PEER REVIEWED

A review of methicillin-resistant *Staphylococcus aureus* (MRSA) in dairy cattle

J. Pinto Ferreira, *DVM, MS, PhD;* **M. T. Correa,** *MS, PhD;* **R. Lyman,** *BA;*
K. L. Anderson, *DVM, PhD, DABVP*
Department of Population Health & Pathobiology, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606
Corresponding author: Dr. K. L. Anderson, Tel.: 919-513-6245; fax: 919-513-6464;
E-mail address: kevin_anderson@ncsu.edu

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged globally as a significant public health/antimicrobial resistance nosocomial problem, both in human and veterinary medicine. In recent years, the so-called livestock-associated MRSA has become an additional focus. A literature review indicates few reports of MRSA in dairy cattle. Work from our laboratory supports previous studies indicating that MRSA is rare in milk of dairy cattle in the United States. Recent and sporadic isolation of MRSA and related staphylococci from cattle in countries other than the United States, and the similarity between some of the human and animal isolates found, provide rationale for monitoring MRSA occurrence in cattle. Considering the importance of *S. aureus* in human infectious disease, its highly contagious behavior among dairy cows, and the current gaps in knowledge about potential human-bovine connections, the epidemiology of MRSA (and other staphylococci) should represent an area of attention by the scientific community.

Key words: dairy, milk, *Staphylococcus aureus*, antibiotic resistance, MRSA

Résumé

Le *Staphylococcus aureus* résistant à la méthicilline (SARM) est devenu globalement un problème nosocomial de santé publique et de résistance antimicrobienne autant en médecine humaine que vétérinaire. Depuis les dernières années, le SARM relié au bétail est devenu un autre sujet d'attention. Une revue de littérature a rapporté peu de cas de SARM chez les bovins laitiers. Des travaux de notre laboratoire supportent les conclusions d'autres études qui indiquent que le SARM est rare dans le lait des vaches laitières des États-Unis. L'isolement récent et sporadique de SARM et autres staphylocoques chez du bétail provenant d'autres pays que les États-Unis de même que la similarité entre certains isolats humains et animaux peuvent justifier la surveillance de SARM chez le bétail. Compte tenu de l'importance de *S. aureus* dans les maladies infectieuses humaines, de son haut degré de contagion chez les vaches laitières, et du manque de connaissance sur la connexion potentielle entre les animaux et les humains, l'épidémiologie de SARM (et autres staphylocoques) devrait représenter un sujet d'intérêt pour la communauté scientifique.

Introduction

Domestication of food animals and the development of antimicrobials can be considered among the most significant achievements of agriculture history. However, the development of antimicrobial resistance, both in humans and animals, might lead to a new era in animal agriculture. Driven largely by human population growth, income growth, and urbanization,[46] concerns have risen about the expected increased need for animal protein at the global level. The correct strategies to achieve this level of production are unclear at this point. Methicillin-resistant *Staphylococcus aureus* (MRSA) and other resistant bacteria are important concerns to consumers of animal food products. MRSA has been described as a nosocomial agent, as a community-associated pathogen, and in recent years, as livestock-associated MRSA (LA-MRSA); other designations include non-typable (NT)-MRSA or MRSA Sequence Type (ST) or Clonal Complex (CC) 398.

The presumptive overuse of antimicrobials has been repeatedly emphasized as the main selection pressure for bacterial resistant strains.[43] According to the US Food and Drug Administration, 90,922 lb (41,328 kg) of cephalosporin's active ingredient and 1,343,131 lb (610,514 kg) of penicillins were sold for food animal industry usage in the US in 2009.[15] Antibiotic therapeutical treatments are a common practice on dairy

farms around the world.[17] However, the situation in dairy cattle challenges the straightforward, simplistic idea that the use of antimicrobials is the sole cause for the development of multi-resistant strains. Despite use of antimicrobials, most reports on MRSA prevalence on dairy farms found no MRSA or very low values (Table 1). At the same time, studies have found no major significant difference in the prevalence of resistant strains between traditional and organic dairy farms.[56]

Mastitis is one of the main reasons for use of antibiotics in the dairy industry, and constitutes its most important cause of economic loss.[9] Of the wide variety of pathogens isolated as causative agents of mastitis, *S. aureus* remains a common and economically significant cause. Although *S. aureus* is recognized as a major contagious mastitis agent worldwide, this does not seem to be true for MRSA (Table 1). Some strains of *S. aureus* produce enterotoxins that are associated with food poisoning,[1] and there is increasing concern about the antibiotic resistance of *S. aureus,* specifically to methicillin or β-lactam antibiotics (i.e. MRSA).

Although hyperproduction of β-lactamase has been suggested as the resistance mechanism,[9] methicillin resistance in *S. aureus* most commonly results from the production of the novel penicillin-binding protein (PBP)-2a, which has a decreased binding affinity for β-lactam antibiotics. PBP-2a requires two to 10 times higher penicillin concentrations for inactivation than PBP-2, and 20 times higher than PBP-1.[28] PBP-2a production is encoded by the chromosomal gene *mec*A found on a large mobile genetic element called the staphylococcal chromosomal cassette mec (SCCmec). There are six major SCC*mec* types (I, II, III, IV, V, and VI) and several subtypes, based on the combination of the cassette chromosome recombinase (ccr) gene complexes and *mec*A regulatory genes, *mec*I and *mec*RI.[60] The presence of the *mec*A gene in MRSA is the specific molecular characteristic that differentiates MRSA from methicillin susceptible *Staphylococcus aureus*, also known as MSSA. *Staphylococcus fleuretti*, a recent putative bacterial species and commensal *Staphylococcus* species of animals, has been reported as the highly probable origin of the *mec*A gene.[47]

The first MRSA occurrence was reported in England by Jevons in 1961[26] soon after the introduction of beta-lactamase resistant penicillins in human medicine (1959). In 1972 a cow in Belgium with mastitis was the first reported MRSA infection in an animal.[12] Despite this historical background, there are many unknowns concerning "MRSA dairy epidemiology" and the potential transmission of MRSA between animals and humans. Moreover, the risk factors involved in trans-infection and the direction of transmission between cattle and humans are not clearly understood.

This article presents an overview of the literature about MRSA in dairy cattle and the potential connections between bovine and human isolates. Suggestions for future research in this area are included.

MRSA Prevalence in Milk, Dairy Products, and Cows

Publications about MRSA and the dairy industry are scarce, especially when compared with literature reports related to MRSA and the swine industry.[54] A search on Pubmed with the words "MRSA" and "dairy" and "cattle" provides only 53 references (accessed September 15, 2011). In Table 1, results of this search and related literature regarding the isolation of MRSA in milk and other dairy food samples are summarized. For this review, we focused on papers published since 2000 independently of the study design. Of the 21 studies, only two were done in the United States.

MRSA Prevalence in Milk Analyzed in the Authors' Laboratory

In 2006 we analyzed 357 *S. aureus* isolates recovered from milk samples submitted to our laboratory for diagnostic bacteriologic testing from 24 dairy herds in North Carolina (NC) and Virginia (see sidebar). Our

Laboratory Methods for Determination of MRSA at NCSU

S. aureus identification was performed in accordance with routine laboratory techniques, including typical colony morphology, Gram's stain, catalase and coagulase tests. DNA extraction was performed according to the UltraClean™ Microbial DNA Isolation Kit instruction manual, with the exception of the initial step. *S. aureus* were plated on Trypticase Soy Agar (TSA) and incubated for 18 hours at 96.8 to 98.6°F (36 to 37°C). A loop-full of this culture was scraped into a 2 mL collection tube, to which 400 µL of MicroBead solution[b] and 20 µL of lysostaphin solution (1 mg/mL) were added. This mixture was incubated at 98.6°F (37°C) for two hours. Polymerase chain reaction (PCR) amplification of the *mec*A gene was performed as described by Lee.[34] Antibiotic resistance pattern (ampicillin, cephalothin, erythromycin, cefoxitin, novobiocin, penicillin G, ceftiofur, sulfamethoxazole trimethropim, streptomycin, tetracycline, sulfisoxazole and pirlimycin) was determined according to Clinical and Laboratory Standards Institute (CLSI) guidelines. According to these guidelines, an isolate is classified as susceptible to cefoxitin, the antibiotic used to test "methicillin-resistance", if the inhibition zone is ≥ 22 mm.

Table 1. Reports of MRSA in bovine milk, mastitis samples, and other dairy products.

Publication year	Ref. no.	Comments
2000	9	De Oliveira *et al* determined the minimum inhibitory concentrations (MIC) for 811 strains of *S. aureus* isolated from cases of bovine mastitis in 11 countries (Denmark, England, Finland, Germany, Iceland, Ireland, Norway, Sweden, Switzerland, United States, and Zimbabwe). Only 12 strains could be phenotypically classified as MRSA, but they were all *mec*A negative. The MIC determinations were performed by a broth microdilution method that adhered to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS). MIC_{90} for oxacillin was 1.0µg/mL.
2003	33	Lee *et al* found an isolation percentage of 1.34% when analyzing 894 milk samples collected from 2001 to 2003 in the Republic of Korea. The susceptibilities of all *mec*A (PCR) positive MRSA isolates were tested by the disk agar method as standardized by the NCCLS. The MICs of oxacillin were also examined according to NCCLS recommendations. Most of the MRSA isolates were random amplified polymorphic DNA (RAPD) type II.
2003	19	Guerin-Faublee *et al* analyzed 119 isolates of *S. aureus* collected between 1998 and 2000 in France from cows with clinical mastitis. For strains with an oxacillin MIC greater than 2 mg/L the *mec*A was identified by PCR. No MRSA was found.
2004	13	Farzana *et al* analyzed 50 raw milk samples collected in 1992 in Pakistan. *S. aureus* was present in all the samples. Resistance to oxacillin and methicillin was assessed by disk diffusion (Bauer-Kirby) test. 10% of the isolates (eight of 77) were methicillin-resistant. Paper does not specify the resistance criteria used. No further molecular work is described.
2005	32	Kwon *et al* found an isolation rate of 0.18% of MRSA in 9,055 milk samples with more than 500,000 somatic cells/mL collected in 1999, 2000, and 2003 in the Republic of Korea. MICs of oxacillin were detected with a microdilution test of the NCCLS for the *S. aureus* isolates. All MRSA isolates harboured SCC*mec* type IV, revealed the same PFGE profile, and showed Sequence Type (ST) 5 with an allelic profile of 1-4-1-4-12-1-10.
2006	49	Turutoglu *et al* analyzed 103 *S. aureus* isolates from milk samples collected from cases of mastitis in herds in Turkey from 2002 to 2004. 18 of the isolates were phenotypically resistant to methicillin (17.5%), using disk diffusion method according to the NCCLS.
2007	39	Nunes *et al* determined the antibiotic susceptibility of 30 isolates of *S. aureus* responsible for subclinical bovine mastitis in Portugal. No MRSA was found, by broth microdilution, following the Clinical and Laboratory Standards Institute (CLSI) standards.
2007	35	Monecke *et al* identified two MRSA from 128 *S. aureus* isolates from cows in Germany and Switzerland based on the expression of the *mec*A gene on a DNA microarray. One isolate was *agr*-type I, CC8 and *spa*-type t068. The other was *agr*-type I, ST398 and t034.
2007	37, 38	Normano *et al* analyzed 437 raw milk, 702 heat-treated milk, 1,578 cheese, 87 curd, 194 ricotta cheese, 350 ice cream, and 349 other dairy products collected between 2003 and 2005 in Italy. The (PCR) *mec*A positive strains were tested for susceptibility using the disk agar diffusion method following the NCCLS guidelines. The isolation rate of MRSA was of 0.16%.
2007	36	Moon *et al* analyzed 3,047 bovine mastitic milk samples from 153 dairy farms in the Republic of Korea, collected from 1997 to 2004. 21 (2.5% of 840) *S. aureus* and 19 (2.4%) coagulase-negative staphylococci were resistant to methicillin. Methicillin resistance was screened by disk diffusion test with an oxacillin disk and confirmed by MIC test with oxacillin. Phenotypic methicillin resistance was defined with an oxacillin MIC $\geq$ 4 µg/mL. 70% of the isolates belonged to four predominant coagulase genotypes (I, II, VII and VIII).
2008	57	Wang *et al* analyzed 72 bovine *S. aureus* isolates obtained from 12 dairy farms in inner Mongolia of China and found no MRSA considering a CLSI oxacillin MIC breakpoint standard of $\geq$4 µg/mL.
2008	18	Graveland *et al* explored the spread of MRSA in veal calf production in the Netherlands, and found surprisingly high prevalences: 32% of the farmers, 8% of the family members, and 28% of the calves were MRSA positive. The presence of the *mec*A gene was confirmed by PCR; a random selection of the *mec*A-positive colonies was confirmed to be MRSA by PCR of the *S. aureus* specific DNA-fragment Martineau. In total 16 different *spa* types were identified; nine different *spa* types in human isolates and 12 different types in veal calves. In calves, the predominant *spa* type found was t011 (80%).

Table 1 continued. Reports of MRSA in bovine milk, mastitis samples, and other dairy products.

Publication year	Ref. no.	Comments
2009	8	Cui *et al* could not find MRSA from 276 cattle nasal swabs and 47 cattle workers collected on four Chinese provinces. Antimicrobial susceptibility was determined via broth microdilution and interpreted according to the CLSI interpretive standards.
2009	55	Studies have also been done using bulk-tank milk samples such as the study by Virgin *et al* where the herd MRSA prevalence in US dairy herds was estimated. In this study, bulk-tank milk samples (n=542) were tested and no positive MRSA was found. To detect MRSA, phenotypic and genotypic methods were used. Phenotypic detection was based on plating on a selective indicator medium, BBL CHROMagar MRSA and, in a parallel assy, on trypticase soy agar with 5% sheep blood and 0.1% esculin (TSA-BE). Genotypic detection was based on PCR using specific *nuc* and *mec*A genes specific primers.
2010	53	Vanderghaeghen *et al* found that nearly 10% of the *S. aureus* isolated from bovine subclinical and clinical mastitis (118 isolates from 118 different farms in Belgium collected from 2006 to 2007) in their study were MRSA, a much higher prevalence than previously published. This suggests that about 10% of the Belgian farms with *S. aureus* mastitis have a MRSA problem. A triplex PCR, targeting a *Staphylococcus*-specific 16S rRNA sequence, the *mec*A gene, and the *S. aureus*-specific region of the thermonuclease gene (nuc) was performed to identify MRSA. Strains proven to be MRSA were tested for susceptibility to non-β-lactam antimicrobial agents by using the disk diffusion method. Results were interpreted according to CLSI guidelines. Strains were ST398, *spa* types t011 or t567, and had SCC*mec*-type Iva or V.
2010	45	Spohr *et al* analyzed the occurrence of MRSA in three dairy farms in Germany with a history of clinical and subclinical MRSA mastitis. Herds were tested twice. In the first investigation, the range of the proportion of positive MRSA cows was 5.1 to 16.7%, and in the second one 1.4 to 10.0%. MRSA was isolated from the noses of four out of seven calves in this study. All *S. aureus* strains from milk samples were tested for antimicrobial susceptibility using disk diffusion according to German national guidelines for veterinary medicine (www.dvg.net). Isolates that were resistant against penicillin G, ampicillin, cloxacillin, cefoperazone, cefquinome, and cephalexine were assumed to be MRSA. All isolates that were suspected to be MRSA were confirmed using an RT-PCR. Confirmation of MRSA-DNA was performed using melting-curve analysis. All MRSA isolates from milk belonged to *spa*-type t011 and SCC*mec*-type V.
2010	24	100 samples of bulk-tank milk and 200 samples of raw-milk cheese were tested in Switzerland. No MRSA was found. *S. aureus* and MRSA diagnosis were confirmed by species-specific 23S rDNA and *mec*A PCR. Phenotypic properties were tested using disk diffusion method, interpreted according to the CLSI guidelines. Etest was additionally used for cefoxitin and oxacillin resistance testing.
2010	20	139 non-duplicate *S. aureus* isolates from bovine mastitis collected in France between 2007 and 2008 were analyzed by Haenni *et al*. One isolate was classified as MRSA. Identification was performed using a triplex PCR targeting 16SrRNA, *mec*A, and *S. aureus*-specific *nuc* genes. Antimicrobial susceptibility was tested by the disc diffusion method on Mueller-Hilton agar and interpreted according to the breakpoints recommended by the Antibiogram Committee of the French Society of Microbiology (www.sfm.asso.fr). MRSA isolate was identical to the human epidemic Geraldine clone, ST5, *spa*-type t002.
2011	4	Prevalence of methicillin resistant *Staphylococci* was established in the animals and staff of a teaching and research farm in Brazil. Nasal swab samples were collected from healthy dairy cattle (n=36) and humans (n=13). Detection of *mec*A gene was performed by PCR. Antimicrobial resistance of *mec*A+ isolates was determined by disk-diffusion method (Kirby-Bauer) according with the CLSI breakpoints. No MRSA was isolated.
2011	22	1.3% (two isolates) was the MRSA prevalence found by Haran *et al* in bulk milk tanks in Minnesota. One isolate was ST5-USA100-spa type 2 (traditionally reported as HA-MRSA) and the other ST8-USA300-t121 (traditionally reported as CA-MRSA).
2011	31	Kumar *et al* found a 13.1% MRSA prevalence when analyzing 107 strains of *S. aureus* from 195 mastitic milk samples in India. Disk diffusion method on Mueller-Hinton agar according to CLSI guidelines was used to determine antibiotic susceptibility profile and molecular confirmation with PCR of 16S rDNA, *nuc*, and *mec*A genes.

laboratory found that 86% (308 of 357) of *S. aureus* isolates were susceptible to all antimicrobials tested and no MRSA was found.[3] In order to screen for the presence of MRSA in NC dairies over time, we analyzed *S. aureus* isolates from our collection of about 3,800 bovine *S. aureus* isolates. Of these, 125 *S. aureus* isolates had been collected from bulk tanks from 19 NC dairies and were further tested. Represented samples were collected over a 13-year period from 1997 to 2009, and the isolates were frozen (at -103°F or -75°C) for variable periods of time in 15% glycerol. No MRSA were isolated from milk or bulk tanks representing a cross-section of dairy herds in NC, and all the isolates were phenotypically susceptible to cefoxitin. The *mec*A gene could not be detected and amplified in any of the PCR reactions.

Analysis and Discussion of MRSA in Dairy Cattle Publications

Most manuscripts reviewed were prevalence reports or case-series studies. For the most part, samples and isolates had not been collected with the specific goal of determining MRSA prevalence, but rather were convenience samples.

Until recently, most reports indicated that MRSA was an agent of minor importance and low prevalence in dairy samples (Table 1). However, comparing isolation rates of different studies is difficult since there is no standardization of sampling and diagnostic methods. In fact, there is not even agreement upon the definitions of CA and LA-MRSA.[30] Our bulk-tank *S. aureus* results agree with those of Virgin *et al.*[55] Both suggest that the prevalence of MRSA on dairies in the US is very low or nonexistent. The *S. aureus* isolates in our investigations had been collected during a 13-year time period, which suggests prevalence has not changed over time. One of the main limitations of our study was that the samples in our database were not randomly collected and potentially do not represent true prevalence. Also, bulk-tank milk samples were used, which might have resulted in a significant number of false negatives, as single bulk-tank culture sensitivity has been reported as not being higher than 60%.[40]

The study by van Griethuysen *et al*[51] raises the concern of the potential loss of the *mec*A gene during storage of MRSA isolates. They reported that the *mec*A gene was lost in 36 (14.4%) of 250 MRSA isolates after two years of storage at -112°F (-80°C) with the Microbank system.[a] In our study, *S. aureus* isolates were not tested for the presence of the *mec*A gene before storage, so we cannot predict the influence of freezing isolates.

Despite the fact that dairy cows have been treated with penicillin for decades,[25] the relatively low resistance rates observed among *S. aureus* isolates from bovine mastitis[3] may be explained by the limited possibility of acquiring resistance genes in a virtually sterile compartment, such as the udder, where no indigenous bacterial flora is present.[58] However, recent reports of increased MRSA prevalence in milk samples from countries like Germany, Belgium, Netherlands or India[18,45,53] are intriguing. The differences in prevalence between the European and American studies might be explained by the different agricultural systems: more traditional, family based farms with multiple animal species are found in Europe (facilitating the transfer of genetic material between species[45]), as opposed to the US where there are larger and more industrial herds. Cattle turnover is higher in the US than in Europe, where cows are traditionally kept longer. Also, national surveys are being conducted in Europe, particularly in the Netherlands in the swine industry, which might lead to the finding of increased prevalence.

A recent finding in the United Kingdom provides another potential explanation for the differences in prevalence presented in Table 1. A novel *mec*A homologue was isolated (and named *mec*A_{LGA251}) from bulk milk.[16] This divergent *mec*A gene was also found in human samples from Scotland, England, and Denmark.[16] It is only 70% identical to *S. aureus mec*A homologues, meaning that routine diagnostic procedures will phenotypically identify these isolates as methicillin-resistant, *but* will fail to confirm the diagnosis at the molecular level.[16] At this point, it seems prudent, especially when analyzing dairy samples, to test them first at the phenotypic level and then run the PCR confirmation methods with primers able to amplify this novel *mec*A_{LGA251}.

Different antimicrobial national policies and regulations could also explain the different prevalence rates. To make possible an accurate comparison of antimicrobial policies, national public health institutions, both human and veterinary, should annually collect data on pounds or kilograms of active antimicrobial agents used by species, route of administration, and specific purpose of use, such as therapeutic, prophylaxis, or growth promotion. Grave *et al*[17] recently compared the sales of veterinary antibacterial agents between 10 European countries and found that 48% of sales of veterinary antibacterial agents were tetracyclines. The authors found a wide variation (8.2-85.5 mg/lb or 18-188 mg/kg – mg of antibacterial drug sold/kg of biomass of slaughtered food animal plus estimated live weight of live dairy cattle) in the usage between countries, and concluded that the difference could not be explained only by differences in the animal species demographics. Speculative explanations include different animal husbandry practices, pharmaceutical drug availability in the market or veterinary prescription habits.[17]

MSSA, CNS, MRSP, and MRSA: Is There a Link?

Considering that the horizontal transfer of genetic material[10] between bacteria has been claimed to be the most significant way for the spread of antibiotic resistance,[59] it is important to analyze the published literature for potential connections between MRSA, MSSA, and other staphylococci. The relative importance of MRSA as a pathological agent differs between human and veterinary medicine. While MRSA has been recognized as responsible for more human deaths in the US than other infectious diseases, including HIV/AIDS,[29] methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), also a coagulase-positive staphylococcus, is of major concern to veterinarians.

S. aureus is considered a major contagious mastitis agent in dairy cattle, but a variety of coagulase-negative staphylococci (CNS) are considered important minor mastitis agents. Importantly, CNS have been suggested as a source of SCCmec in the farming environment,[60] and the transfer of SCCmec from CNS to *S. aureus* could change sensitive into resistant strains,[41,60] i.e., MRSA.

Two recent human reports suggest the highly probable transfer of methicillin resistance between *Staphylococcus haemolyticus* (a CNS) and *S. aureus*. *S. haemolyticus* was the probable donor of the SCCmec element to *S. aureus* that led to a MRSA outbreak in a neonatal intensive care unit in Sweden.[5] Chlebowicz *et al*[7] reported the *in vivo* conversion of MRSA ST-398 to MSSA during a "community-acquired" human infection in which a mother and her daughter suffered from pneumonia and umbilicus phlegmon, respectively.

At this point, the potential exchange of genetic material between CNS and *S. aureus* can speculatively be considered as one of the reasons for the increased prevalence of MRSA reported in some recent publications.

Human and Cattle Interspecies MRSA Transmission

Juhasz-Kasaznayitzky *et al*[27] reported that MRSA (ST1) were isolated from cows with subclinical mastitis and from a person who worked with these animals. The bovine and human isolates were undistinguishable by phenotyping and genotyping methods, possibly representing the first documented case of direct transmission of MRSA between cows and humans. The direction of transmission could not be determined.[27] More recently, other cases of cattle-human interspecies MRSA transmission have been reported. In the study by Haenni *et al*[20] in France (Table 1), the MRSA isolate identified had identical characteristics to the human Geraldine clone (ST5, *spa*-type t002, and the same virulence genes, resistance pattern, and SCC*mec* cassette type I). In Italy, a recent case of MRSA ST398 necrotizing fasciitis in a dairy farmer might represent another case of MRSA cattle-to-human transmission.[44] This study lacks information about MRSA colonization of animals on the farm, and this assumption is only based on the absence of other risk factors.[44]

Risk Factors

From a prevention standpoint, it is important to know which factors increase the risk of MRSA carriage in people and animals. This knowledge could be essential for development of appropriate biosecurity measures and public or agricultural policies applied to farm or hospital environments. Hanselman *et al*[21] isolated MRSA from 27 of 417 (6.5%) attendees at the Annual American College of Veterinary Internal Medicine Conference held in Baltimore, Maryland in 2005. In this study, colonization was more common for large-animal (15 of 96 or 15.6%) personnel than for small-animal personnel (12 of 271 or 4.4%). Employment in large animal veterinary practice versus academic or general practice was, in fact, the only variable significantly associated with colonization, with an odds ratio (OR) close to 3. It is important to highlight that the attendees at this meeting may be more representative of referral environments, and therefore may not be representative of field environments. In a study by Van Loo *et al*[52] performed in the Netherlands, contact with cattle had an OR of 20, indicating that those who have contact with cattle are 20 times more likely to be infected with NT-MRSA than the typable-MRSA controls (using PFGE). These studies suggest that colonization or infection with MRSA might be an occupational hazard for dairy farmers and veterinarians.[21]

Direction of Transmission

"Who infects whom?" is a question that still represents a challenge for the scientific community. In 1975, when current molecular epidemiological tools were unavailable, Devriese[11] suggested a human origin for the 68 MRSA isolates from Belgian dairy herds. Lee,[33] considering the high prevalence (over 50%) of MRSA among human *S. aureus* isolates in Korea and the low prevalence of MRSA in animals (including cattle), suggested that animal isolates may have transferred from humans to animals. Turkyilmaz *et al*[48] analyzed 16 isolates of *S. aureus* recovered from mastitic bovine milk in Turkey. Fourteen of the 16 isolates were classified as ST239-SCC*mec* type III, a lineage that is associated with hospital associated clones which seems to suggest a human origin of the bovine isolates, transferred initially from the hospital. A human to animal transfer of MRSA was also suggested by results of the study by Haenni *et al*.[20]

Nearly 35 years have passed between the Devriese *et al*[11] and Haenni *et al*[20] studies, and both suggest

the same conclusion that the origin of bovine MRSA isolates is human. However, direction of infection is still unknown.

Comparison of Human and Bovine MRSA Isolates

Using various molecular tools, other authors have studied the similarity between human and bovine isolates. Feßler *et al*[14] investigated the genetic relationship of 25 MRSA ST-398 isolates from bovine mastitis (collected from 17 dairy farms in Germany) and two isolates from farm personnel. The two human isolates were indistinguishable genotypically (ApaI PFGE, *spa* typing, SCC*mec* typing, and direct repeat unit (*dru*) typing) and phenotypically (broth microdilution antimicrobial resistance pattern) from mastitis isolates from the same farm.[14] Hata *et al*[23] first reported and analyzed four bovine milk MRSA isolates obtained in Japan between May 1998 and May 2005 and evaluated their relationship with nine human MRSA isolates, where three of the bovine isolates showed identical genotypes to the human isolates.

Brodly *et al*[6] reported that the human MRSA252 strain uniquely shares multiple DNA sequence blocks with three different etiological agents of contagious bovine mastitis, including *S. aureus*, but not with other human isolates. Turutoglu *et al*[50] sequenced the *mec*A genes of three MRSA isolated from bovine mastitis cases and found a very high homology with human MRSA isolates; all three bovine *mec*A genes were identical to those found in human MRSA isolates, except for a one-base substitution at nucleotide position 757. In addition to isolating MRSA from individual cows, calves, and bulk-tank samples (Table 1), Spohr and co-workers[45] isolated MRSA from nasal and oropharyngeal swabs taken from herd workers on seven of nine farms tested. All isolates were the same *spa* type, *spa*-type t011.

Considering that dairy cows are food producing animals and as such have limited contact with humans compared to companion animals, the similarity between human and bovine isolates is somewhat surprising. The literature seems to suggest that food (milk and other dairy products) may not be the most likely bridge between dairy cattle and humans. The role of wildlife[43] and the importance of environmental contamination and airborne transmission of MRSA between farms and neighboring residential buildings is currently unknown.[42] Alvarado *et al*[2] evaluated the concentration and seasonal variation of airborne fungi and antibiotic resistant bacteria in a dairy farm in the southwestern US. *S. aureus* was the predominant bacteria present, and more than half of the *S. aureus* found were resistant to one or more antibiotics. Currently, without an explanation for the similarity or no similarity of bovine and human MRSA isolates, there appears to be a need for further MRSA surveillance in animals.

Conclusions

Despite an interesting historical background, MRSA references associated with dairy literature are scarce. Considering the small number of MRSA isolates found in the dairy industry, MRSA in dairy products seems to be a minor consumer or public health concern. The common use of pasteurization and the low levels of MRSA found in raw milk should also be seen as a reason for little concern.[37,45] Recent and sporadic isolation of MRSA and related staphylococci from cattle in countries other than the US, and the similarity between some of the human and animal isolates found, provide rationale for monitoring MRSA occurrence in cattle.

Considering the importance of *S. aureus* as a human infectious disease agent, its highly contagious typical behavior among dairy cows, and the current gaps in knowledge about the potential human-bovine connections, the epidemiology of MRSA (and other staphylococcal species) in the dairy industry should represent a future area of attention by the scientific community.

Future collaborative research should include longitudinal studies, addressing the persistence of MRSA colonization in humans and cattle and exploring all the potential sources and reservoirs. The legal framework that regulates these types of collaborations must be made more reasonable and encouraging for researchers. International organizations should provide guidelines for the ideal uniformization of methods and protocols. Molecular characterization of MRSA isolates (SCC*mec* typing, *spa* typing, *dru* typing *mec*A PCR, MLST, PFGE) is needed to enable tracking of the path of dissemination of MRSA. Cost-benefit analysis addressing surveillance and antimicrobial policy analysis should complement the epidemiological studies.

Endnotes

[a]Pro-lab Diagnostics, Austin, TX
[b]Mo Bio Laboratories, Inc, 2746 Loker Ave West, Carlsbad, CA

Conflict of Interest

No conflict of interest is recognized.

Acknowledgements

This work was supported in part by funds from the College of Veterinary Medicine, North Carolina State University, when the first author was in the Department of Population Health and Pathobiology.

References

1. Akineden O, Annemuller C, Hassan AA, Lammler C, Wolter W, Zschock M. Toxin genes and other characteristics of *Staphylococcus aureus* isolates from milk of cows with mastitis. *Clin Diagn Lab Immunol* 2001;8:959-964.
2. Alvarado CS, Gandara A, Flores C, Perez HR, Green CF, Hurd WW, Gibbs SG. Seasonal changes in airborne fungi and bacteria at a dairy cattle concentrated animal feeding operation in the southwest United States. *J Environ Health* 2009;71:40-44.
3. Anderson KL, Lyman RL, Bodeis-Jones SM, White DG. Genetic diversity and antimicrobial susceptibility profiles among mastitis-causing *Staphylococcus aureus* isolated from bovine milk samples. *Am J Vet Res* 2006;67:1185-1191.
4. Aquino GD, Maluta RP, de Avila FA. Prevalence of methicillin-resistant staphylococci on a farm: staff can harbour MRS when animals do not. *Zoonoses Public Health* 2012;59:1-3.
5. Berglund C, Soderquist B. The origin of a methicillin-resistant *Staphylococcus aureus* isolate at a neonatal ward in Sweden-possible horizontal transfer of a staphylococcal cassette chromosome mec between methicillin-resistant *Staphylococcus haemolyticus* and *Staphylococcus aureus*. *Clin Microbiol Infect* 2008;14:1048-1056.
6. Brody T, Yavatkar AS, Lin Y, Ross J, Kuzin A, Kundu M, Fann Y, Odenwald WF. Horizontal gene transfers link a human MRSA pathogen to contagious bovine mastitis bacteria. *PLoS One* 2008;3:e3074.
7. Chlebowicz MA, Nganou K, Kozytska S, Arends JP, Engelmann S, Grundmann H, Ohlsen K, van Dijl JM, Buist G. Recombination between ccrC genes in a type V (5C2&5) staphylococcal cassette chromosome mec (SCCmec) of *Staphylococcus aureus* ST398 leads to conversion from methicillin resistance to methicillin susceptibility in vivo. *Antimicrob Agents Chemother* 2010;54:783-791.
8. Cui S, Li J, Hu C, Jin S, Li F, Guo Y, Ran L, Ma Y. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from swine and workers in China. *J Antimicrob Chemother* 2009;64:680-683.
9. De Oliveira AP, Watts JL, Salmon SA, Aarestrup FM. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Europe and the United States. *J Dairy Sci* 2000;83:855-862.
10. de Vries LE, Christensen H, Skov RL, Aarestrup FM, Agerso Y. Diversity of the tetracycline resistance gene tet(M) and identification of Tn916- and Tn5801-like (Tn6014) transposons in *Staphylococcus aureus* from humans and animals. *J Antimicrob Chemother* 2009;64:490-500.
11. Devriese LA, Hommez J. Epidemiology of methicillin-resistant *Staphylococcus aureus* in dairy herds. *Res Vet Sci* 1975;19:23-27.
12. Devriese LA, Van Damme LR, Fameree L. Methicillin (cloxacillin)-resistant *Staphylococcus aureus* strains isolated from bovine mastitis cases. *Zentralbl Veterinarmed B* 1972;19:598-605.
13. Farzana K, Shah SNH, Jabeen F. Antibiotic resistance pattern against various isolates of *Staphylococcus aureus* from raw milk samples. *Journal of Research (Science), Bahauddin Zakariya University, Multan, Pakistan* 2004;15:145-152.
14. Feßler A, Scott C, Kadlec K, Ehricht R, Monecke S, Schwarz S. Characterization of methicillin-resistant *Staphylococcus aureus* ST398 from cases of bovine mastitis. *J Antimicrob Chemother* 2010;65:619-625.
15. Food and Drug Administration, Department of Health and Human Services. Summary report on antimicrobials sold or distributed for use in food-producing animals. 2009.
16. Garcia-Alvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK, Kearns AM, Pichon B, Hill RL, Larsen AR, Skov RL, Peacock SJ, Maskell DJ, Holmes MA. Meticillin-resistant *Staphylococcus aureus* with a novel *mec*A homologue in human and bovine populations in the UK and Denmark: a descriptive study. *Lancet Infect Dis* 2011;11:595-603.
17. Grave K, Torren-Edo J, Mackay D. Comparison of the sales of veterinary antibacterial agents between 10 European countries. *J Antimicrob Chemother* 2010;65:2037-2040.
18. Graveland H, Wagenaar JA, Heesterbeek H, Mevius D, van Duijkeren E, Heederik D. Methicillin resistant *Staphylococcus aureus* ST398 in veal calf farming: human MRSA carriage related with animal antimicrobial usage and farm hygiene. *PLoS One* 2010;5:e10990.
19. Guerin-Faublee V, Carret G, Houffschmitt P. In vitro activity of 10 antimicrobial agents against bacteria isolated from cows with clinical mastitis. *Vet Rec* 2003;152:466-471.
20. Haenni M, Galofaro L, Ponsin C, Bes M, Laurent F, Madec JY. Staphylococcal bovine mastitis in France: enterotoxins, resistance and the human geraldine methicillin-resistant *Staphylococcus aureus* clone. *J Antimicrob Chemother* 2011;66:216-218.
21. Hanselman BA, Kruth SA, Rousseau J, Low DE, Willey BM, McGeer A, Weese JS. Methicillin-resistant *Staphylococcus aureus* colonization in veterinary personnel. *Emerg Infect Dis* 2006;12:1933-1938.
22. Haran P, Godden S, Boxrud D, Jawahir S, Bender J, Sreevatsan S. Characterization of methicillin-resistant *Staphylococcus aureus* from bulk-tank milk from MN dairy farms. Proc 2nd ASM-ESCMID Conference on Methicillin-Resistant Staphylococci in Animals: Veterinary and Public Health Implications. September 2011; p. 35.
23. Hata E, Katsuda K, Kobayashi H, Uchida I, Tanaka K, Eguchi M. Genetic variation among *Staphylococcus aureus* strains from bovine milk and their relevance to methicillin-resistant isolates from humans. *J Clin Microbiol* 2010;48:2130-2139.
24. Huber H, Koller S, Giezendanner N, Stephan R, Zweifel C. Prevalence and characteristics of meticillin-resistant *Staphylococcus aureus* in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland, 2009. *Euro Surveill* 2010;15:1-4.
25. Hunter P, Dawson S, French G, Goossens H, Hawkey P, Kuijper E, Nathwani D, Taylor D, Teale C, Warren R, Wilcox M, Woodford N, Wulf M, Piddock LJV. Antimicrobial-resistant pathogens in animals and man: prescribing, practices, and policies. *J Antimicrob Chemother* 2010;65 Suppl 1:i3-i7.
26. Jevons MP. "Celbenin"-resistant staphylococci. *Br Med J* 1961;1:124.2.
27. Juhasz-Kaszanyitzky E, Janosi S, Somogyi P, Dan A, Graaf-van Bloois Lvd, Duijkeren Ev, Wagenaar JA. MRSA transmission between cows and humans. *Emerg Infect Dis* 2007;13:630-632.
28. Kaszanyitzky E, Jnosi S, Egyed Z, Agost G, Semjn G. Antibiotic resistance of staphylococci from humans, food and different animal species according to data of the Hungarian resistance monitoring system in 2001. *Acta Vet Hung* 2003;51:451-464.
29. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM, Craig AS, Zell ER, Fosheim GE, McDougal LK, Carey RB, Fridkin SK. Active bacterial core surveillance (ABCs) MRSA investigators. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *J Am Med Assoc* 2007;298:1763-1771.
30. Kock R, Becker K, Cookson B, van Gemert-Pijnen J, Harbarth S, Kluytmans J, Mielke M, Peters G, Skov R, Struelens M, Tacconelli E, Navarro Torne A, Witte W, Friedrich A. Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe. *Euro Surveill* 2010;15:1-9.
31. Kumar R, Yadav BR, Singh RS. Antibiotic resistance and pathogenicity factors in *Staphylococcus aureus* isolated from mastitic Sahiwal cattle. *J Biosci* 2011;36:175-188.
32. Kwon NH, Park KT, Moon JS, Jung WK, Kim SH, Kim JM, Hong SK, Koo HC, Joo YS, Park YH. Staphylococcal cassette chromosome mec (SCCmec) characterization and molecular analysis for methicillin-resistant *Staphylococcus aureus* and novel SCCmec subtype IVg isolated from bovine milk in Korea. *J Antimicrob Chemother* 2005;56:624-632.
33. Lee JH. Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. *Appl Environ Microbiol* 2003;69:6489-6494.
34. Lee JH. Occurrence of methicillin-resistant *Staphylococcus aureus* strains from cattle and chicken, and analyses of their *mec*A, *mec*R1 and *mec*I genes. *Vet Microbiol* 2006;114:155-159.

35. Monecke S, Kuhnert P, Hotzel H, Slickers P, Ehricht R. Microarray based study on virulence-associated genes and resistance determinants of *Staphylococcus aureus* isolates from cattle. *Vet Microbiol* 2007;125:128-140.
36. Moon JS, Lee AR, Kang HM, Lee ES, Kim MN, Paik YH, Park YH, Joo YS, Koo HC. Phenotypic and genetic antibiogram of methicillin-resistant staphylococci isolated from bovine mastitis in Korea. *J Dairy Sci* 2007;90:1176-1185.
37. Normanno G, Corrente M, Salandra Gl, Dambrosio A, Quaglia NC, Parisi A, Greco G, Bellacicco AL, Virgilio S, Celano GV. Methicillin-resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product in Italy. *Int J Food Microbiol* 2007;117:219-222.
38. Normanno G, Firinu A, Virgilio S, Mula G, Dambrosio A, Poggiu A, Decastelli L, Mioni R, Scuota S, Bolzoni G, Di Giannatale E, Salinetti AP, La Salandra G, Bartoli M, Zuccon F, Pirino T, Sias S, Parisi A, Quaglia NC, Celano GV. Coagulase-positive staphylococci and *Staphylococcus aureus* in food products marketed in Italy. *Int J Food Microbiol* 2005;98:73-79.
39. Nunes SF, Bexiga R, Cavaco LM, Vilela CL. Technical note: antimicrobial susceptibility of Portuguese isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis* in subclinical bovine mastitis. *J Dairy Sci* 2007;90:3242-3246.
40. Radostitis OM, Gay CC, Hinchcliff KW, Constable PD. In: *Veterinary medicine--A textbook of the disease of cattle, horses, sheep, pigs and goats*. 10th ed. New York: Saunders Elsevier, 2007;700.
41. Robinson DA, Enright MC. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2003;47:3926-3934.
42. Smith TC, Moritz ED, Leedom Larson KR, Ferguson DD. The environment as a factor in methicillin-resistant *Staphylococcus aureus* transmission. *Rev Environ Health* 2010;25:121-134.
43. Smith TC, Pearson N. The emergence of *Staphylococcus aureus* ST398. *Vector Borne Zoonotic Dis* 2011;11:327-339.
44. Soavi L, Stellini R, Signorini L, Antonini B, Pedroni P, Zanetti L, Milanesi B, Pantosti A, Matteelli A, Pan A, Carosi G. Methicillin-resistant *Staphylococcus aureus* ST398, Italy. *Emerg Infect Dis* 2010;16:346-348.
45. Spohr M, Rau J, Friedrich A, Klittich G, Fetsch A, Guerra B, Hammerl JA, Tenhagen BA. Methicillin-resistant *Staphylococcus aureus* (MRSA) in three dairy herds in southwest Germany. *Zoonoses Public Health* 2011;58:252-261.
46. Thornton PK. Livestock production: recent trends, future prospects. *Philosophical Transactions of the Royal Society Biological Sciences* 2010;365:2853-2867.
47. Tsubakishita S, Kuwahara-Arai K, Sasaki T, Hiramatsu K. The origin and molecular evolution of the determinant of methicillin-resistance in staphylococci. *Antimicrob Agents Chemother* 2010;54:4352-4359.
48. Turkyilmaz S, Tekbiyik S, Oryasin E, Bozdogan B. Molecular epidemiology and antimicrobial resistance mechanisms of methicillin-resistant *Staphylococcus aureus* isolated from bovine milk. *Zoonoses Public Health* 2010;57:197-203.
49. Turutoglu H, Ercelik S, Ozturk D. Antibiotic resistance of *Staphylococcus aureus* and coagulase-negative staphylococci isolated from bovine mastitis. *Bulletin of the Veterinary Research Institute in Pulawy* 2006;50:41-45.
50. Turutoglu H, Hasoksuz M, Ozturk D, Yildirim M, Sagnak S. Methicillin and aminoglycoside resistance in *Staphylococcus aureus* isolates from bovine mastitis and sequence analysis of their *mec*A genes. *Vet Res Commun* 2009;33:945-956.
51. van Griethuysen A, van Loo I, van Belkum A, Vandenbroucke-Grauls C, Wannet W, van Keulen P, Kluytmans J. Loss of the *mec*A gene during storage of methicillin-resistant *Staphylococcus aureus* strains. *J Clin Microbiol* 2005;43:1361-1365.
52. van Loo I, Huijsdens X, Tiemersma E, de Neeling A, van de Sande-Bruinsma N, Beaujean D, Voss A, Kluytmans J. Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. *Emerg Infect Dis* 2007;13:1834-1839.
53. Vanderhaeghen W, Cerpentier T, Adriaensen C, Vicca J, Hermans K, Butaye P. Methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 associated with clinical and subclinical mastitis in Belgian cows. *Vet Microbiol* 2010;144:166-171.
54. Vanderhaeghen W, Hermans K, Haesebrouck F, Butaye P. Methicillin-resistant *Staphylococcus aureus* (MRSA) in food production animals. *Epidemiol Infect* 2010;138:606-625.
55. Virgin JE, Van Slyke TM, Lombard JE, Zadoks RN. Short communication: methicillin-resistant *Staphylococcus aureus* detection in US bulk tank milk. *J Dairy Sci* 2009;92:4988-4991.
56. Walther C, Perreten V. Methicillin-resistant *Staphylococcus epidermidis* in organic milk production. *J Dairy Sci* 2007;90:5351.
57. Wang Y, Wu CM, Lu LM, Ren GW, Cao XY, Shen JZ. Macrolide-lincosamide-resistant phenotypes and genotypes of *Staphylococcus aureus* isolated from bovine clinical mastitis. *Vet Microbiol* 2008;130:118-125.
58. Werckenthin C, Cardoso M, Martel JL, Schwarz S. Antimicrobial resistance in staphylococci from animals with particular reference to bovine *Staphylococcus aureus*, porcine *Staphylococcus hyicus*, and canine *Staphylococcus intermedius*. *Vet Res* 2001;32:341-362.
59. Zaneveld JR, Nemergut DR, Knight R. Are all horizontal gene transfers created equal? prospects for mechanism-based studies of HGT patterns. *Microbiol* 2008;154(Pt 1):1-15.
60. Zhang Y, Agidi S, LeJeune JT. Diversity of staphylococcal cassette chromosome in coagulase-negative staphylococci from animal sources. *J Appl Microbiol* 2009;107:1375-1383.