Methicillin-sensible Staphylococcus aureus and Methicillin-Resistant Staphylococcus aureus isolated in raw milk from dairy sheep farms located in Central Italy

G. Giacinti, D. Sagrafoli, A. Tammaro, E. Bovi, N. Marri, G. Giangolini, V. Carfora, G. Cordaro, A. Ianzano, S. Lorenzetti, A. Caprioli, S. Amatiste

Istituto Zooprofilattico del Lazio e della Toscana "M. Aleandri"
National Reference Centre for ovine and caprine milk and dairy products quality (CReLDOC)

Summary

The aim of this study was to estimate the presence of methicillin–sensible Staphylococcus aureus (MSSA) and methicillin–resistant Staphylococcus aureus (MRSA) in bulk tank milk (BTM) samples from dairy sheep farms located in Central Italy. Between January and May 2012, a total of 286 BTM samples were examined for Staphylococcus aureus (S. aureus). Estimated MSSA herd prevalence was 53.5% (n. 153/286), while estimated MRSA herd prevalence was 0.35% (n. 1/286). Using disk diffusion method, MSSA isolates showed resistances mainly towards those classes of antimicrobials: tetracycline, sulfonomides, ampicillin and trimethoprimsulfamethoxazole, while the unique MRSA isolate detected was resistant, not only to all the beta-lactams tested, but also to erythromycin, streptomycin, kanamycin and tetracycline. This latter isolate belonged to spa type t127, sequence type 1, clonal complex 1, SCCmec type IVa, a lineage usually considered as a human communityassociated MRSA.
Enterotoxigenic S. aureus isolates were detected in 65/153 (42.5%) samples tested positive for S. aureus. Enterotoxin production was assessed in 191 out of 697 (27.4%) S. aureus isolates, and among the enterotoxin producing isolates, SEC was the most frequently detected (163/191, 85.3%), followed by SEA (13/191, 6.8%) and SEB (8/191, 4.2%). The high prevalence of S. aureus and enterotoxigenic S. aureus found in BTM samples, and the significant isolation of a MRSA from a dairy sheep farm, underline the importance of adopting control measures against S. aureus mastitis and monitoring/surveillance programs in order to minimize the risks for animal and public health.

Introduction

Staphylococcus aureus (S. aureus) is a common cause of mastitis in dairy farms and in humans is responsible for food poisoning, frequently associated with consumption of raw milk dairy products (1). S. aureus contamination can occur throughout all the small ruminants dairy food chain, while intra-farm spreading mainly occurs following milking procedures through milking equipment such as teat cups, towels, and milkers hands (2).
In the last years, MRSA have been isolated from both companion and food animals, with the emergence, in Europe, of Livestock-Associated methicillin-resistant strains (LA-MRSA), mostly belonging to the clonal complex (CC) 398 (3, 4, 5). Horizontal transmission of LA-MRSA to people working in close-contact with animals in positive herds is of particular concern for the zoonotic risk of both infection and/or colonization (6, 7). Furthermore, the detection in food of multidrug-resistant bacteria, such as MRSA, is also of concern for Public Health due to their potential spread through the dairy food chain (8, 9).
However, to date, little information is available on MRSA prevalence in raw milk from sheep farms. CC398 strains were recently isolated from three bulk milk samples (1.31%) from 229 dairy sheep farms along with a mecC- MRSA (ST130) strain in Spain (10). Similarly, MRSA CC398 strains were isolated from nasal swabs of dead sheep at slaughterhouse in Denmark (11), while a MRSA CC80 lineage was isolated from the nares of healthy sheep in Tunisia (12).
The objective of this study was to estimate the prevalence of MSSA and MRSA in bulk tank milk samples from dairy sheep farms located in Central Italy. We also investigated the enterotoxins production of S. aureus isolates by using Reverse Passive Latex Agglutination (RPLA) assay and

antimicrobials susceptibility of the MSSA and MRSA isolates by using disk diffusion method. Detected MRSA isolates were further genotypically characterized.

Material and methods

Between January and June 2012 a total of 286 bulk tank milk (BTM) samples collected from 286 sheep farms located in Lazio region were supplied to the Laboratories of the Istituto Zooprofilattico Sperimentale del Lazio e 356 della Toscana "M. Aleandri", by commercial milk testing companies responsible for the quality assurance testing of the bulk milk of the dairy farms in Lazio region. The selected farms accounted for about 11%% of the sheep farms of the region, and were distributed in five provinces.
The milk samples, collected by trained technicians, were transported to the laboratory in ice-cooled containers and analysed within 24 h after collection.
All the samples were analysed for the enumeration of coagulase-positive staphylococci (CPS) using Baird–Parker agar plus rabbit plasma fibrinogen (BP-RPF) according to ISO 6888-2: 1999 and Amd1: 2003 (ISO 6888-2 and Amd1, 2003). Genomic DNA was obtained from Staphylococcus spp. colonies subcultured on blood agar (5% defibrinated bovine blood) by using InstaGeneTM Matrix (Bio-Rad, Milano, Italy) as reported by Bianchi et al. 2014 (13). From each positive sample, suspected colonies (up to 5) were further analysed.
S. aureus identification was performed by a modified species-specific PCR, using primers targeting femA gene (132 BP) (14).
S. aureus detected isolates (n. 679) were screened for methicillin resistance using the cefoxitin disk diffusion method according to the criteria of Clinical Laboratory Standard Institute.
A representative subset of S. aureus detected isolates were tested, by using the Kirby Bauer disk diffusion method, for phenotypic susceptibility to β-lactams and other drugs representative of the most relevant antimicrobial classes used for Staphylococcus spp. infections: ampicillin, amoxacillin-clavulanic acid, cefotaxime, tetracycline, sulfonamides, chloramphenicol, gentamicin, trimethoprim-sulfamethoxazole, clindamycin, enrofloxacin, erythromycin, kanamycin. Results were interpreted according to the criteria of the Clinical Laboratory Standards Institute (CLSI).
Phenotipically suspected MRSA isolates were further confirmed by molecular methods. DNA was extracted from the isolates using a commercial kit (QIAamp DNA Mini Kit; Qiagen GmbH, Hilden, Germany). The detection of mecA and blaZ genes by PCR assay was performed using primers and protocols described by Martineau et al. 2000 (15) and Strommenger et al. 2003 (16). MRSA isolates were genotyped by spa typing, multilocus sequence typing (MLST) and by typing/subtyping of the staphylococcal cassette chromosome mec (SCCmec) using multiplex PCR methods as previously described (17).
All S. aureus isolates (n. 679) were tested for staphylococcal enterotoxins production (SEA-SED) by reverse passive latex agglutination (RPLA) method using the kit SET-RPLA,according to the manufacturer's instructions.

Results and discussion

S. aureus was detected in 53.5% of the milk samples (153/286) with an average content of 2.53 log cfu/ml and a concentration ranging between 1.2 and 4.63 log cfu/ml. A lower prevalence was observed in previous works carried out in Swiss and in Iran in raw sheep milk, where prevalences of 33% and 10% were found respectively (18, 19).
The presence of MRSA was found in a single sample out of 153 tested positive for S. aureus. Overall, the estimated MRSA herd-level prevalence was 0.35% (1/286), suggesting current negligible presence among dairy sheep farms of Central Italy. The MRSA isolate belonged to spa type t127, sequence type 1, clonal complex 1, SCCmec type IVa, a lineage usually considered as a human community-associated MRSA, but previously described in cases of dairy cattle mastitis (20,

21) and in pigs (22), suggesting its ability to adapt to different mammalian hosts. This isolate also harboured a SCCmec element, and showed a co-resistance pattern previously described in both human and animal spa type t127 ST1 isolates (22). Although the prevalence was low, the isolation of MRSA from raw sheep milk is of some interest. The origin of this isolate remain yet unclear; more accurate and targeted studies aiming at collecting other samples from animals, humans and environment at farm level would be helpful to identify the source of contamination. Using disk diffusion method, MSSA isolates showed resistances mainly towards those classes of antimicrobials: tetracycline, sulfonomides, ampicillin and trimethoprim-sulfamethoxazole. These findings can be probably explained by the common use of these antimicrobials in sheep dairy farms.
In this study, enterotoxigenic S. aureus isolates were detected in 65/153 (42.5%) samples tested positive for S. aureus. Enterotoxins production was assessed in 191 out of 697 (27.4%) isolates, and among the enterotoxin producing isolates, SEC was the most frequently detected (163/191, 85.3%), followed by SEA (13/191, 6.8%) and SEB (8/191, 4.2%). Seven isolates were able to produce more than one toxin, such as SEA-SEC, SEC-SED and SEB-SEC. The high presence of SEC producing isolates is consistent with other studies (23, 24). The 357 frequent detection of enterotoxins producing isolates indicates that a potential food safety risk associated with dairy products does exist, in particular when proper strategies to avoid S. aureus growth and enterotoxins formation in foods are not implemented (25).

Conclusion

The high prevalence of S. aureus and enterotoxigenic S. aureus found in BTM samples, and the significant isolation of a MRSA from a dairy sheep farm, underline the importance of adopting control measures against S.aureus contamination, also through frequent bacteriological/quality analyses of bulk tank/individual milk samples. In this regard, the implementation of monitoring/surveillance programs at national and/or regional level would be useful to minimize the risks for animal and public health.

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