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Detection of methicillin-resistant *Staphylococcus aureus* in dairy cow farms

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common pathogens causing nosocomial infections worldwide. Animal-associated MRSA hazard has been recently identified, but less information is currently available regarding MRSA in cattle. The aim of this study was to estimate the presence of MRSA in samples of bulk milk, environmental dust, conjunctival and nasal swabs of workers obtained from thirty dairy cow farms. A total of 200 *S. aureus* strains were identified using phenotypic and molecular approaches. Three other species (*Staphylococcus epidermidis*, *Staphylococcus xylosus* and *Staphylococcus saprophyticus*) were found. In five *S. aureus* strains isolated from environmental dust and one *S. epidermidis* strain derived from human samples, *mec*A gene was detected showing a specific fragment at 527 bp. Moreover, 66 *S. aureus* strains were distinguished by susceptibility to 15 antimicrobial agents. The highest resistance profile was ascribed to ampicillin, amoxicillin and penicillin G, both in workers and bulk milk samples. Generally, a multiple resistance to 4 up to 10 antibiotics was detected. *S. aureus mec*A^{+} strains and *S. epidermidis mec*A^{+} strain showed multiple resistance to 13 and 11 antibiotics, respectively. The obtained results suggested that the low number of MRSA strains, probably of human origin, was due to the appropriate hygienic practices adopted by the dairy cow farms.

1. Introduction

Staphylococcus aureus is a human and animal pathogen involved in multiple disease processes including skin and soft tissue infections, sepsis, osteomyelitis and pneumonia (Crago et al., 2012). In dairy cows it is considered one of the most important mastitis agents (Tenhagen, Köster, Wallmann, & Heuwieser, 2006) which mainly spread at milking. The probability of udder infection increases if the animal has direct contact with the reservoirs of pathogens or indirect contact via fomites (Capurro, Aspán, Ericsson Unnerstad, Persson Waller, & Artursson, 2010). Then, measures to control mastitis range from the improvement of milking hygiene to the use of antimicrobial agents, but, the well-documented resistance of *S. aureus* to antibiotics may have two relevant effects, the first is a reduction in cure rates after treatment, and the second is the potential impact of transmission of resistant bacteria to humans via the food chain (Tenhagen, Hansen, Reinecke, & Heuwieser, 2009). Although resistance in human infections is mainly caused by human antibiotic use, the treatment of farm animals with antimicrobials can be the main source of resistance for some infections (EFSA, 2008).

The acquisition of resistance to most classes of antimicrobial agents has made treatment and control of staphylococcal infections increasingly difficult. The widespread use of methicillin and other semi-synthetic penicillins led to the emergence of methicillin-resistant *S. aureus* (MRSA), which is present in both the health care and community environments (Stevens, 2003). It is known that MRSA can cause nosocomial infections worldwide (Shahraz et al., 2012). Methicillin-resistance is conferred by presence of the *mec*A gene, which encodes for production of an altered penicillin binding protein (PBP2a or PBP2′), with a low affinity for all beta-lactam antimicrobials (Kwon et al., 2006).

The emerging problem of MRSA colonization in food producing animals and the links with human infection have an impact both on food production and on health of people that work with animals with possible risks of disease for the general population. Isolation of MRSA from animals was first reported in 1972 following its

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detection in milk from mastitic cows (Devriese, Vandamme, & Fameree, 1972). Then, occasional reports have been published in domestic animals including dogs, cats, cattle, pigs, sheep, chickens, rabbits and horses (O'Mahony et al., 2005). Studies on farms and in slaughterhouses have reported high prevalence of MRSA colonization at pig farms level (Weese & van Duijkeren, 2010), and a new strain (ST398) has recently been detected in production animals, among which pigs have been recognized as an important source of infection for pig farmers (Commission Decision 2008/55/EC). Indeed, less information is available regarding MRSA colonization in cattle and only few studies investigated extra-mammary sites such as milking liners, hands and nostril of milkers, and the environment of the animals in general (Foti, Fisichella, Conte, Passantino, & Giacopello, 2012; Haveri, Hovinen, Roslof, & Pyörälä, 2008; Spohr et al., 2011; Zadoks et al., 2002). The detection of MRSA carriers is important for the prevention and follow-up of these infections. In addition to hospital-associated MRSA infections, community-acquired infections caused by MRSA are of an increasing concern (Witte et al., 2008). Well timed distinction of *S. aureus* from coagulase negative staphylococci (CoNS) and methicillin-susceptibility results have important therapeutic, prognostic and economic impact (Jukes et al., 2010). Many approaches have been implemented to type and differentiate MRSA for monitoring and tracking MRSA spread in different contexts (van Belkum et al., 2007; Collery et al., 2008). Random Amplification of Polymorphic DNA-PCR (RAPD PCR), ribotyping, biotyping, pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST) and *spa* typing are the main techniques used to monitor the mammary persistent chronic infection caused by a strain strain or more than one. In particular, genomic analysis techniques allow the investigation of the population structure and the development of evolutionary hypotheses.

The purpose of this study was to evaluate the rate of MRSA in some dairy cow farms located in Abruzzo region, central Italy, both in bulk milk and livestock environment. Moreover, as MRSA infections in dairy cattle have been ascribed to human-to-animal transfer (Juhász-Kaszanyitzky et al., 2007), the livestock workers were investigated by nasal and conjunctival swabs. Analysis of antibiotic resistance pattern and molecular detection of *mec*A gene in *S. aureus* strains using sensitive and specific PCR techniques were also performed.

2. Materials and methods

2.1. Dairy cow farms selection

This study involved thirty dairy cow farms (Fig. 1) located in Chieti province (Abruzzo region, Italy). Herd size ranged from 10 to 40 lactating cows of Frisona and Bruna alpina breeds. They were all family-run farms, except for three of them having staff workers to animals. Dairy outbuildings were connected only to five farms and just only one was located near to a pig breeding farm.

2.2. Sampling and Staphylococcus aureus detection

In the selected dairy cow farms, bulk milk samples ($n = 30$) were collected from the tank containing the milk obtained in the evening milking. Then, dust samples ($n = 30$) from the environment were

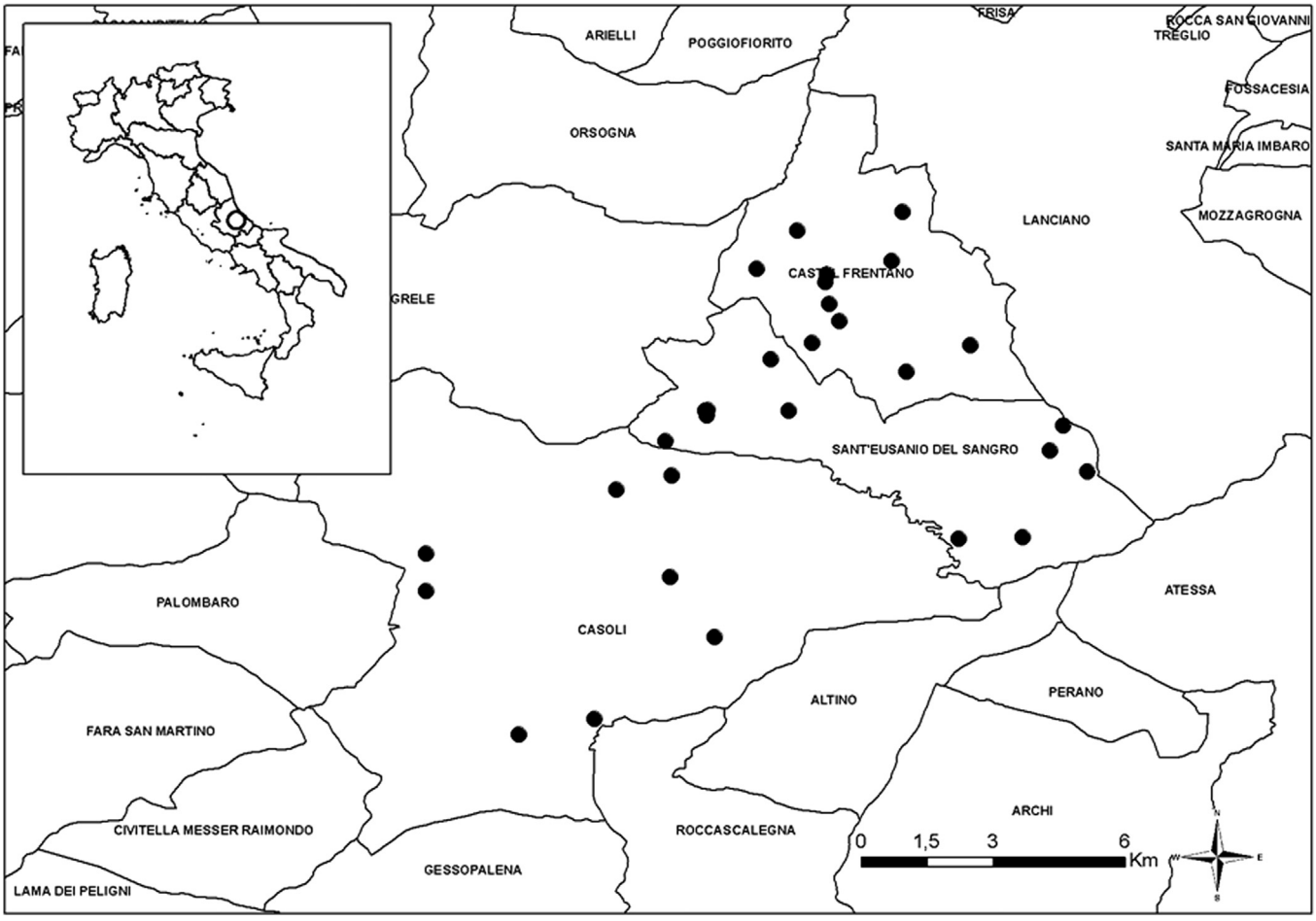

Fig. 1. Map of dairy cow farms located in Chieti province, (Abruzzo region, Italy).