Characterization of a methicillin-resistant *Staphylococcus aureus* carrying *mecC* gene isolated from raw sheep milk in Italy

G. Giacinti[1a], V. Carfora[1b], R. Amoruso[1b], M. Iurescia[1b], D. Sagrafoli[1a], N. Marri[1a], G. Giangolini[1a], S. Amatiste[1a], A. Franco[1b], A. Caprioli[1b], A. Battisti[1b]

[1] Istituto Zooprofilattico del Lazio e della Toscana "M. Aleandri"
[a] Centro di Referenza Nazionale per la Qualità del Latte e dei Prodotti Derivati degli ovini e dei caprini
[b] Centro di Referenza Nazionale per l'Antibioticoresistenza

Keywords: methicillin-resistant *Staphylococcus aureus*, *mecC,* characterization

Introduction

In the last years, the emergence of Livestock-Associated methicillin-resistant *Staphylococcus aureus* (MRSA) has been increasingly reported worldwide, with a raising concern for the risks of zoonotic transmission. Recently, MRSA with a divergent *mecA* homologue, named *mec*C, has been detected in different animals species and human beings in different European countries (4,6). We report the characterization of a *mecC*-positive MRSA strain isolated in a sheep bulk tank milk sample from a dairy sheep farm in Central Italy.

Material and methods

Between January and June 2012 a total of 286 bulk tank milk samples were collected from 286 dairy sheep farms located in Central Italy. All the samples were analysed 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. *S. aureus* identification was performed by PCR, using primers targeting the *femA* gene (5) and positive isolates screened for methicillin resistance by the cefoxitin disk diffusion test according to the criteria of Clinical Laboratory Standards Institute (CLSI). Cefoxitin-resistant isolates were tested for the presence of *mec*A, *mecC* and *bla*Z genes by PCR assays using primers and protocols as previously described (3). 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 (2). *Mec*-positive isolates were tested for phenotypic susceptibility to several antimicrobials by the broth microdilution method and screened by PCR analysis for the presence of specific immune-evasion and virulence genes as previously described (3).

Results

One cefoxitin resistant *S. aureus* isolate from a sample collected in a semi-extensive dairy sheep herd tested positive for the *mecC* gene. The *mecC*-positive isolate belonged to *spa* type t843, ST(CC)130, SCC*mec* type IX, and was resistant to all the beta-lactams, while was susceptible to all the other antimicrobials tested. The *mec*C-MRSA was *blaZ* negative and was also negative for the presence of genes coding for the Panton–Valentine leucocidin (PVL), the toxic shock syndrome toxin 1 (TSST-1), nine tested *S. aureus* enterotoxins, and for the immune evasion cluster (IEC) genes.

Discussion

The detection of MRSA in ovine milk and derived dairy products is a sporadic event (3). The MLST analysis revealed that our *mecC* isolate belonged to *spa* type t843, ST(CC)130, SCC*mec* type IX, which is a common lineage of *mecC*-MRSA reported among humans, cattle and sheep in Europe (1,6). Noteworthy, our *mecC*-positive isolate was negative for the presence of specific immune-evasion and virulence genes prominent among CA-MRSA, a finding consistent with a possible animal-host origin/adaptation of this strain (6). As for the antimicrobial resistance, the *mecC*-positive isolate was resistant only to beta-lactams, as already reported for other similar *mecC* isolates (1,6), thus suggesting that this lineage may not have been extensively subjected to antibiotic pressure.

Conclusion

This study represents the first report of *mecC*-positive MRSA isolation in Italy and would confirm that, among livestock animals, sheep might act as *mecC*-MRSA reservoir. In this regard, it should

be considered that the prevalence of *mecC*-MRSA could be underestimated because they are difficult to identify by routine methods. The implementation of specific monitoring/surveillance programs at national and/or regional level would help in better understanding the "MRSA epidemiology" in dairy small ruminant herds.

Bibliografia

1. Ariza-Miguel J et al., 2014. Methicillin-Resistent Staphylococcus aureus Harboring mecC in Livestock in Spain. J. Clin. Microbiol. 52,4067-4069.
2. Battisti A et al., 2010. Heterogeneity among methicillin-resistant *Staphylococcus aureus* from Italian pig finishing holdings. Vet. Mic. 142,361–366.
3. Carfora V et al., 2016. Methicillin-resistant and methicillin-susceptible Staphylococcus aureus in dairy sheep and in-contact humans: An intra-farm study. J. D. Sci. 99 4251–4258.
4. Garcia-Alvarez L et al., 2011. Meticillin-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, 595–603.
5. Mehrotra M et al., 2000. Multiplex PCR for detection of genes for Staphylococcus aureus enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. J. Clin. Microbiol. 38, 1032–1035.
6. Paterson GK et al., 2014. The emergence of mecC methicillin-resistant Staphylococcus aureus. Trends Mic. 22, 42-7.