, and its pathogenicity is mainly related to a combination of genetic characteristics mediating virulence, invasive capacity, immune evasion, and antibiotic resistance (Chua et al., 2014). *Staphylococcus aureus* is considered one of the most significant etiological agents of IMI in dairy ruminants (Contreras et al., 2007), causing both clinical and subclinical mastitis and resulting in substantial economic losses due to reduced milk production and quality (Bergonier et al., 2003). In the last years, emergence of multidrug-resistant livestock-associated methicillin-resistant *S. aureus* (LA-MRSA) has been increasingly reported worldwide (Guardabassi et al., 2013). From a public health perspective, there is concern about the risk of zoonotic transmission of LA-MRSA strains by direct contact of people working with animals (Pan et al., 2009; Guardabassi et al., 2013), including those working in dairy farms (Juhász-Kaszanyitzky et al.,

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2007; Spohr et al., 2011; Alba et al., 2015, Feltrin et al., 2015) and also by their possible introduction in the community through the food chain (Kluytmans, 2010).

Currently, MRSA clonal complex (CC) sequence type (ST)398 is the most prevalent lineage among LA-MRSA (Cuny et al., 2013), although other MRSA lineages (e.g., ST1, ST5, ST9, ST97, ST130, ST433) have been found in farmed animals worldwide (Guardabassi et al., 2013). In Italy, LA-MRSA also frequently belongs to CC398 (Battisti et al., 2010; Luini et al., 2015; Normanno et al., 2015), but other clones such as CC97 and CC1 represent major LA-MRSA lineages often detected in the Italian pig and dairy cattle industry (Franco et al., 2011; Alba et al., 2015; Feltrin et al., 2015). Additionally, some lineages of MRSA isolated from dairy cattle in Italy show multidrug resistance and contain virulence and immunomodulatory genes associated with the ability to colonize humans (Alba et al., 2015).

Currently, investigations into the diffusion and epidemiology of MRSA in dairy sheep farms are few (Fessler et al., 2012; Harrison et al., 2013; Petersen et al., 2013), and only scarce information is available about the presence and diffusion of MRSA in ovine milk (Ariza Miguel et al., 2014; Caruso et al., 2015; Pexara et al., 2015) and sheep dairy products (Normanno et al., 2007; Shanehbandi et al., 2014; Carfora et al., 2015).

The aim of this study was to investigate the intra-farm prevalence and circulation of MRSA and methicillin-susceptible *S. aureus* (MSSA) in an Italian dairy sheep farm previously identified as MRSA-positive (first isolation in the year 2012). We investigated the genetic characteristics and relatedness of the MRSA isolates from human and animal sources within the farm to gain further insight into possible transmission patterns and for epidemiological and risk assessment purposes.

MATERIALS AND METHODS

Dairy Sheep Farm Characteristics and History

The study was carried out on a farm located in the province of Rome, central Italy, with a semi-extensive Comisana dairy sheep herd. At the time of the investigation (May 2014), the herd included 556 ewes in late lactation, 250 dry ewes, and 150 lambs under 6 mo of age. The ewes were usually milked twice daily using a milking machine. Handling of animals was carried out by workers wearing dedicated coveralls and boots but not using gloves. Teat-washing before milking and treatment with antibiotics at dry-off were not performed.

In 2012, the farm was already included in a survey on the presence of *S. aureus* in bulk tank milk (BTM) samples from dairy sheep farms in central Italy. At that

time, the farm was the only one from which a BTM sample tested positive for MRSA out of 286 dairy sheep farms tested (1/286, 0.35%). The isolate, denominated BTM-A, belonged to ST(CC)1 and *spa* type t127, and harbored staphylococcal cassette chromosome *mec* (SCC*mec*) type IVa. In 2013, another MRSA isolate belonging to the same lineage, designated BTM-B, was isolated from a BTM sample obtained from the same farm (Carfora et al., 2015).

Intra-Farm Study: Sample Collection

In May 2014, 556 individual milk samples were collected from all lactating ewes of the herd to investigate intra-farm MRSA and MSSA prevalence, and a BTM sample was taken at the end of the milking procedures. Two weeks later, the following samples were collected from animals that tested positive for the presence of MRSA in individual milk samples: nasal swabs from both nares, half-udder milk samples, and udder skin samples. A wound swab was also collected from one animal that presented a hock abrasion.

Nasal samples were taken by using cotton-tipped swabs (Amies Agar Gel with Charcoal, Laboindustria s.p.a., Padova, Italy), whereas the udder skin was sampled by using Sodibox wipes (Sodibox, Névez, France). During the same visit, samples were collected from 3 individuals working in close contact with the animals: the farm owner and 2 milkers. These samples included nasal swabs, hand skin samples, and oropharyngeal swabs. Human nasal samples were taken by means of cotton-tipped swabs from both anterior nares, whereas hand samples were taken by using Sodibox wipes. None of the subjects reported recent hospitalization or the presence of a healthcare worker in the household. No skin or wound infections or any recent antimicrobial treatment was reported.

All human samples were obtained voluntarily and the farm owner consented to animal sampling (in Italy, MRSA infection in animals is not a notifiable disease). All procedures followed were in accordance with ethical standards of the relevant national and institutional committees on experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Farm workers gave oral informed consent to participate in the study.

All collected samples were transported to the laboratory in ice-cooled containers and subjected to analyses within 24 h of collection.

Isolation and Identification of S. aureus

For individual, bulk tank, and half-udder milk samples, 10 μ L of milk was directly spread on Baird-Parker agar plus rabbit plasma fibrinogen plates (bioMérieux,

Marcy l'Etoile, France) and on a chromogenic agar selective for MRSA, BBL CHROMagar MRSA (BD Diagnostics, Sparks, MD) and then incubated for 24 to 48 h at 37°C. Bulk tank milk samples (1 mL) were also enriched in 9 mL of Mueller-Hinton broth supplemented with 6.5% NaCl (Battisti et al., 2010). After incubation at 37°C for 24 h, 10 µL of culture was spread on BBL CHROMagar and incubated for further 24 to 48 h at 37°C. Coagulase-positive colonies (up to 5 per sample) and presumptive MRSA colonies growth on BBL CHROMagar (1 colony per sample) were subcultured and confirmed as *S. aureus* by biochemical and molecular assays as previously reported (Carfora et al., 2015). Swabs and samples taken by Sodibox wipes were tested using both nonselective and MRSA-selective media according to Battisti et al. (2010). Suspected colonies were confirmed as *S. aureus* by biochemical and molecular assays as previously described (Battisti et al., 2010).

SCC Analysis

All individual milk samples were analyzed for SCC determination by using a fluoro-opto-metric method (Fossomatic 5000 series, Foss Electric, Hillerød, Denmark) and according to the International Dairy Federation (1995) method.

MRSA and MSSA Genotypic and Phenotypic Characterization

All *S. aureus* colonies were tested for the presence of the *mecA/mecC* and *blaZ* genes according to Stegger et al. (2012) and Martineau et al. (2000), respectively. Genotyping of *mec*-positive *S. aureus* (1 isolate per sample) and of *mec*-negative *S. aureus* (1 isolate per sample) was performed by *spa* typing, multilocus sequence typing, and typing and subtyping of SCC*mec* as previously described (Alba et al., 2015).

Eleven *S. aureus* isolates, representative of the different types of samples and sources tested (Table 1), were screened by PCR analysis for the presence of specific immune evasion, virulence, and antimicrobial resistance (AMR) genes. These included the immune evasion cluster (IEC) genes *sak* (staphylokinase) and *scn* (staphylococcal complement inhibitor precursor), as reported by van Wamel et al. (2006), the tetracycline (*tetK*, *tetL*, *tetM*, and *tetO*) and erythromycin (*ermA*, *ermB*, and *ermC*) resistance genetic determinants according to Trzcinski et al. (2000) and Martineau et al. (2000), respectively. The presence of 9 selected *S. aureus* enterotoxins (SE; *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *ser*) and 2 staphylococcal-like enterotoxins (SEI; *selj* and *selp*) genes was assessed by using 2 multiplex

PCR protocols, as previously described (Kérouanton et al., 2007; Bianchi et al., 2014). A further characterization of these isolates involved *Sma*I pulsed-field gel electrophoresis (PFGE), performed according to Alba et al. (2015).

The same 11 isolates were also tested for their antimicrobial susceptibility by the broth microdilution method (Trek Diagnostic Systems, Westlake, OH). The following drugs were tested: penicillin, cefoxitin, ciprofloxacin, chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, streptomycin, linezolid, quinopristin/dalfopristin, fusidic acid, mupirocin, rifampicin, tetracycline, tiamulin, sulfamethoxazole, trimethoprim, and vancomycin. Minimum inhibitory concentrations were determined, and results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST; <http://www.eucast.org>), using epidemiological cut-offs for the categorization of “microbiological resistance” or “non-wild-type” isolates. Results for the quality control documents were within published ranges.

Two selected MRSA isolates (1 of human and 1 of ovine origin) were tested by microarray for the detection of a variety of pathogenicity and virulence-associated genes, AMR genes, and strain- or host-specific markers of *S. aureus* using the *S. aureus* Genotyping DNA Microarray (Alere Technologies GmbH, Germany), as previously described (Franco et al., 2011). The results were interpreted according to the manufacturer's instructions (<http://www.alere-technologies.com/>).

RESULTS

MRSA and MSSA Isolation and Identification

We detected *S. aureus* in 12 of 556 individual milk samples tested (2.16%, 95% CI: 1.24–3.74%), with 10 (10/556; 1.8%, 95% CI: 0.98–3.28%) being positive for MSSA and 2 (2/556; 0.34%, 95% CI 0.1–1.3%) being positive for MRSA. None of the *S. aureus*-positive ewes exhibited clinical signs of mastitis. Somatic cell counts in the 12 *S. aureus*-positive milk samples ranged between 78,000 and 16,135,000 cells/mL, with a geometric mean of 2,691,534 cells/mL. The geometric mean SCC of *S. aureus*-negative individual milk samples was 398,107 cells/mL. The BTM sample, taken at the end of the milking procedures, also tested MSSA and MRSA positive, after both enrichment and by direct plating.

Among the additional samples collected from the 2 MRSA-positive animals, MRSA was also isolated from the udder skin and from a single half-udder milk sample of both animals. The nasal and the hock abrasion swabs tested negative for MRSA, whereas an MSSA isolate was detected from the nasal swab of one animal.

Methicillin-resistant *S. aureus* isolates were also detected from 2 out of 3 persons examined, in particular, from the nasal swab of the farm owner and from the hands and nasal swab of milker A.

Characterization of MRSA and MSSA

All suspected MRSA isolates were positive for both *mecA* and *blaZ* genes, but negative for *mecC*. All belonged to ST(CC)1 and *spa* type t127 and harbored SCC*mec* type IVa. In contrast, all the MSSA isolates were *mecA-mecC-blaZ*-negative and belonged to CC130, ST700, *spa* type t528. Eleven *S. aureus* isolates were further characterized by PCR analysis for the presence of IEC, virulence, and AMR genes, and for their antimicrobial susceptibility. They comprised 9 MRSA isolates, including the 3 BTM isolates (BTM-C, BTM-B, BTM-A), 2 isolates from individual milk samples (SH-A_M and SH-B_M), 2 isolates from udder skin samples (SH-A_S and SH-B_S), 2 human isolates (HU-O and HU-A), and 2 MSSA isolates (SH-C and SH-D) from individual milk samples (Table 1).

Analysis by PCR for IEC and virulence genes (Figure 1) revealed that the *sak* and *scn* genes were present only in the human isolates and in 1 MRSA isolate detected from 1 udder skin sample (SH-B_S). All 9 MRSA isolates harbored the *seh* gene, whereas the 2 MSSA isolates

harbored the *sei* gene. No other tested SE or SEI genes were detected.

Regarding the presence of AMR genes other than *mecA* and *blaZ*, the 11 selected isolates except 1 MSSA (SH-C) harbored the *tet(K)* gene, whereas the *erm(C)* gene was present in all 9 CC1 MRSA isolates but not in the 2 CC130 MSSA isolates (Figure 1).

All MRSA selected isolates displayed an identical resistance phenotype, being resistant to cefoxitin, penicillin, erythromycin, clindamycin, streptomycin, kanamycin, and tetracycline, with 1 isolate (SH-A_M) being also resistant to trimethoprim. Conversely, the 2 MSSA isolates were susceptible to all tested antimicrobials except for tetracycline resistance in SH-C.

Analysis by PFGE (Figure 1) of the 11 selected isolates identified 3 different pulsotypes (A, B, and C). Pulsotype A included the 2 CC1 MRSA isolates of human origin (HU-O and HU-A) and the ovine MRSA isolate SH-B_S from an udder skin sample. Pulsotype B included all the other CC1 MRSA isolates of ovine origin (BTM-A, BTM-B, BTM-C, SH-A_M, SH-B_M, SH-A_S), whereas pulsotype C included the 2 MSSA isolates (SH-C and SH-D). Pulsotype A and B were closely related (96.3% similarity), differing from each other in only one band, whereas pulsotype C (CC130), as expected, showed only 48.2% similarity with the other pulsotypes.

Table 1. Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates detected in the investigated dairy sheep farm and selected isolates for further characterization

Sample ID	Source	Year	MRSA/MSSA isolate	ST (CC) ¹	<i>spa</i> type	Selected isolate	Isolate ID ²
12009514	Bulk tank milk	2012	MRSA	1	t127	Yes	BTM-C
13074730	Bulk tank milk	2013	MRSA	1	t127	Yes	BTM-B
14041623	Bulk tank milk	2014	MRSA	1	t127	Yes	BTM-A
14041623	Bulk tank milk	2014	MSSA	700 (130)	t528	No	—
14041593/1	Individual milk sheep 1	2014	MSSA	700 (130)	t528	No	—
14041594/7	Individual milk sheep 7	2014	MSSA	700 (130)	t528	No	—
14041594/10	Individual milk sheep 10	2014	MSSA	700 (130)	t528	No	—
14041594/26	Individual milk sheep 26	2014	MSSA	700 (130)	t528	No	—
14041594/27	Individual milk sheep 27	2014	MSSA	700 (130)	t528	No	—
14041592/36	Individual milk sheep 36	2014	MSSA	700 (130)	t528	Yes	SH-C
14041592/49	Individual milk sheep 49	2014	MSSA	700 (130)	t528	Yes	SH-D
14041593/111	Individual milk sheep 111	2014	MSSA	700 (130)	t528	No	—
14041592/131	Individual milk sheep 131	2014	MRSA	1	t127	Yes	SH-B _M
14041592/134	Individual milk sheep 134	2014	MRSA	1	t127	Yes	SH-A _M
14041592/135	Individual milk sheep 135	2014	MSSA	700 (130)	t528	No	—
14041593/188	Individual milk sheep 188	2014	MSSA	700 (130)	t528	No	—
14045174/131	Left half-udder milk sheep 131	2014	MRSA	1	t127	No	—
14045174/134	Right half-udder milk sheep 134	2014	MRSA	1	t127	No	—
14048310/131	Udder skin sheep 131	2014	MRSA	1	t127	Yes	SH-A _S
14048310/134	Udder skin sheep 134	2014	MRSA	1	t127	Yes	SH-B _S
14048310/134 NS	Nasal swab sheep 134	2014	MSSA	700 (130)	t528	No	—
14041626	Nasal swab, owner	2014	MRSA	1	t127	Yes	HU-O
14048310/A-NS	Nasal swab, milker A	2014	MRSA	1	t127	No	—
14048310/A-H	Hands, milker A	2014	MRSA	1	t127	Yes	HU-A

¹ST (CC) = sequence type (clonal complex).

²Only the identity of the 11 selected isolates is reported.

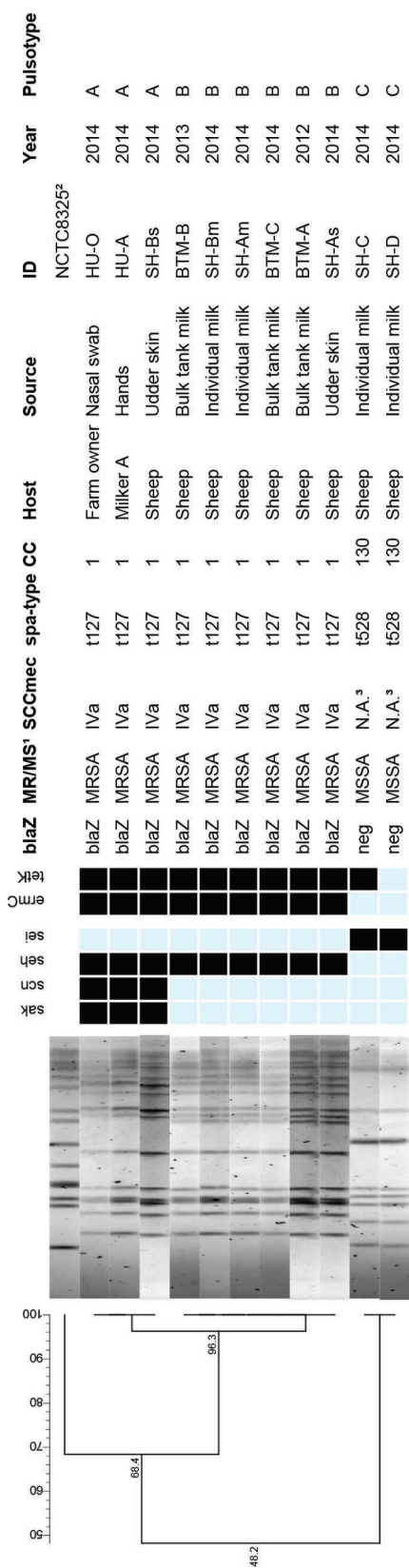


Figure 1. Dendrogram of *Sma*I pulsed field gel electrophoresis (PFGE) macrorestriction patterns, with selected virulence and antimicrobial resistance marker genes and elements of 9 methicillin-resistant *Staphylococcus aureus* (MRSA) and 2 methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates from sheep and human. MR/MS = methicillin resistant/methicillin susceptible; NCTC 8325 = *S. aureus* reference isolate; CC = clonal complex; NA = not applicable; SCCmec = staphylococcal cassette chromosome mec; blaZ = positive for the *blaZ* gene; neg = negative for *blaZ*. Black shading represents positive, gray (blue) represents negative. Color version available online.

The microarray characterization carried out on 2 selected isolates belonging to pulsotypes A (HU-A) and B (SH-B_M) confirmed that the genes of the IEC were present only in isolate HU-A, whereas the *hly* gene lacking the phage insertion (undisrupted *hly*) was present only in isolate SH-B_M. Apart from these differences, the gene profiles of the 2 isolates were identical (Table 2).

DISCUSSION

This work represents the first study investigating the prevalence and circulation of MRSA within a dairy sheep farm. The overall *S. aureus* prevalence observed was 2.16% and, even though the study was conducted on a farm already known to be MRSA-positive, we found a very low intra-farm MRSA prevalence, with only 2 positive animals out of 556 tested (0.34%). Similarly, Cortimiglia et al. (2015) reported a low MRSA prevalence (1.3%) on a dairy goat farm in Italy, whereas prevalence rates ranging from 0 to 29% were reported within dairy cattle herds (Vanderhaeghen et al., 2010; Schlotter et al., 2014; Luini et al., 2015).

As for the results of the SCC analysis, *S. aureus*-positive individual milk samples were associated with a SCC geometric mean of 2,691,534 cells/mL, suggesting the occurrence of IMI (Paape et al., 2007).

Analysis of human samples showed the presence of MRSA in 2 out of the 3 persons attending the herd. Molecular characterization of animal and human isolates showed that all MRSA belonged to ST(CC)1, *spa* type t127, and harbored a SCCmec type IVa, a major lineage in humans and dairy cattle in Italy (Alba et al., 2015). In particular, in recent years, ST(CC)1 SCCmec IVa has been increasingly detected in bulk milk and in cases of mastitis in dairy cattle (Alba et al., 2015; Luini et al., 2015) and also occasionally isolated from goat milk (Cortimiglia et al., 2015) and dairy products (Carfora et al., 2015). Conversely, all MSSA isolates belonged to CC130, ST700, *spa* type t528, a clonal complex frequently isolated from sheep in Europe (Porrero et al., 2012; Eriksson et al., 2013; Smith et al., 2014).

The MRSA isolates belonged to 2 closely related PFGE types (pulsotypes A and B) and showed an almost identical core genome, with the same pathogenicity and host- and strain-associated genotypic and phenotypic antimicrobial resistance profiles. These core genome and multidrug-resistance profiles (resistance toward β-lactams, tetracyclines, macrolide-lincosamide-streptogramin B antimicrobials, aminoglycosides) were very similar to those already described in ST(CC)1, *spa*-type t127 isolates detected in Italian holdings of farmed ruminants (Alba et al., 2015; Cortimiglia et al., 2015; Luini et al., 2015). As in cattle MRSA, these isolates also carried genes and acquired resistance genes

Table 2. Microarray profile of the 2 selected methicillin-resistant *Staphylococcus aureus* (MRSA) isolates (HU-A¹ and SH-B_M¹)

Gene group	Target gene
<i>S. aureus</i> -species markers	<i>gapA, katA, coA, nuc1, spa, sbi</i>
Regulatory	<i>sarA, saeS, vraS, agrB-III, agrD-III, hld</i>
Enterotoxins	<i>seh</i>
Hemolysins and leukocidins	<i>lukD, lukE, lukF, lukS, lukY, lukX, hlgA</i>
Immune-evasion	<i>hysA1, hysA2, isaB, isdA, imrP, mprF</i> (COL+MW2)
Staphylococcal superantigen/enterotoxin-like genes	<i>setC/selr, set6/ssl1, set7/ssl2, set8/ssl3, set9/ssl4, set3/ssl5, set21/ssl6, set1/ssl7, set12/ssl8, set5/ssl9, set4/ssl10, set2/ssl11, setB1, setB2, setB3</i>
Capsule	<i>cap8, capH8, capI8, capJ8, capK8</i>
Biofilm/adhesion	<i>clfB, clfA, cna, ebh, ebpS, eno, icaA, icaC, icaD, fib, fnbA, fnbB, bbp, sdrC, sdrD, sasG, vwb</i>
Type I restriction modification system	<i>hsdS2</i> (MW2+MSSA476), <i>hsdS3, hsdSX</i> (CC25)
Hemolysins	<i>hl, hla, hlb, hllll, undisrupted hlb²</i>
Immune evasion cluster genes (β -converting phage)	<i>sak³, scn³</i>
Proteases, others	<i>aur, splA, splB, sspA, sspB, sspP, Q2FXCO</i>
Antimicrobial resistance gene group	Genotype
Methicillin-resistance genes	<i>mecA, ugpQ, delta mecR, ccrA-2, ccrB-2</i>
Penicillinase (BlaZ gene cluster system)	<i>blaZ, blaI, blaR</i>
Macrolide-lincosamide-streptogramin B antibiotics	<i>erm(C)</i>
Aminoglycosides	<i>aphA3+sat</i>
Tetracyclines	<i>tet(K)</i>
Efflux systems	<i>sdrM</i>

¹Prototype strain = clonal complex (CC) 1 MRSA IV, West Australian (WA) MRSA 1/57.

²Only SH-B_M tested positive.

³Only HU-A tested positive.

(such as *sat* and *aphA3*, associated with Tn5405-like elements) that are considered markers of human origin (Alba et al., 2015). In this regard, it is interesting to note that the IEC genes (*sak* and *scn*) were only present in pulsotype A isolates. The presence of these genes is a typical feature of human-associated CC1 clones, and represents another marker of CC1 MRSA and MSSA isolates from Italian dairy cattle and sheep (Alba et al., 2015), different from what had been found in CC1 from pigs (Franco et al., 2011; Alba et al., 2015). Overall, the results of the genetic characterization of the ST(CC)1 LA-MRSA isolated in this study on a dairy sheep farm are in agreement with those of previous studies on CC1 *S. aureus* in ruminants, suggesting the capacity for transmission between humans and animals.

Possible pathways of human-to-animal transmission include direct contact between farm workers and animals or indirect exposure through the farm environment. Zoonotic transmission between sheep and in-contact humans has already been hypothesized for isolates belonging to other MRSA lineages and sharing identical PFGE or multiple-locus variable number tandem repeat analysis (MLVA) patterns (Fessler et al., 2012; Petersen et al., 2013) and confirmed by whole-genome sequencing (Harrison et al., 2013).

Some hypotheses on the origin of the 2 related (>96% similarity) ST(CC)1, *spa*-type t127 MRSA pulsotypes identified in this study can be formulated. Probably, the presence of a “human” pulsotype A (SH-B_S) on the ud-

der skin of a sheep might be due to a contamination of human origin, caused by the habit of the farm workers to milk ewes with bare hands. The repeated exposure of ewes may have favored an occasional udder colonization or infection by a human MRSA strain, which might have differentiated over time and undergone an adaptation process in the ovine host, such as the loss of the human-associated IEC genes or genetic rearrangements causing slight changes in the PFGE macrorestriction patterns.

However, the hypothesis of independent introduction of 2 closely related strains into the farm through different sources cannot be ruled out, considering that the core genome of ST(CC)1 *spa* type t127 MRSA described in Italy is not so heterogeneous (Alba et al., 2015).

Exposure of dairy animals is likely to result in colonization and even infection, because it is known that ST(CC)1 MRSA is capable of causing mastitis in cattle (Juhász-Kaszanyitzky et al., 2007; Pilla et al., 2012; Luini et al., 2015) and mastitis or colonization in small ruminants (Caruso et al., 2015; Cortimiglia et al., 2015). In this regard, severe measures should be implemented at the farm level to minimize the risk of animal colonization, infection (e.g., mastitis), and within-herd transmission, thus reducing occupational exposure (Alba et al., 2015; Feltrin et al., 2015). Moreover, dairy farming may represent a source of LA-MRSA and a hazard for the community through unpasteurized milk or dairy products (Normanno et al., 2007; Carfora et

al., 2015). In this perspective, the capacity of MRSA to persist over years at the farm level, as shown in this study for sheep and as already on goat dairy farms (Stastkova et al., 2009; Cortimiglia et al., 2015), is of concern. In fact, on the sheep farm studied, the presence of the same MRSA strain (e.g., same ST, CC, *spa* type, SCC*mec* type, PFGE pattern, resistance, and virulence profile) in BTM samples was detected 1 and 2 yr after the first sampling. Indeed, the occurrence of subclinical forms of MRSA infection in dairy sheep could remain unobserved or underestimated, because milk samples are usually tested for *S. aureus* only when an overt problem of mammary infection affects the herd (Luini et al., 2015). In this respect, the results of our study confirm that testing BTM samples may represent a sensitive screening method to identify MRSA-positive dairy herds, even if colonized at low intra-farm prevalence rates (Ariza-Miguel et al., 2014; Cortimiglia et al., 2015; Traversa et al., 2015).

Our findings highlight the possible zoonotic potential of the ST(CC)1 LA-MRSA detected in the dairy sheep farm under investigation. Although a low intra-farm prevalence of MRSA was observed, biosecurity measures and good hygiene practices could be useful to prevent MRSA spread at the farm level and minimize exposure in the community. Further focused studies may help in better understanding “MRSA epidemiology” in dairy small ruminant herds, including the risk factors involved in animal colonization and infection and in within-herd transmission.

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