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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 μL of milk was directly spread on Baird-Parker agar plus rabbit plasma fibrinogen plates (bioMerieux,