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Occurrence of methicillin-resistant *Staphylococcus aureus* in dairy cattle herds, related swine farms, and humans in contact with herds

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ABSTRACT

In this study we investigated the circulation of methicillin-resistant *Staphylococcus aureus* (MRSA) in 2 dairy cattle farms (farm A and B), previously identified as MRSA-positive in bulk tank milk samples, and epidemiologically related to swine farms. Collected specimens included quarter milk samples and nasal swabs from dairy cows, pig nasal swabs collected at both the farm and slaughterhouse level, environmental dust samples, and human nasal swabs from the farms' owners and workers. The prevalence of MRSA was estimated at the herd level by testing quarter milk samples. The prevalence of MRSA was 4.8% (3/63; 95% confidence interval = 0–10.2%) and 60% (33/55; 95% confidence interval = 47.05–72.95) in farm A and B, respectively. In farm A, MRSA was also isolated from humans, pigs sampled at both farm and slaughterhouse level, and from environmental samples collected at the pig facilities. The dairy cattle facilities of farm A tested negative for MRSA. In farm B, MRSA was isolated from environmental dust samples in both the cattle and pig facilities, whereas nasal swabs collected from cows and from humans tested negative. Sixty-three selected MRSA isolates obtained from different sources in farm A and B were genetically characterized by multilocus sequence typing, *spa*-typing, ribosomal spacer-PCR, and also tested for the presence of specific virulence genes and for their phenotypical antimicrobial susceptibility by broth microdilution method. Different clonal complex (CC) and *spa*-types were identified, including CC398, CC97, and CC1, CC already reported in livestock animals in Italy. The MRSA isolates from quarter milk of farm A and B mostly belonged to CC97 and CC398, respectively. Both lineages were also identified in humans in farm A. The CC97 and CC398 quarter milk isolates were also identified as genotype GTBE and GTAF by ribosomal spacer-PCR respectively, belonging to distinct clusters with specific virulence and resistance patterns. The GTBE and GTAF clusters also included swine, environmental, and human isolates from both farms. A high heterogeneity in the genetic and phenotypic profiles was observed in environmental isolates, in particular from farm B. These results demonstrate the possibility of a dynamic sharing and exchange of MRSA lineages or genotypes between different species and farm compartments in mixed-species farms. The risk of transmission between swine and related dairy cattle herds should be considered. Our findings also confirm the zoonotic potential of livestock-associated MRSA and underline the importance of applying biosecurity measures and good hygiene practices to prevent MRSA spread at the farm level and throughout the food production chain.
Key words: dairy cow, pig, methicillin-resistant *Staphylococcus aureus*, molecular typing, zoonosis

INTRODUCTION

The emergence of livestock-associated (**LA**) methicillin-resistant *Staphylococcus aureus* (**MRSA**) among and within livestock species is a relevant issue from both human and animal health perspectives (Voss et al., 2005). Currently, clonal complex (**CC**) 398, including several *spa*-types, is the most prevalent LA-MRSA lineage in Europe and, although it does not have a high host specificity, it is mainly found as a nasal colonizer of pigs in countries with a high density of swine farms (EFSA, 2010). In Italy, other major LA-MRSA lineages, such as CC1 and CC97, have also been found to colonize and cause infection in livestock (Alba et al.,

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2015; Feltrin et al., 2015; Luini et al., 2015; Carfora et al., 2016). Zoonotic transmission of LA-MRSA strains from livestock to humans, with subsequent severe infections, have been reported (Soavi et al., 2010; Lozano et al., 2011). Moreover, it has been demonstrated that people living and working in close contact with farm animals are particularly exposed to MRSA colonization (van Loo et al., 2007; Van den Broek et al., 2009; Van Cleef et al., 2011; Carfora et al., 2016), possibly contributing to the MRSA spread throughout the food chain (Kluytmans, 2010; Wendlandt et al., 2013).

Livestock-associated MRSA can colonize the udder and cause IMI in dairy ruminants (Cortimiglia et al., 2015; Luini et al., 2015; Carfora et al., 2016), sometimes leading to clinical mastitis (**CM**) and relevant economic losses (Feßler et al., 2010; Vanderhaeghen et al., 2010). However, the epidemiology of MRSA in dairy cattle is yet to be fully investigated and the rate of the infection is not clear (Vanderhaeghen et al., 2010). A high variability of inter-herd (Haran et al., 2012; Kreausukon et al., 2012; Paterson et al., 2012) and intra-herd (Vanderhaeghen et al., 2010; Feßler et al., 2012; Luini et al., 2015) prevalence has been reported so far. Moreover, on the base of the genetic relatedness observed among isolates detected from different sources, different patterns of transmission between and within investigated farms have been described (Feßler et al., 2012). In a previous study (Locatelli et al., 2016), we demonstrated a clear exposure-response relationship between the number of swine and swine herds present in a territory with the MRSA status of dairy cattle herds. Moreover, it has been already demonstrated that environmental dust carried by the wind or contaminated items can act as a passive MRSA spreader (Friese et al., 2012; Merialdi et al., 2013), allowing a possible transmission between swine and close-proximity dairy herds.

The aims of the current work were (1) to assess the presence of MRSA in individual quarter milk samples from 2 dairy farms of northern Italy previously identified as MRSA-positive and geographically or epidemiologically related to swine farms; (2) to investigate the MRSA circulation by testing environmental, human, and swine specimens from the same farms; (3) to characterize the MRSA isolated from different sources by molecular methods; and (4) to evaluate the genetic relatedness of the MRSA isolates and to hypothesize possible dynamics of transmission.

MATERIALS AND METHODS

MRSA-Positive Farm Characteristics

The study was carried out in 2 dairy farms (farm A and B) located in northern Italy. In March 2010, a survey was performed to assess the presence of MRSA in dairy cattle herds located in a highly productive area of northern Italy. Bulk tank milk samples from 27 dairy farms were collected and analyzed at the laboratory of the Department of Health, Animal Science and Food Safety, University of Milan, as previously described (Locatelli et al., 2016). At that time, 2 different farms, named farm A and B, were found to be positive for MRSA. The studied dairy farms were not epidemiologically related, were located in different municipalities, had no exchange of living animals, and had no common workers or veterinarians. Both farms were geographically or epidemiologically related to swine facilities. In both cases, pig and dairy cattle facilities were situated within less than 100 m and the farms shared the same service passages, without any physical division.

Farm A included dairy and pig herds in close proximity, although 2 different owners managed these activities. The dairy herd comprised 180 lactating cows, milked twice a day and reared in freestall facilities. The swine herd was a farrow-to-finish herd consisting of 3,135 animals. The 2 owners and 2 employees represented all the staff. The cattle owner (owner 1) managed the dairy herd and normally milked twice daily, helped by an employee milker. The milking routine procedures included wearing disposable gloves, predipping followed by cleaning with paper wipes, and postdipping. The manager of the swine herd (owner 2) was also occasionally involved in all the other activities within the family property, including daily milking procedures.

Farm B included 55 lactating cows reared in freestall facilities and the farmers (father and son) milked twice a day. They did not use gloves and applied only a postmilking teat dip. The swine facility was located close by and was a finishing unit composed of 5 barns harboring about 4,000 fattening pigs. The swine unit was managed by a third person, whereas a caretaker, living nearby, was entrusted with the feeding operations and animal care. The owners of the dairy cows and the swine caretaker had free access to the whole external area outside both farms, without any restriction.

Samples Collection and MRSA Identification

Between April and July 2010, quarter milk samples, animal and human nasal swabs, and environmental dust samples were collected from the 2 dairy farms and the respective neighboring swine farms. All samples were analyzed at the laboratory of the Department of Health, Animal Science and Food Safety of the University of Milan. The owners voluntarily accepted to participate to the survey, but agreed to sample only part of the animals.