

Risk Factors for the Occurrence of Methicillin-Resistant *Staphylococcus aureus* in Dairy Herds: An Update

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Abstract

In dairy cows, *Staphylococcus aureus* is a major mastitis pathogen and methicillin-resistant *S. aureus* (MRSA) has been reported from dairy farms around the world. The risk of foodborne zoonotic infections with bovine MRSA strains seems to be low since MRSA prevalence is low in dairy herds and milk is commonly heat treated before consumption. However, bovine mastitis caused by MRSA is an important issue in veterinary medicine since treatment options with non- β -lactam antibiotics are limited. For the development of effective MRSA prevention strategies, it is necessary to know which factors increase the risk for MRSA transmission into and within dairy herds. Therefore, the aim of this review is to summarize the risk factors for the occurrence of MRSA in dairy herds and to identify the respective knowledge gaps. MRSA was more frequently detected in conventional dairy farms than in organic farms and in larger farms than in smaller farms. Dairy farms housing pigs along with cattle are more frequently affected by MRSA. Moreover, humans carrying MRSA can probably infect dairy cows. Consequently, pigs and humans may introduce new MRSA strains into dairy herds. MRSA transmission within dairy herds was associated with improper milking hygiene procedures. Furthermore, methicillin-resistant coagulase-negative staphylococci (MR-CoNS) were repeatedly isolated from dairy farms. This is an important issue since MR-CoNS may transfer resistance genes to *S. aureus*. The role of antimicrobial exposure as a risk factor for the occurrence of MRSA within dairy herds needs to be further investigated.

Keywords: methicillin, staphylococcus, MRSA, dairy, milk

Introduction

STAPHYLOCOCCUS AUREUS is considered a contagious mastitis pathogen that enters the mammary gland through the teat canal. In most cases there is one predominant *S. aureus* strain that affects multiple cows and spreads from cow to cow within dairy herds (Zadoks *et al.*, 2000; Barkema *et al.*, 2006; Keefe, 2012). Thus, the primary risk period for *S. aureus* transmission is during the milking process. The usual routes of transmission are milkers' hands, udder cloths, and milking equipment such as teat liners.

The overall prevalence of mastitis pathogens is highly variable and differs between herds and regions. To date, the most common pathogens causing clinical mastitis seem to be environmental streptococci and coliform bacteria followed by *S. aureus* (Ruegg, 2018). In some studies, *S. aureus* is still the most prevalent pathogen isolated from mastitis milk samples (Østerås, 2018).

In *S. aureus*, methicillin resistance is mediated by a *mecA*- or *mecC*- gene. This gene is located on a mobile genetic element called "staphylococcal cassette chromosome *mec*" (SCC*mec*). The gene is responsible for the production of an altered penicillin-binding protein 2a (PBP2a). The PBP2a has a lower affinity for β -lactam antimicrobials than the normal PBP. Thus, *mecA*/*mecC*-positive staphylococci are resistant to most β -lactam antibiotics (Holmes and Zadoks, 2011; Miragaia, 2018).

While cure rates for lactational *S. aureus* treatments are low, dry cow therapy (DCT) is typically more effective (Keefe, 2012). Most frequently recommended dry cow antibiotics for the treatment of methicillin-sensitive *S. aureus* (MSSA) infections contain β -lactams (Tenhagen *et al.*, 2006; Saini *et al.*, 2012a). Especially cloxacillin is extensively used on dairy farms and cure rates for dry cow treatment of *S. aureus* infections with cloxacillin were reported to range up to 98% (Makovec and Ruegg, 2003a; Tenhagen *et al.*, 2006;

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Saini *et al.*, 2012c). Although, there are no studies on antibiotic treatment outcomes for mastitis caused by methicillin-resistant *S. aureus* (MRSA), cloxacillin, and other β -lactams are probably ineffective. Consequently, culling might be the only chance to remove MRSA from dairy herds. In addition, MRSA in dairy cows is of human health concern since people working on dairy farms were shown to carry similar MRSA strains as their cows (Juhász-Kaszanyitzky *et al.*, 2007; Hata *et al.*, 2010; Spohr *et al.*, 2011; Lim *et al.*, 2013; Locatelli *et al.*, 2017). In these studies, the direction of transmission remained unclear. MRSA transmission from cows to consumers of milk seems unlikely due to commonly practiced heat treatment. However, the consumption of raw milk is a possible source of infection (Al-Ashmawy *et al.*, 2016; Parisi *et al.*, 2016). This might be an issue since many dairy farmers and their families consume raw milk and the number of raw milk vending machines is increasing in Europe (www.milkmaps.com). Thus, MRSA in dairy herds represents a possible health hazard for both humans and cattle. The objective of this review is to summarize the risk factors for the occurrence and spread of MRSA in dairy herds and to identify the respective knowledge gaps.

Prevalence and Epidemiology of MRSA in Dairy Herds

The detection of *S. aureus* in dairy cows is demanding due to its intermittent shedding patterns in milk (Barkema *et al.*, 2006; Keefe, 2012). Comparison of MRSA prevalence studies is additionally challenging because of differences in types of samples, inoculum volumes, (pre-) enrichment, and detection methods.

MRSA prevalence (*mecA/mecC*) in bulk tank milk (BTM) has been previously reported to range from 0% to 20% (Table 1). A study from Sicily found a significantly higher MRSA prevalence of 43.8% in BTM from dairy farms (Antoci *et al.*, 2013). This high prevalence was presumably caused by the preselection of dairy farms that had been tested positive for MRSA in previous years. The average MRSA

prevalence from all other BTM samples in Table 1 is $\sim 2.9\%$. The majority of studies (76%) are from Europe. MRSA prevalence was significantly lower in BTM samples from the United States with $\sim 0.3\%$ (3/980) (Virgin *et al.*, 2009; Haran *et al.*, 2012; Cicconi-Hogan *et al.*, 2014). Compared with Europe, MRSA prevalence was also lower in pig herds from the United States (Sun *et al.*, 2015; Abreu *et al.*, 2019). As shown in Table 2, the MRSA prevalence of *S. aureus* mastitis isolates was reported to be between 0% and 49%. The average MRSA prevalence of all individual milk samples in Table 2 is $\sim 4.5\%$. The MRSA prevalence within individual dairy herds is shown in Table 3. The highest within-herd prevalence of MRSA was 39.7% (31/78) in Japan, 44% (11/25) in Sweden, and 60% ($n=33/55$) in a herd from Italy (Hata, 2016; Locatelli *et al.*, 2017; Unnerstad *et al.*, 2018).

The overall MRSA prevalence in dairy herds is low, compared with other animal species, especially pigs. However, reports from Korea and Germany indicate that MRSA prevalence rates might be increasing over time. In Germany, the prevalence of MRSA-positive BTM samples increased from 4.1% in 2009 over 4.7% in 2010 to 9.7% in 2014 (Tenhagen *et al.*, 2014, 2018). The German studies included BTM samples from all over Germany. The studies were performed under similar conditions within the framework of a national monitoring program. In Korea, MRSA prevalence was up to 6% until 2003 and 13.9% in 2011–2012 (Kwon *et al.*, 2005; Moon *et al.*, 2007; Song *et al.*, 2016). The Korean studies tested mastitis milk samples from different regions in Korea and their comparability is therefore difficult to evaluate. The authors of the last study concluded that the prevalence of MRSA in mastitis milk has continuously increased in Korea (Song *et al.*, 2016). In conclusion, there is some evidence that MRSA prevalence might be increasing in some countries.

In Europe, livestock-associated MRSA (LA-MRSA) belonging to clonal complex 398 (CC398) are the predominant MRSA strains in dairy herds. They were repeatedly isolated from milk samples (Fessler *et al.*, 2010; Vanderhaeghen *et al.*, 2010; Kreausukon *et al.*, 2012; Paterson *et al.*, 2012; Tavakol *et al.*, 2012; Tenhagen *et al.*, 2014, 2018; Luini *et al.*,

TABLE 1. METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* PREVALENCE IN BULK TANK MILK FROM DAIRY COWS

References	MRSA in BTM % (n=MRSA/n=samples)	Year(s) of collection	Country
Antoci <i>et al.</i> (2013)	43.8 (21/48)	2010	Italy
Cicconi-Hogan <i>et al.</i> (2014)	0.03 (1/288)	2009–2011	United States
Cortimiglia <i>et al.</i> (2016)	3.8 (32/844)	2012–2013	Italy
Haran <i>et al.</i> (2012)	1.3 (2/150)	2009	United States
Kreausukon <i>et al.</i> (2012)	4.4 (28/635)	2009–2010	Germany
Locatelli <i>et al.</i> (2016)	4.0 (9/224)	2011	Italy
Obaidat <i>et al.</i> (2018)	20.0 (16/80)	2015–2016	Jordan
Papadopoulos <i>et al.</i> (2018)	10.0 (1/10)	2016	Greece
Parisi <i>et al.</i> (2016)	2.5 (12/486)	2012–2013	Italy
Paterson <i>et al.</i> (2012)	0.5 (7/1500)	2012	United Kingdom
Paterson <i>et al.</i> (2014)	2.4 (11/465)	2011–2012	United Kingdom
Ronco <i>et al.</i> (2018)	0.0 (0/94)	2016	Denmark
Tenhagen <i>et al.</i> (2014)	4.4 (28/635)	2009–2010	Germany
Tenhagen <i>et al.</i> (2018)	9.7 (36/372)	2014	Germany
Virgin <i>et al.</i> (2009)	0.0 (0/542)	2007	United States
Visciano <i>et al.</i> (2014)	0.0 (0/30)	—	Italy
Vyletřlova <i>et al.</i> (2011)	2.8 (20/703)	—	Czech Republic, Slovakia

MRSA was defined as *mecA/mecC*-positive *S. aureus* strains.
BTM, bulk tank milk; MRSA, methicillin-resistant *S. aureus*.

TABLE 2. METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* PREVALENCE OF *STAPHYLOCOCCUS AUREUS* ISOLATES DETECTED IN INDIVIDUAL MILK SAMPLES FROM MORE THAN ONE FARM

References	Total No. of milk samples (n)	% MRSA of <i>S. aureus</i> isolates (n = MRSA/n = S. aureus)	Year(s) of collection	Country
Ahangari <i>et al.</i> (2017)	—	1.3 (1/75)	2014–2015	Iran
Aslantas and Demir (2016)	330	4.5 (5/112)	2008–2010	Turkey
Bao <i>et al.</i> (2016)	121	9.6 (5/52)	—	China
Bardiau <i>et al.</i> (2013)	—	4.4 (19/430)	2005–2008	Belgium
Bengtsson <i>et al.</i> (2009)	987	0 (0/211)	2002–2003	Sweden
Bervoets (2009)	—	0 (0/550)	—	Canada
Dan <i>et al.</i> (2018)	186	16.3 (16/98)	—	China
da Costa Krewer <i>et al.</i> (2015)	2064	0 (0/126)	—	Brazil
de Jong <i>et al.</i> (2018)	—	1.6 (3/192)	2009–2012	Europe
Gindonis <i>et al.</i> (2013)	—	1.5 (2/135)	2005–2006	Finland
Haenni <i>et al.</i> (2011)	—	0.7 (1/139)	2007–2008	France
Huber <i>et al.</i> (2010)	—	1.4 (2/142)	2009	Switzerland
Jamali <i>et al.</i> (2014)	207	11.6 (5/43)	2008–2010	Iran
Jamali <i>et al.</i> (2015)	1035	13 (21/162)	2006–2013	Iran
Kamal <i>et al.</i> (2013)	35	9.1 (3/33)	2011–2012	Egypt
Kumar <i>et al.</i> (2010)	185	7.8 (10/128)	2007–2008	India
Kwon <i>et al.</i> (2005)	9055	6.0 (15/248)	1999, 2000, 2003	Korea
Lee (2003)	894	1.3 (12/265)	2001–2003	Korea
Li <i>et al.</i> (2015)	214	0.8 (1/121)	—	China
Luini <i>et al.</i> (2015)	—	9.2 (15/163)	2006–2013	Italy
Mekonnen <i>et al.</i> (2018)	—	0 (0/79)	2014–2016	Ethiopia
Moon <i>et al.</i> (2007)	3047	1.6 (13/835)	1997–2004	Korea
Oliveira <i>et al.</i> (2016)	552	32.3 (21/65)	—	Brazil
Pu <i>et al.</i> (2014)	450	49.6 (49/103)	2008	China
Qu <i>et al.</i> (2018)	—	4 (15/96)	2014–2017	China
Riva <i>et al.</i> (2015)	383	20.0 (7/35)	2012	Italy
Rola <i>et al.</i> (2015)	115	0 (0/71)	2009–2013	Poland
Ronco <i>et al.</i> (2018)	—	1.6 (1/63)	2016	Denmark
Ruegg <i>et al.</i> (2015)	—	0 (0/35)	2010	United States
Saini <i>et al.</i> (2012b)	—	0.1 (1/1810)	—	Canada
Shrivastava <i>et al.</i> (2018)	400	23.0 (57/248)	—	India
Song <i>et al.</i> (2016)	649	13.9 (23/165)	2011–2012	Korea
Turkyilmaz <i>et al.</i> (2010)	—	17.2 (16/93)	2002–2006	Turkey
Unnerstad <i>et al.</i> (2013)	8757	0.8 (4/534)	2010–2011	Sweden
Vanderhaeghen <i>et al.</i> (2010)	—	9.3 (11/118)	2006–2007	Belgium
Vyletélóva <i>et al.</i> (2011)	724	1.7 (3/180)	—	Czech Republic, Slovakia

MRSA was defined as *mecA/mecC*-positive *S. aureus* strains.
MRSA, methicillin-resistant *S. aureus*.

TABLE 3. METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* PREVALENCE IN SINGLE DAIRY HERDS

References	% MRSA prevalence (n = MRSA/n = number of cows)	Year(s) of collection	Country
Hata (2016)	39.7 (31/78)	2005	Japan
Locatelli <i>et al.</i> (2017)	Farm A 4.8 (3/63), Farm B 60.0 (33/55)	2010	Italy
Magro <i>et al.</i> (2018)	12.5 (3/24)	—	Italy
Matyi <i>et al.</i> (2013)	5.3 (7/133)	—	United States
Falk (2018)	13.2 (139/1050)	2018	Israel
Schlotter <i>et al.</i> (2014)	28.6 (16/56)	2013	Germany
Silva <i>et al.</i> (2014)	11.0 (4/36)	—	Brazil
Spohr <i>et al.</i> (2011)	Farm A 7.5 (12/160), Farm B 16.7 (7/42), Farm C 5.1 (4/78)	2008	Germany
Unnerstad <i>et al.</i> (2018)	44 (11/25)	2012	Sweden

MRSA was defined as *mecA/mecC*-positive *S. aureus* strains.
MRSA, methicillin-resistant *S. aureus*.

2015; Cortimiglia *et al.*, 2016; Parisi *et al.*, 2016; Locatelli *et al.*, 2017; Ronco *et al.*, 2018). Studies from Brazil, China, and Israel also found LA-MRSA CC398 in mastitis milk samples (Silva *et al.*, 2014; Falk, 2018; Yi *et al.*, 2018). Furthermore, LA-MRSA CC398 was found in nasal swabs and in udder cleft swabs from dairy cows (Antoci *et al.*, 2013; Nemeghaire *et al.*, 2014; van Duijkeren *et al.*, 2014). The predominant LA-MRSA in Southeast Asia is multilocus sequence type 9 (ST9). It was also detected in milk samples (Wang *et al.*, 2012; Tenhagen *et al.*, 2018). In most studies, predominant MRSA strains were found within herds, suggesting a contagious transmission from cow to cow (Moon *et al.*, 2007; Holmes and Zadoks, 2011; Schlotter *et al.*, 2014; Luini *et al.*, 2015; Song *et al.*, 2016). However, a study from Italy reported a high heterogeneity of MRSA CC, *spa*-types, and genotypes within two dairy herds (Locatelli *et al.*, 2017). The authors concluded that the environment could act as a reservoir of these MRSA strains.

In 2011, a new *mecA* homolog (*mecA_{LGA251}*) was identified in isolates from milk samples that were phenotypically resistant to methicillin but tested negative for the *mecA* gene (Garcia-Alvarez *et al.*, 2011). This new *mecA* homolog is also known as *mecC* and is often carried by strains belonging to clonal complex 130 (CC130). Zoonotic transmission has been reported for *mecC*-CC130 MRSA (Harrison *et al.*, 2013). As of this writing, *mecC*-positive milk samples have been reported from Finland, the United Kingdom, Germany, and Sweden (Garcia-Alvarez *et al.*, 2011; Gindonis *et al.*, 2013; Unnerstad *et al.*, 2013; Paterson *et al.*, 2014; Schlotter *et al.*, 2014). In a review about MRSA in human and bovine mastitis, the authors additionally reported *mecC*-positive bovine *S. aureus* isolates from Portugal, Denmark, and France (Holmes and Zadoks, 2011). However, according to the authors, these findings had not been published and were based on personal communications.

Risk Factors for the Occurrence of MRSA in Dairy Herds

Improper milking hygiene

Proper milking hygiene and especially the use of post-milking teat disinfectants are important control strategies for *S. aureus* mastitis (Barkema *et al.*, 2006). In the past several decades, progressive use of milking hygiene procedures and other recommendations from the National Mastitis Council 5- and 10-point plan have led to a reduction in the prevalence of contagious mastitis pathogens in many countries (Makovec and Ruegg, 2003b; Barkema *et al.*, 2006; Ruegg, 2018).

A recent case study from Brazil reported a high MRSA prevalence (12.2%) in mastitis milk samples from one herd (Guimaraes *et al.*, 2017). The authors observed a lack of pre- and postdipping procedures, udder towels were used on more than one cow, and the use of gloves was inappropriate. On the farm with the highest overall MRSA prevalence (60%) in Italy, milkers were not using gloves (Locatelli *et al.*, 2017). In a study from Sicily, the milking hygiene score was negatively correlated with MRSA prevalence (Antoci *et al.*, 2013). The authors concluded that improper milking hygiene procedures may be a risk factor for MRSA transmission within dairy herds.

Contact with pigs

The most frequently detected bovine MRSA strain in Europe (CC398) was initially associated with pigs (Armand-

Lefevre *et al.*, 2005; Voss *et al.*, 2005; Huijsdens *et al.*, 2006). In all studies on LA-MRSA CC398 in farm animals, pigs were most frequently affected and prevalence rates were up to 89% (Porrero *et al.*, 2012; Abreu *et al.*, 2019). Thus, it was assumed that pigs may transfer MRSA to bovines. A recent study on 844 dairy herds from Italy has not found any association between the MRSA status and the presence of any other animal species on the same farm (Cortimiglia *et al.*, 2016). In contrast, two studies from the Netherlands have found that 64% (9/14) and 47% (28/60) of MRSA-positive farms harbored cows and pigs (Olde Riekerink *et al.*, 2009; Tavakol *et al.*, 2012). Another Italian study has reported that both the number of pigs and the number of pig herds close to the dairy farms were associated with the MRSA status (Locatelli *et al.*, 2016). The authors have not only reported CC398 but also CC97 MRSA strains. An Italian study which analyzed CC97 MRSA isolates from pigs and cattle reported that all strains were very similar and that the detected clone spreads among pig and dairy cattle holdings in Italy (Feltrin *et al.*, 2016). One MRSA-affected dairy farm from Germany also housed dairy cows and pigs. The same *spa*-type (t011) was found in the dairy cows and in the pig stall environment. The authors concluded that transmission might occur between the two livestock holdings (Spohr *et al.*, 2011). Therefore, certain MRSA strains, especially those of CC398, can probably spread between pigs and cows. Possible routes of transmission between the stables are dust (wind), rodents, people working with both species, and equipment used in both parts of the farm (van de Giessen *et al.*, 2009; Graveland *et al.*, 2010; Visciano *et al.*, 2014).

Humans carrying MRSA

Epidemiological investigations have suggested that sequence types of bovine and human *S. aureus* strains are usually different, and the risk of zoonotic and reverse zoonotic transmission is low (Holmes and Zadoks, 2011; Fitzgerald, 2012; Fluit, 2012). This seems to be different for MRSA, where the majority of isolates are considered LA-MRSA strains that infect or colonize both, humans and cattle.

Additionally, several reports of community and health care-associated MRSA (CA-/HA-MRSA) strains in dairy cows were published (Table 4). A case report from Australia has found a CA-MRSA strain (ST1, t127-IV), also known as WA-MRSA-1, in a milk sample of a subclinical mastitis case (Abraham *et al.*, 2017). According to the authors, WA-MRSA-1 is one of the most prevalent CA-MRSA strains circulating in Australia. Whole-genome sequencing has proved that both MRSA strains carried similar resistance and virulence genes. The authors concluded that transmission might have occurred from humans to the dairy cow. Unfortunately, the authors could not obtain samples from the farm personnel to confirm this hypothesis. Molecular analysis of human and bovine ST1-MRSA strains in Italy showed several human-associated genetic features in bovine isolates (Alba *et al.*, 2015). Other cases of CA-MRSA ST1, t127 in cattle, were reported from Germany, Italy, Switzerland, and Hungary (Juhasz-Kaszanyitzky *et al.*, 2007; Huber *et al.*, 2010; Pilla *et al.*, 2012; Tenhagen *et al.*, 2018). The authors from Italy assumed that humans were probably the source of infection, since the infected cow was kept on a closed farm (Pilla *et al.*, 2012). HA-MRSA was found in dairy cows in

TABLE 4. REPORTS OF COMMUNITY AND HEALTH CARE-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES IN SAMPLES FROM DAIRY COWS

References	MLST/spa-type/SCCmec type of MRSA	Year(s) of collection	Country
Abraham <i>et al.</i> (2017)	ST1/t127/IV	2015	Australia
Bardiau <i>et al.</i> (2013)	ST8/t008/IV	2005–2008	Belgium
Haenni <i>et al.</i> (2011)	ST5/t002/I	2007–2008	France
Haran <i>et al.</i> (2012)	ST8/t121/IVa, ST5/-/II	2009	United States
Hata <i>et al.</i> (2010)	ST5/t002/II, ST89/t5266/IIIa	1998–2005	Japan
Huber <i>et al.</i> (2010)	ST1/t127/IV	2009	Switzerland
Juhász-Kaszanyitzky <i>et al.</i> (2007)	ST1/t127/IV	2002–2004	Hungary
Luini <i>et al.</i> (2015)	ST1/t127/IV, ST8/t3092/V	2006–2013	Italy
Magro <i>et al.</i> (2018)	ST22/-/-	—	Italy
Monecke <i>et al.</i> (2007)	ST8/t068/-	—	Switzerland, Germany
Nam <i>et al.</i> (2011)	ST72/t324/IVa	2003–2009	Korea
Parisi <i>et al.</i> (2016)	ST1/t127/IVa, ST5/t688/V, ST8/-/IVa, V	2012–2013	Italy
Pilla <i>et al.</i> (2012)	ST1/t127/IV	—	Italy
Song <i>et al.</i> (2016)	-/t148/IVa	2011–2012	Korea
Tenhagen <i>et al.</i> (2018)	ST1/t127/-, ST22/t790/-	2014	Germany
Turkyilmaz <i>et al.</i> (2010)	ST239/t030/III, ST8/t190/IV	2002–2006	Turkey

MRSA was defined as *mecA/mecC*-positive *S. aureus* strains.

MLST, multilocus sequence typing; MRSA, methicillin-resistant *S. aureus*; SCCmec, staphylococcal cassette chromosome *mec*.

Germany (ST22), Japan (ST5), the United States (ST5), and Turkey (ST239) (Hata *et al.*, 2010; Turkeyilmaz *et al.*, 2010; Haran *et al.*, 2012; Tenhagen *et al.*, 2018). In Korea CA-MRSA (ST72, t324-IVa) and HA-MRSA (t148-IVa) were detected in milk samples (Nam *et al.*, 2011; Song *et al.*, 2016). A study from France reported the human-associated epidemic Geraldine-MRSA clone (ST5, t002-I) in a bovine milk sample (Haenni *et al.*, 2011). In conclusion, CA- and HA-MRSA may be transferred to dairy cows. In light of the increasing numbers of CA- and HA-MRSA isolates in samples from cattle, the relevance of reverse zoonotic MRSA transmission might be underestimated.

Production system

A study from the United States has reported that *S. aureus* isolates from organic farms were phenotypically more susceptible to antimicrobials than isolates from conventional farms (Tikofsky *et al.*, 2003). In contrast, a study from Denmark has not found a significant difference in susceptibility to penicillin between *S. aureus* isolates from organic and conventional farms (Bennedsgaard *et al.*, 2006).

Currently, only two MRSA (*mecA/mecC*) prevalence studies have differentiated between organic and conventional production systems. One study from the United States tested BTM from 192 organic and 100 conventional farms for the *mecA/mecC* gene (Cicconi-Hogan *et al.*, 2014). The authors only found one MRSA isolate in all farms and concluded that MRSA prevalence is low independent of the production system. The other study included 372 conventional and 303 organic BTM samples from Germany (Tenhagen *et al.*, 2018). The MRSA prevalence was lower in organic herds (1.7%) than in conventional herds (9.7%). Consequently, there is some evidence that cows from conventional farms are more likely to carry MRSA than cows from organic farms.

Herd size

Two studies reported positive correlations between herd size and MRSA prevalence. In Germany, the prevalence of

MRSA in BTM was higher on conventional farms with a larger herd size than on small farms (Tenhagen *et al.*, 2018). An Italian study found the highest *S. aureus* prevalence (68.5%) in BTM samples from Sondrio province, where farms are small (median value 20 animals) (Cortimiglia *et al.*, 2016). In contrast, the highest MRSA prevalences of 10.8% and 6.4% were reported from the provinces of Cremona and Lodi, where the median herd size was the highest in this study (325 and 278 cows/herd, respectively). In another Italian study, the average size of dairy herds tended to be positively correlated with MRSA status ($p=0.08$) (Locatelli *et al.*, 2016). On larger farms, more cows contribute to the BTM, increasing the likelihood of a positive BTM with a given cow level prevalence. Higher numbers of trading contacts and a higher use of third-generation cephalosporins may also contribute to a higher MRSA prevalence in BTM from large dairy herds (Saini *et al.*, 2012a). However, smaller farms are probably more likely to keep multiple animal species, including pigs. This is also considered a risk factor for the presence of MRSA in a dairy herd.

Methicillin-Resistant Coagulase-Negative-Staphylococci

Coagulase-negative staphylococci (CoNS) are a diverse group of predominantly opportunistic pathogens. In several studies, CoNS were the most frequently detected organisms from milk samples (Pitkälä *et al.*, 2004; Sampimon *et al.*, 2009; Tenhagen *et al.*, 2009; Oliveira *et al.*, 2016). Molecular studies suggest that CoNS carry fewer virulence genes than *S. aureus* and are therefore considered less pathogenic (Åvall-Jääskeläinen *et al.*, 2018). In China, 73% (82/112) of non-aureus staphylococci carried the *mecA* gene and MRSA prevalence was 4% (15/96) (Qu *et al.*, 2018). A study from the United States has reported 11 methicillin-resistant coagulase-negative staphylococci (MR-CoNS) in BTM from 288 farms and just 1 single MRSA isolate (Cicconi-Hogan *et al.*, 2014). In contrast, in 3047 mastitis milk samples from Korea, the authors reported 12 MR-CoNS and 13 MRSA

isolates (Moon *et al.*, 2007). This would be in line with the lower virulence of the MR-CoNS. The last VetPath study from Europe reported that 7 of 165 CoNS isolates from mastitis milk samples carried the *mecA* gene (4.2%) and 1.6% (3/192) of *S. aureus* isolates were classified as MRSA (de Jong *et al.*, 2018). In Finland, two studies reported that 5.2% (17/324) and 1.8% (2/110) of the CoNS isolates were *mecA* positive and MRSA prevalence among *S. aureus* isolates was 1.5% (2/135) (Gindonis *et al.*, 2013). A study from Portugal did not find MRSA but 9.3% (19/204) of mastitis milk samples were positive for MR-CoNS (Seixas *et al.*, 2014). In conclusion, MR-CoNS have been detected in MRSA affected dairy herds and the prevalence of methicillin resistance was generally higher than in *S. aureus*.

A study from Belgium reported that SCCmec types in bovine MR-CoNS ($n=101$) differed from those mostly detected in LA-MRSA CC398 (Vanderhaeghen *et al.*, 2013). The authors assumed that the SCCmec of MR-CoNS is probably not a reservoir of resistance determinants for LA-MRSA CC398. However, it is well known that resistance genes can be transferred between staphylococcal species (Morikawa *et al.*, 2012; Chlebowicz *et al.*, 2014; Ray *et al.*, 2016). The *in vivo* transfer of SCCmec was the most probable explanation for identical SCCmec in *S. aureus* and *Staphylococcus epidermidis* in an infected patient, although transmission could not be reproduced *in vitro* (Bloemendaal *et al.*, 2010). *In vitro*, the transfer of SCCmec was achieved through transformation (incorporation of DNA from the environment) (Morikawa *et al.*, 2012), through plasmids (Ray *et al.*, 2016), conjugation (sexual transfer) (Tsubakishita *et al.*, 2010), and transduction (bacteriophage transfer) (Chlebowicz *et al.*, 2014). All these studies were performed under laboratory conditions. To the best of our knowledge, it remains unclear which mechanism(s) of SCCmec transfer occur *in vivo*. In conclusion, MR-CoNS could act as a reservoir of resistance genes that may be transferred to MSSA in dairy cows. The role of SCCmec transfer for the development of new MRSA strains needs to be further investigated.

The Amount of Antibiotics Used on Dairy Farms

The use of antibiotics is associated with the development of antibiotic resistance (Chantziaras *et al.*, 2014). Every time bacteria are exposed to antimicrobial agents, selection pressure will cause antibiotic resistance to increase (Lam *et al.*, 2014). A meta-analysis reported a significant association between antimicrobial exposure and the number of MRSA isolates in humans (Tacconelli *et al.*, 2008).

For dairy cows, mastitis is the leading cause of antibiotic treatment. Blanket DCT with long-acting β -lactam antimicrobials, especially cloxacillin, is still commonly applied to prevent and cure intramammary *S. aureus* infections (Oliver *et al.*, 2011; Saini *et al.*, 2012a; Oliveira *et al.*, 2016). Therefore, it is hypothesized that the large-scale use of β -lactams in dairy cows is a possible risk factor for the selection of new MRSA strains (Saini *et al.*, 2012c).

A study from Germany found a lower MRSA prevalence in organic herds (1.7%) than in conventional herds (9.7%) (Tenhagen *et al.*, 2018). Organic farmers are considered to use fewer antibiotics. In a study from the Netherlands, veal calves were more often MRSA carriers when treated with antibiotics (Graveland *et al.*, 2010).

Unfortunately, most studies that included the amount of antibiotics used on dairy farms only performed phenotypic resistance testing and did not detect the *mecA/mecC* gene. This matters, since phenotypic testing was shown to lead to false-negative (Pu *et al.*, 2014; Guimaraes *et al.*, 2017) and false-positive results in previous studies (Cicconi-Hogan *et al.*, 2014; da Costa Krewer *et al.*, 2015; Li *et al.*, 2015; de Jong *et al.*, 2018; Wang *et al.*, 2018). In Thailand, milk samples from 78 cows on 18 farms were tested for phenotypic oxacillin resistance (Suriyasathaporn *et al.*, 2012). The authors reported higher numbers of methicillin resistant staphylococci on farms with high antibiotic use (21%) than on farms with normal use of antibiotics (5.9%). High antibiotic use was defined as more than two treatment periods per cow per year and normal use as no more than two treatment periods per cow per year. One study from Canada has found a positive correlation between intramammary and systematically administered penicillin treatments and phenotypic penicillin resistance in 89 dairy herds (Saini *et al.*, 2012c).

A study from the United States included 2778 mastitis isolates for phenotypic antibiotic susceptibility testing over a 6-year period, from 1994 to 2000. The proportion of isolates, which were phenotypically susceptible to β -lactam antimicrobials, did not change during the period (Erskine *et al.*, 2002). Another study from the United States has not reported a higher proportion of *S. aureus* isolates that were phenotypically resistant to any antimicrobial drug (Makovec and Ruegg, 2003a). In a literature review about the impact of antibiotic use in dairy cows on antimicrobial resistance, the authors concluded that there is no evidence for increasing resistance rates due to antibiotic treatment (Oliver *et al.*, 2011).

In conclusion, there is an ongoing debate about the role of antimicrobial exposure as a risk factor for the occurrence of MRSA in dairy cows. It was suggested that antimicrobial resistance is low in milk because the total number of bacteria in the udder is low in comparison to the intestinal tract, skin, or mucous membranes. For this reason, resistance levels through intramammary treatment might be lower than in other parts of the body after oral or parenteral application of antibiotics (Lam *et al.*, 2014).

Association of MRSA with a High Somatic Cell Count in Milk

The somatic cell count is the number of cells present in milk (cells/mL). Beside some epithelial cells, the majority of somatic cells are cells from the immune system (Harmon, 1994). Therefore, a higher somatic cell count is considered a reflection of an inflammatory response in the mammary gland. The most reliable somatic cell count cutoff value for mastitis detection is between 200,000 and 250,000 cells/mL (Laevens *et al.*, 1997; Schepers *et al.*, 1997; Schukken, 2007).

A German study has reported that quarters harboring MRSA had a higher somatic cell count than other quarters (Spohr *et al.*, 2011). In a case report about MRSA in a Brazilian dairy herd, the bulk milk somatic cell count was 628,000 cells/mL (Guimaraes *et al.*, 2017). In Sicily, a negative correlation between somatic cell count and MRSA status in BTM from 45 dairy farms was reported (Antoci *et al.*, 2013). A study from Italy detected higher somatic cell counts ($286,000 \pm 212,000$ cells/mL) in BTM from MRSA-affected farms in comparison to farms with negative test

results ($236,000 \pm 231,000$ cells/mL) (Locatelli *et al.*, 2016). However, this difference was not significant ($p=0.38$). Two Italian studies sampled milk from MRSA-infected cow(s) continuously over the entire lactation. The somatic cell count in MRSA-infected quarters fluctuated between 300,000 and 6,000,000 cells/mL in one study and between 1000 and 1,800,000 cells/mL in the other study (Pilla *et al.*, 2012; Magro *et al.*, 2018). In one study, the authors reported that fluctuation was not related to the shedding of MRSA (Pilla *et al.*, 2012). A Swedish case study reported somatic cell counts between 12,000 and 2,885,000 cells/mL in MRSA-positive milk samples (Unnerstad *et al.*, 2018). In China, 5 MRSA isolates have been reported among 121 quarter milk samples. All 5 MRSA were isolated from clinically healthy cows with a somatic cell count $<300,000$ cells/mL (Bao *et al.*, 2016). In a case report from Japan, the authors have reported a low bulk tank somatic cell count of 114,000 cells/mL in a MRSA-affected herd (Hata, 2016). The somatic cell count in a German dairy herd with high MRSA prevalence was even lower with 51,600 cells/mL (Schlotter *et al.*, 2014). Thus, a higher somatic cell count in milk is probably not a reliable indicator for the occurrence of MRSA in dairy herds.

Additional Risk Factors for Udder Infections Caused by *S. aureus* in Dairy Cows That Have Not Been Addressed in Studies on MRSA

Some studies have suggested that older cows are more likely to be *S. aureus* infected (Pyörälä and Pyörälä, 1998; Barkema *et al.*, 2006). Moreover, a study found higher rates of phenotypic penicillin resistance in animals from the third and following lactations, than in animals from the first and second lactation (Sol *et al.*, 2000). In addition, a larger mammary gland size was shown to be predisposing for *S. aureus* infections and hind quarters were more frequently affected (Deluyker *et al.*, 2005). Furthermore, it has been known that purchasing infected replacement heifers and people that have visited many farms per day (e.g., veterinarians, artificial insemination technicians, and cattle traders) might introduce new *S. aureus* strains into dairy herds (Middleton *et al.*, 2002). Moreover, some studies have found multiple different *S. aureus* strains within dairy herds, suggesting that in some cases *S. aureus* might be regarded as a sporadic environmental pathogen (Sommerhäuser *et al.*, 2003; Zadoks *et al.*, 2011). *S. aureus* has been detected in environmental samples, such as, flies, bedding materials, and feedstuff (Roberson *et al.*, 1998; Capurro *et al.*, 2010; Zadoks *et al.*, 2011). Further studies are needed to confirm these findings for MRSA in dairy farms.

Conclusion

The risk factors for the transmission of MRSA into dairy herds are direct or indirect contact with pigs and humans carrying MRSA. Within dairy herds, MR-CoNS may transfer resistance genes to MSSA. Moreover, improper milking hygiene procedures enhance the spread of MRSA within herds as is well known for MSSA. There is some evidence that conventional dairy farms and farms with a larger herd size are more often affected by MRSA. The association of antimicrobial exposure and MRSA prevalence in dairy herds needs to be further investigated. High amounts of β -lactam antibiotics have been used for dry cow treatment and mastitis

therapy on dairy farms. Nevertheless, MRSA prevalence is low in dairy cows. Furthermore, it is not known whether additional risk factors for *S. aureus* transmission in dairy herds differ from those of MRSA. According to our findings, a higher somatic cell count in milk is probably not a reliable indicator for the occurrence of MRSA in dairy herds.

The risk of foodborne zoonotic MRSA infections through consumption of milk seems to be low. Milk is usually heat treated before marketing and consumption and MRSA prevalence is low in milk from dairy cows. However, MRSA prevalence should be carefully monitored, since some studies suggest increasing levels of resistance.

In veterinary medicine, MRSA emerge as mastitis pathogens in dairy cows and spread within herds. Dry cow treatment with β -lactam antibiotics, as an important part of *S. aureus* control programs, is probably ineffective in curing MRSA infections. Therefore, segregation and culling of infected cows often remains the only option for removing MRSA from dairy herds. In conclusion, we stress the need for a continuous MRSA monitoring in dairy herds and the development of MRSA prevention strategies.

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