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Mini Review

Livestock-associated methicillin-resistant *Staphylococcus aureus* in animals and humans

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**A B S T R A C T**

Since 2004 MRSA emerged in animals, particularly in pigs and veal calves. This new MRSA variant was since its first appearance referred to as Livestock Associated-MRSA (LA-MRSA). In Europe and Northern America, LA-MRSA belongs predominantly to clonal complex (CC) 398 whereas in Asia ST9 seems to be dominant in pigs. Persons in direct contact with LA-MRSA-positive animals have an increased risk of becoming MRSA positive. The risk of carriage is mainly related with the intensity of animal contact and with MRSA prevalence among animals on the farm. In contrast with its success in animals, it seemed that MRSA CC398 is a poor persistent colonizer in humans. MRSA ST398 can, however, cause serious (invasive) infections and outbreaks, although, only incidentally reported so far. Farm hygiene and antimicrobial use contributed to MRSA occurrence in animals. Therefore these two determinants should in principle be incorporated into MRSA-control programmes in animal production. Like any other microorganism, LA-MRSA is expected to be able to adapt to new hosts and may change over time in the potential to colonize and to produce toxins. Also, the current circulating clone CC398 may be replaced by another clone in Western countries or emerge in countries where this clone is currently low-prevalent. Ongoing MRSA surveillance in humans and animals is needed to detect changes in epidemiology and to implement effective control measures.

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**Introduction**

*Staphylococcus aureus* (*S. aureus*) is a Gram-positive bacterium which belongs to the commensal flora of humans and various animal species (Vanderhaeghen et al., 2010b). Multiple body sites can be colonized in humans, but the anterior nares are the most frequent colonized sites (Wertheim et al., 2005). Approximately 20% of healthy human individuals are persistent *S. aureus* carriers, about 30% are intermittent carriers and around 50% are never colonized with *S. aureus* (Kluytmans and Struelens, 2009). In humans, *S. aureus* is regarded as the most important cause of nosocomial infections with clinical conditions ranging from minor skin infections to severe, life-threatening infections (Lowy, 1998; Kluytmans and Struelens, 2009).

In animals, *S. aureus* is one of the three major pathogenic *Staphylococcus* species, together with *S. hyicus* and the *Staphylococcus intermedius* group – SIG (*S. pseudintermedius*, *S. intermedius*, and *S. delphinus*) with *S. hyicus* and SIG more restricted in host species compared to *S. aureus*. *S. aureus* can cause intramammary infections in cattle and small ruminants (Vanderhaeghen et al., 2010b). It can also cause joint problems in chickens (Butterworth et al., 2001) and it is increasingly reported in surgical site infections in small companion animals and horses (Catry et al., 2010).

**Methicillin-resistant *Staphylococcus aureus* (MRSA)**

Soon after the introduction of penicillin, around 1945, the majority of the *S. aureus* population had become resistant to penicillin through the production of beta-lactamase, an enzyme that hydrolyzes penicillin. In the late 1950s, the beta-lactamase-resistant methicillin was introduced in human medicine. However, soon after introduction, the first methicillin-resistant isolates of *S. aureus* were reported (Robinson and Enright, 2003).

Methicillin resistance is caused by the acquisition of the mecA gene. This gene encodes an alternative penicillin-binding protein, called PBP2A, which has a low affinity for beta-lactam antibiotics (Vanderhaeghen et al., 2010b). The mecA gene is part of a large mobile genetic element, the staphylococcal cassette chromosome

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mec (SCCmec). SCCmec can be integrated at a specific site in the chromosome of methicillin-susceptible S. aureus (MSSA) which is located at the 3 prime end of an open reading frame of a gene with an unknown function (orfX) (Grundmann et al., 2006). SCCmec carries a set of cassette chromosome recombinase genes (ccrA, ccrB or ccrC) for excision and integration into the host chromosome. According to combinations of the mecA and ccr gene complexes contained by the bacterial genome, molecular typing of MRSA strains has revealed that eleven major SCCmec types and several subtypes and composites of two or more SCC elements have emerged worldwide (IWG-SCC, 2009). In addition to the difference in these gene complexes, the various SCCmec elements differ from each other in the antibiotic resistance markers to antimicrobials other than beta-lactams (Grundmann et al., 2006).

Epidemiology of MRSA in Europe and definitions of MRSA groups

The prevalence of MRSA infections in humans varies widely between European countries (Tiemersma et al., 2004). Especially in hospital settings MRSA occurrence is generally high in southern Europe where the proportion of MRSA among invasive (blood and liquor) S. aureus isolates up to 50% is documented (Fig. 1). In contrast, in northern Europe MRSA prevalence is very low, <1%, due to strict MRSA infection control policies (Tiemersma et al., 2004).

Traditionally MRSA has been considered a hospital-associated pathogen (HA-MRSA). Infections with HA-MRSA were supposed to be nosocomial if they emerged at least 48 h after admission. Prolonged hospital stay, care in intensive-care units, prolonged antibiotic treatment, surgical interventions and/or close contact with infected or colonized MRSA-positive individuals are risk factors for attracting HA-MRSA (McCarthy et al., 2010).

Until the 1990s, infections with MRSA were rarely observed in extramural communities. However, since the mid 1990s, MRSA strains were increasingly documented in healthy people without healthcare-associated risk factors. These cases were referred as community-associated MRSA (CA-MRSA). Close contact between humans in sport settings, schools, day-care centers, the military and prisons are considered to be risk factors (Catry et al., 2010). Analysis of the genetic background of these CA-MRSA strains has shown a clear distinction from typical HA-MRSA. CA-MRSA and HA-MRSA belong to different sequence types and in addition carry different SCCmec types. Furthermore the carriage of virulence factors such as Panton-Valentine leukocidin (PVL) is merely associated with CA-MRSA strains (Vanderhaeghen et al., 2010b).

Recently, MRSA has been found to be emerging in livestock (Kock et al., 2010). Animals can act as reservoirs of MRSA, and the bacterium can be transmitted to humans in close contact with MRSA colonized animals. MRSA from this reservoir has been referred to as Livestock Associated-MRSA (LA-MRSA), to distinguish it from HA-MRSA and CA-MRSA types (Smith and Pearson, 2010).

MRSA control in humans

The large MRSA prevalence differences between countries can partly be explained by differences in level of screening, isolation and treatment of patients and staff in hospitals. For example, in the Netherlands and Scandinavian countries a pro-active system has been applied, called the “search and destroy” policy. This strategy consists of active screening of high-risk patients and exposed healthcare workers for MRSA carriage. Risk patients involved hospitalized patients who are repatriated from countries with high MRSA rates and contacts of MRSA patients (Kluymans and Struelens, 2009). People who have had contact with live pigs or veal calves, were included to the risk group since July 2006 and November 2007, respectively (Vandenbroucke-Grauls and Beaune, 2006). Strict implementation of transmission prevention measures for these risk groups and treatment of carriage using topical application of mupirocin nasal cream and washing with disinfecting

agents, such as chlorhexidine is part of the “search and destroy policy” (Kluftmans and Struelens, 2009). The full Dutch strategy is described in the national guidelines (www.wip.nl). In addition, the restrictive use of antimicrobials in humans in the Netherlands contributes to the low MRSA prevalence in the general population (Coenen et al., 2009).

Emergence of LA-MRSA in livestock and other animals

From 1970 to 2000, MRSA was rarely isolated from animals, and if so, these strains were generally supposed to be of human origin, as shown by bio-typing. Therefore, it was thought that until the end of the 20th century, the animal husbandry reservoir was of little relevance to MRSA causing diseases in humans. It was assumed that MRSA was a problem caused by antimicrobial use in human medicine (Catry et al., 2010).

In 1975 the first report on MRSA isolated from cows with mastitis was published (Devriese and Hommez, 1975). This sporadic case was followed by only a few other cases in the next 25 years. From 2000s onwards reports became more frequent and in 2007 transmission of MRSA (ST1; spa-type t1172) between cows and humans was reported (Juhasz-Kaszanyitzky et al., 2007). The initial case of LA-MRSA in a human was described in 2005 in a 6-month-old girl admitted to a hospital for invasive surgery in the Netherlands. The girl remained MRSA positive despite sever decolonization attempts. The girl’s parents, who lived on a swine farm, were also found to be colonized with MRSA (Voss et al., 2005). Since neither the girl nor her family had a history of traveling or admission to a foreign hospital and the MRSA-isolate was non-typeable by standard pulsed-field gel electrophoresis (PFGE), further investigations began into the source of the MRSA in regional pigs and pig farmers (Huijtdens et al., 2006). An additionally study on the presence of MRSA in pigs at slaughterhouse confirmed that LA-MRSA are widely spread in the Dutch pig population (de Neeling et al., 2007).

Genotyping showed that the LA-MRSA strains as found in pigs and pig farmers were non-typeable by PFGE as these were resistant to digestion with the routinely used enzyme Smal, and therefore referred to as non-typeable MRSA (NT-MRSA). Later studies showed that these isolates are typeable if another enzyme is used (Argudín et al., 2010). The strains belonged to clonal complex CC398, with the majority of strains belonging to ST398. Risk factors for humans acquiring this LA-MRSA were pig and cattle farming (van Loo et al., 2007). From 2005 onwards LA-MRSA is more frequently reported in different food production animals including cattle (Vanderhaeghen et al., 2010a), pigs (Huijtdens et al., 2006; van Duijkeren et al., 2008) and poultry (Nemati et al., 2008; Mulders et al., 2010), also in other European countries outside the Netherlands (Lewis et al., 2008) as well as in the Americas (Smith et al., 2009) and Asia (Guardabassi et al., 2009; Wagenaar et al., 2009). The EU-baseline study on MRSA prevalence in swine production showed that in most European countries MRSA can be found on pig farms (European Food Safety Authority, 2009). In contrast to studies in Europe and the Americas, ST398 does not appear to be the dominant MRSA strain in Asian pigs. Several studies have shown that the predominant strain as found in Asian pigs belonged to ST9 (Cui et al., 2009; Guardabassi et al., 2009; Wagenaar et al, 2009). ST72 is the predominant MLST type found in meat products in Korea (Lim et al., 2010; Ko et al., 2011).

MRSA has also been found in companion animals, but these strains generally differ from those in livestock. The reason for this is that the transmission route is thought to be from humans to companion animals and therefore human epidemic clones are found in these animal species, i.e., MRSA in companion animals is primarily a humanosis (Morgan, 2008).

Molecular aspects of MRSA ST398

MRSA isolates of ST398 possess some typical features. As aforementioned, the strains are non-typeable with standard PFGE using Smal digestion. This is due to the presence of a restriction/methylation system leading to protection from Smal digestion (Bens et al., 2006). The strains carry SCCmec element IV or V (Smith and Pearson, 2010). SCCmec cassette types II and III have also been reported but this may be the result of misidentification (Jansen et al., 2009). Many different spa-types have been documented in ST398. In 2010 there were 25 different spa-types identified related to ST398 but new spa-types are continuously being reported (Vanderhaeghen et al., 2010b). It is suggested that ST398 has been evolved by multiple introductions of the SCCmec element. Studies have shown that strains with identical spa-types can carry different SCCmec elements (van Duijkeren et al., 2008). It has been suggested that coagulase-negative staphylococci in the farming environment could serve as a source of SCCmec (Hansen and Ericsson, 2006).

The transfer of staphylococcal toxin genes to isolates of CC398 seems to be uncommon (Smith and Pearson, 2010). Generally, certain important virulence factors (for instance Panton-Valentine Leukocidin (PVL), tst and LukM) are absent (Vanderhaeghen et al., 2010b). Despite the lack of these virulence factors, MRSA ST398 strains have been found to cause disease in both animals (van Duijkeren et al., 2007; Cuny et al., 2008; Vanderhaeghen et al., 2010a) and humans (Ekkelenkamp et al., 2006; Pan et al., 2009).

ST398 strains are generally resistant to tetracycline, and resistance against macrolides, lincosamides, aminoglycosides and trimethoprim is documented. Fluoroquinolone resistance has also been reported; though to a lesser extent (Vanderhaeghen et al., 2010b).

Public health consequences of LA-MRSA

Persons in direct contact with MRSA-positive animals have an increased risk of becoming MRSA positive. This has been documented for individuals working in companion animal and equine clinics, and livestock production environments (Morgan, 2008). It has been shown that MRSA ST398 has limited host specificity: it is able to colonize and to cause infections in various hosts. So far, the mechanisms of host adaptation are poorly understood (Cuny et al., 2010). However, incidentally reported so far, MRSA ST398 can cause serious (invasive) infections and outbreaks (Kluftmans, 2010).

There is a potential risk of MRSA introduction from the animal reservoir into hospitals with humans as vector. Therefore, in the Netherlands, pig and cattle farmers were included as risk groups as defined by the “Search and Destroy” policy. Consequently the annual number of people at submission to the hospital, suspected of MRSA colonization and requiring MRSA screening, has increased in the Netherlands due to the emergence of LA-MRSA. This is a huge burden for the health care system (van Rijen et al., 2008; Wassenberg and Bonten, 2010). Identification of risk factors and knowledge about persistence of LA-MRSA in humans is essential for successful continuation of the “Search and Destroy” policy. Improved understanding of the mechanisms underlying transmission and persistence, and the role of exposure in LA-MRSA carriage in both animals and humans could have a significant impact on antibiotic and infection control policies in the hospitals. It also provides information for evidence-based guidance on the development of new strategies and preventive measures for the control of MRSA.
Risk factors for animal and human LA-MRSA carriage

Few studies investigated risk factors for the occurrence of ST398 in animals and humans. A high risk of animal to human transmission of ST398 has been reported in pig farming (Lewis et al., 2008; Smith et al., 2009; van den Broek et al., 2009). A direct association between MRSA carriage in animals and MRSA carriage in humans was observed in veal calf farming (Graveland et al., 2010). It was demonstrated that LA-MRSA carriage among veal farmers and their family members and employees was strongly associated with intensity of animal contact and with the number of MRSA-positive animals on the farm. However, a positive farmer clearly contributed to the risk of MRSA carriage in family members. On the other hand, a longitudinal study among farmers, employees and family members over a 10-week period showed that persistent carriers of ST398 were rarely observed. MRSA prevalence rapidly decreased during absence of animal contact, during holidays and in between production cycles, which suggests that LA-MRSA is a poor persistent colonizer in most humans (Graveland et al., 2010). The observed decrease in prevalence was strongest during the holiday period.

Veterinarians occupationally exposed to pigs also are at increased risk for becoming MRSA carriers (Wulf et al., 2008).

In human medicine, a causal relationship between the usage of antimicrobials and the presence of MRSA has been demonstrated (Tacconelli et al., 2008). It is hypothesized that a similar relationship may occur in animals (de Neeling et al., 2007; Wulf and Voss, 2008). In pig farming the use of standard antimicrobial medication of the pigs seems to be a risk factor for MRSA carriage (van Duijkeren et al., 2008). In veal farming, it is shown that calves were more often found MRSA positive when they had been treated with antibiotics (Graveland et al., 2010). A similar association was found in veal. Further quantification is necessary. Farm hygiene appeared to be associated with a lower prevalence of MRSA among veal calves (Graveland et al., 2010).

Human to human transmission of MRSA ST398

Few studies have examined transmissibility, and from these it appears that in hospital settings, ST398 transmits less frequently than most HA-MRSA strains (van Rijen et al., 2008; Wassenberg and Bonten, 2010; Wassenberg et al., 2011). A large Dutch multi-center study in hospital settings has shown that the relative risk on transmission of MRSA ST398, as compared to HA-MRSA, was 0.28 (Wassenberg et al., 2011). Based on these data, the genotype-specific single admission reproduction number ($R_0$ value) was estimated. For LA-MRSA this was 0.16 which is clearly lower than 0.93 as found for HA-MRSA. Taken these $R_0$ values together, this resulted in a $R_0$ ratio between HA-MRSA and LA-MRSA of 5.9 (95% CI 2.24–23.81), indicating that LA-MRSA is 5.9 times less transmissible than HA-MRSA (Bootsma et al., 2011). However, these data should be interpreted with caution since several assumptions about homogeneity between HA-MRSA and LA-MRSA carriers are made and data of other potential risk factors and patient characteristics are lacking or ignored in these calculations. Despite the lower human to human transmission likelihood of LA-MRSA, a positive association between MRSA carrier status of family members and MRSA carriage of the farmer was demonstrated in veal farming (Graveland et al., 2011). Especially this finding in children, suggests that MRSA carriage in children, who are generally low exposed, is more strongly determined by contact with highly exposed family members or through environmental contamination than by direct animal contact.

Generally, transmission is dependent of host and environmental characteristics. This explains why differences may occur in human to human transmission in and outside hospital settings. This is also recognized by Wassenberg et al. (2011), who mention that farmers usually belong to a healthy population (as compared to hospitalized patients with non-ST398 MRSA) and they therefore, in hospital settings, may be less likely to transmit the pathogen to other patients. Despite the fact that the risk for transmission in clinical populations is considerably lower for ST398, public health aspects of ST398 carriage, and rapid emergence in livestock and human populations closely associated with livestock, require further attention.

Conclusions

LA-MRSA seems to be recently introduced into production animals and is predominantly present in pigs and veal calves. However, other animals can be MRSA carrier as well due to overgrowth towards other species.

It has been shown that persons in direct (occupational) contact to LA-MRSA-positive animals have an increased risk for LA-MRSA carriage. The risk for LA-MRSA carriage in humans is mainly related to exposure to MRSA-positive animals. However, the positive association between MRSA carrier status of family members and MRSA carriage of the farmer indicates that human to human transmission cannot be excluded. It has been shown that MRSA ST398 has limited host specificity; it is able to colonize and to cause infections in various hosts.

Farm hygiene (cleaning and disinfection of stables between production cycles) seems to be associated with a lower prevalence of MRSA. Antimicrobial use contributes to MRSA presence in animals. These two determinants of ST398 MRSA may be incorporated into control programmes aiming for a reduction of MRSA in livestock.

Like any other microorganism, LA-MRSA is expected to be able to adapt to new hosts and may change over time in the potential to colonize and to produce toxins. Also, the current circulating clone CC398 may be replaced by another clone in Western countries or emerge in countries where this clone is currently low-prevalent. Ongoing MRSA surveillance in humans and animals is needed to detect changes in epidemiology and to implement effective control measures.

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