J. Dairy Sci. 100:1–7
https://doi.org/10.3168/jds.2017-12940

Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* carrying *mecA* or *mecC* and methicillin-susceptible *Staphylococcus aureus* in dairy sheep farms in central Italy

G. Giacinti,* V. Carfora,† A. Caprioli,† D. Sagrafoli,* N. Marri,* G. Giangolini,* R. Amoruso,† M. Iurescia,† F. Stravino,† S. Dottarelli,† F. Feltrin,† A. Franco,† S. Amatiste,* and A. Battisti†[1]
*National Reference Centre for Ovine and Caprine Milk and Dairy Products Quality (CRELDOC), and
†General Diagnostic Department, National Reference Laboratory for Antimicrobial Resistance, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" Via Appia Nuova 1411, 00178 Rome, Italy

ABSTRACT

Between January and May 2012, a total of 286 bulk tank milk samples from dairy sheep farms located in central Italy were tested for the presence of *Staphylococcus aureus*. One hundred fifty-three samples were positive for *S. aureus* (53.5%), with an average count of 2.53 log cfu/mL. A total of 679 *S. aureus* colonies were screened for methicillin resistance by the cefoxitin disk diffusion test, and 104 selected cefoxitin-susceptible isolates were also tested for their susceptibility to other antimicrobials representative of the most relevant classes active against *Staphylococcus* spp. by using the Kirby-Bauer disk diffusion method. Two methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, carrying respectively the *mecA* and the *mecC* genes, were detected in 2 samples from 2 different farms (prevalence 0.7%). The *mecA*-positive MRSA isolate was *blaZ* positive, belonged to *spa* type t127, sequence type (ST)1, clonal complex (CC)1, carried a staphylococcal cassette chromosome *mec* (SCC*mec*) type IVa, and was phenotypically resistant to all the β-lactams tested and to erythromycin, streptomycin, kanamycin, and tetracycline. The *mec*C-positive MRSA isolate was negative for the chromosomally or plasmid-associated *blaZ* gene but positive for the *blaZ* allotype associated with SCC*mec* XI (*blaZ*-SCC*mec*XI), belonged to *spa* type 843, ST(CC)130, carried a SCC*mec* type XI, and was resistant only to β-lactams. Both MRSA were negative for the presence of specific immune-evasion and virulence genes such as those coding for the Panton-Valentine leucocidin, the toxic shock syndrome toxin 1, and the immune evasion cluster genes. Regarding the presence of the major *S. aureus* enterotoxin genes, the *mec*C-positive MRSA tested negative, whereas the ST (CC)1 *mecA*-positive MRSA harbored the *seh* gene. Among the 104 methicillin-susceptible *S. aureus* isolates examined for antimicrobial susceptibility, 63 (60.58%) were susceptible to all the antimicrobials tested, and 41 (39.42%) were resistant to at least 1 antimicrobial. In particular, 23 isolates (22.12%) were resistant to tetracycline, 16 (15.38%) to sulfonomides, 14 (13.46%) to trimethoprim and sulfamethoxazole, and 9 (8.65%) to ampicillin, whereas only 1 isolate was resistant to both fluoroquinolones and aminoglycosides. The high prevalence of *S. aureus* found in bulk tank milk samples and the isolation of MRSA, although at a low prevalence, underlines the importance of adopting control measures against *S. aureus* in dairy sheep farms to minimize the risks for animal and public health. Moreover, this study represents the first report of *mecC*-positive MRSA isolation in Italy and would confirm that, among livestock animals, sheep might act as a *mecC*-MRSA reservoir. Although this lineage seems to be rare in dairy sheep (0.35% of farms tested), because *mecC*-positive MRSA are difficult to detect by diagnostic routine methods employed for *mecA*-positive livestock-associated MRSA, diagnostic laboratories should be aware of the importance of searching for the *mecC* gene in all the *mecA*-negative *S. aureus* isolates displaying resistance to oxacillin, cefoxitin, or both.

Key words: sheep milk, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *mecA*/*mecC*

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 a common cause of IMI in dairy ruminants, causing both clinical and subclinical

Received March 27, 2017.
Accepted June 17, 2017.
[1]Corresponding author: antonio.battisti@izslt.it

forms accompanied with relevant economic losses due to reduced milk production and quality (Bergonier et al., 2003).

In the last years, the emergence of livestock-associated (**LA**) methicillin-resistant *Staphylococcus aureus* (**MRSA**) has been increasingly reported worldwide, with a raising concern for the risks of zoonotic transmission, especially for people with occupational livestock exposure (Hanselman et al., 2006; Vanderhaeghen et al., 2010; Fessler et al., 2012; Guardabassi et al., 2013), but also for the possible introduction of these strains in the community through the food chain (Kluytmans, 2010). Clonal complex (**CC**)398 is the most prevalent LA-MRSA lineage in Europe, although in Italy other major LA-MRSA lineages, such as CC1 and CC97, have spread and have also been found to colonize and cause infections in livestock (Alba et al., 2015; Feltrin et al., 2015; Luini et al., 2015; Carfora et al., 2016). In the last decade, MRSA clones with a divergent *mecA* homolog, named *mec*C (formerly *mecA*LGA251), have been detected in different animal species and human beings in different European countries, with isolates mainly belonging to CC130, CC1943, and CC425 (García-Álvarez et al., 2011; Paterson et al., 2014; Angen et al., 2017). Zoonotic transmission of *mecC*-MRSA has been previously reported (Harrison et al., 2013; Petersen et al., 2013), although data on the prevalence, animal reservoir, and epidemiology of *mecC*-MRSA are still limited (Harrison et al., 2013; Petersen et al., 2013).

In recent years, our research group has been investigating the presence and the characteristics of *S. aureus*, particularly MRSA, from sheep dairy products and sheep farms of central Italy (Carfora et al., 2015; Carfora et al., 2016), an area where the milk and cheese manufacturing industry is well developed and the consumption of raw milk dairy products of ovine origin is quite popular.

In this paper we report data on the prevalence of *S. aureus* in the bulk tank milk (**BTM**) samples collected from dairy sheep farms located in central Italy. The antimicrobial resistance profiles of methicillin-susceptible *S. aureus* (**MSSA**) isolates are also reported, together with the genotypic characteristics of *mecA* and *mecC*-positive MRSA strains.

MATERIALS AND METHODS

Sample Collection

Between January and June 2012, a total of 286 BTM samples were collected from 286 dairy sheep farms located in central Italy (Lazio region). The milk samples, collected by trained technicians, were transported to the laboratory in ice-cooled containers and analyzed within 24 to 48 h after collection.

S. aureus Isolation and Identification

All collected samples were analyzed for the enumeration of coagulase-positive staphylococci using Baird-Parker agar with rabbit plasma fibrinogen supplement according to ISO 6888–2: 1999 and Amd1: 2003 (ISO 6888–2: 1999/Amd1; ISO, 2003). Coagulase-positive colonies were identified as *Staphylococcus* spp. by microscopic observation, Gram staining, and catalase determination. Considering the composite nature of BTM samples, multiple suspected colonies (up to 5) were further analyzed from each positive sample. Genomic DNA was obtained from *Staphylococcus* spp. colonies previously subcultured on blood agar (5% defibrinated bovine blood) by using InstaGene Matrix (Bio-Rad, Milano, Italy), as reported by Bianchi et al. (2014). *Staphylococcus aureus* identification was performed by a modified species-specific PCR, using primers targeting the *femA* gene (Mehrotra et al., 2000).

Screening for Methicillin Resistance by Cefoxitin Disk Diffusion Test

A total of 679 *S. aureus* colonies were screened for methicillin resistance by the cefoxitin disk diffusion test according to the criteria of Clinical Laboratory Standards Institute (**CLSI**). The results were interpreted following the Performance Standards for Antimicrobial Susceptibility Testing, Twenty-Third Informational Supplement (CLSI, 2013a).

Molecular Characterization

Cefoxitin-resistant isolates were tested for the presence of the *mec*A/*mec*C and *bla*Z genes by PCR assays using primers and protocols described by Stegger et al. (2012) and Martineau et al. (2000), respectively. The MRSA isolates were further genotyped by *spa* typing, multilocus sequence typing (**MLST**) and by typing/subtyping of the staphylococcal cassette chromosome *mec* (**SCC*mec***) using multiplex PCR methods as previously described (Battisti et al., 2010; Shore et al., 2011). The *MecC*-positive isolates were also tested by PCR analysis for the presence of the *blaZ* allotype associated with SCC*mec* XI (*blaZ*-SCC*mec*XI), as reported by García-Álvarez et al. (2011).

The *Mec*A/*mec*C-positive isolates were also screened by PCR analysis for the presence of specific immune evasion and virulence genes. These included the genes coding for the Panton-Valentine leucocidin (**PVL**) and