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■ Prevalence of methicillin-resistant *Staphylococcus aureus* in milk and dairy products

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■ Παρουσία ανθεκτικών στη μεθικιλίνη *Staphylococcus aureus* σε γάλα και γαλακτοκομικά προϊόντα

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ABSTRACT. *Staphylococcus aureus* is an opportunistic Gram positive pathogen and the causative agent of many human and animal diseases. It is also an important human foodborne pathogen. Certain strains of *S. aureus* can produce staphylococcal enterotoxins (SEs) in foods and cause staphylococcal food poisonings (SFP). In recent years *S. aureus* has been increasingly associated with antibiotic resistance. Methicillin-resistant *S. aureus* (MRSA) includes those strains that have acquired genes conferring resistance to methicillin and essentially all other beta-lactam antibiotics. MRSA was initially reported as a nosocomial pathogen in human hospitals (or hospital-associated MRSA, HA-MRSA). Since the 1990s, community-acquired or community-associated MRSA (CA-MRSA) infections have also been reported to affect people having no epidemiological connection with hospitals. More recently, MRSA has been isolated from most food-producing animals and foods of animal origin, raising public health concerns. MRSA strains have been isolated from cows' or small ruminants' milk and various dairy products in many countries. The MRSA prevalence in milk and dairy products recorded in different countries or even regions of the same country differs significantly. High MRSA prevalence have been recorded in milk produced in most African countries, for instance as high as 60.3% in Ethiopia. The MRSA prevalence in Asian countries varies from high e.g. 28.3% in Iran to low (e.g. in Korea and Japan). In most European countries, the MRSA prevalence in milk and dairy products has been generally found to be low. In the US and Canada, zero to low MRSA prevalence estimates have been reported. The investigation of MRSA prevalence in milk may serve as a tool for assessing both the sanitary conditions employed in dairy herds and the health risks that humans may encounter when infected with antibiotic-resistant strains.

Keywords: dairy products, methicillin-resistant *Staphylococcus aureus*, milk, MRSA, prevalence

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ΠΕΡΙΛΗΨΗ. Ο *Staphylococcus aureus* είναι ένα θετικό κατά Gram, ευκαιριακά παθογόνο βακτήριο, που μπορεί να προσβάλλει τους ανθρώπους και τα ζώα. Είναι επίσης και σημαντικό τροφιμογενές παθογόνο βακτήριο καθώς ορισμένα στελέχη του *S. aureus* μπορούν να παράγουν σταφυλοκοκκικές εντεροτοξίνες στα τρόφιμα και να προκαλέσουν σταφυλοκοκκική τοξίνωση. Τα τελευταία χρόνια παρατηρείται ολοένα αυξανόμενη παρουσία στελεχών *S. aureus* ανθεκτικών στα ευρέως χρησιμοποιούμενα αντιβιοτικά. Στους ανθεκτικούς στη μεθικιλίνη *S. aureus* (methicillin resistant *S. aureus*, MRSA) ανήκουν στελέχη τα οποία, μέσω λήψης συγκεκριμένων γονιδίων, έχουν αναπτύξει ανοχή στη μεθικιλίνη και σε όλα τα β-λακταμικά αντιβιοτικά. Ο MRSA αρχικά χαρακτηριζόταν ως ένα ενδονοσοκομειακό παθογόνο βακτήριο (hospital-associated MRSA, HA-MRSA). Από το 1990 όμως άρχισαν να καταγράφονται και περιστατικά σταφυλοκοκκικών λοιμώξεων από MRSA σε ανθρώπους που δεν είχαν καμία επιδημιολογική σύνδεση με νοσοκομεία (community-acquired or community-associated MRSA, CA-MRSA). Πιο πρόσφατα, έχει περιγραφεί απομόνωση MRSA στελεχών από τα περισσότερα παραγωγικά ζώα καθώς και από τρόφιμα ζωικής προέλευσης, αυξάνοντας τις ανησυχίες για τη δημόσια υγεία. Στελέχη MRSA έχουν απομονωθεί από αγελαδινό, πρόβειο ή γίδινο γάλα και διάφορα γαλακτοκομικά προϊόντα σε πολλές χώρες. Η παρουσία του MRSA στο γάλα και τα γαλακτοκομικά προϊόντα που καταγράφεται σε διαφορετικές χώρες ή ακόμη και στις διάφορες περιοχές της ίδιας χώρας, διαφέρει σημαντικά. Υψηλά ποσοστά παρουσίας του MRSA καταγράφονται στο γάλα που παράγεται στις περισσότερες αφρικανικές χώρες, φτάνοντας και το 60,3%, ποσοστό που καταγράφηκε στην Αιθιοπία. Στις ασιατικές χώρες η συχνότητα της παρουσίας του MRSA ποικίλλει από υψηλά ποσοστά (π.χ. 28,3% στο Ιράν), έως και χαμηλά ποσοστά (π.χ. στην Κορέα και την Ιαπωνία). Στις περισσότερες ευρωπαϊκές χώρες, στελέχη MRSA σε γάλα και γαλακτοκομικά προϊόντα έχουν βρεθεί γενικά σε χαμηλά ποσοστά. Σύμφωνα με έρευνες που πραγματοποιήθηκαν στις ΗΠΑ και τον Καναδά, έχουν καταγραφεί μηδενικά έως χαμηλά ποσοστά παρουσίας του MRSA. Η διερεύνηση της παρουσίας του MRSA στο γάλα μπορεί να χρησιμεύσει για την αξιολόγηση τόσο των συνθηκών υγιεινής που εφαρμόζονται στις εκτροφές των ζώων γαλακτοπαραγωγής, αλλά και των κινδύνων για την υγεία που μπορεί να αντιμετωπίσουν οι καταναλωτές ύστερα από λοιμώξεις με στελέχη ανθεκτικά στα αντιβιοτικά.

Λέξεις ευρετηρίασης: ανθεκτικός στη μεθικιλίνη *Staphylococcus aureus*, γάλα, γαλακτοκομικά προϊόντα, MRSA, παρουσία

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium, widely prevalent in nature. It can be isolated from many environmental sites (e.g., dust, water, air, faeces) and is part of the normal bacterial flora of mammals. Approximately 20 to 60% of humans are permanent or intermittent carriers of *S. aureus* and relevant sites include the anterior nares, axillae, perineum and vagina (Duquette and Nuttall, 2004; Leonard and Markey, 2008; Kluytmans, 2010).

Apart from the widespread prevalence in healthy people, *S. aureus* is also a very dangerous opportunistic pathogen. It is a very common cause of human skin and soft tissue infections and the most common causative agent of hospital-acquired infections (Waldvogel, 1990). Clinical signs range from minor skin conditions (e.g., pimples, boils and impetigo) to severe disease, such as cellulitis and post-operative wound infections. In humans, *S. aureus* can also cause pneumonia, bacteraemia, meningitis, sepsis and pericarditis (Horan et al., 1988).

S. aureus is also an important food-borne pathogen. Staphylococcal food poisoning is caused by ingestion of food containing one or more preformed enterotoxins (SEs) produced by *S. aureus*. Staphylococcal food poisoning ranks third among reported food-borne

diseases in the world (Boerema et al., 2006). In 2006, *S. aureus* toxins were responsible for 49% of 482 human food-borne outbreaks caused by bacterial toxins and 4% of all reported outbreaks reported by EU Member States (EFSA, 2007). Symptoms have a rapid onset and include nausea, vomiting and diarrhoea (Jablonski and Bohach, 1997).

There are five classical enterotoxins (SEA, SEB, SEC, SED and SEE), six new types of enterotoxins (SEG, SEH, SEI, SER, SES, SET) and ten staphylococcal-like (SeI, designated as SE/I to SE/IV) proteins. It is known that about 95% of staphylococcal food poisoning cases are caused by the classical enterotoxin types (Bergdoll, 1983). The remaining 5% of outbreaks may therefore be associated with other enterotoxins (Bergdoll and Wong, 2006). SEH have clearly been involved in food poisoning outbreaks (Pereira et al., 1996; Jorgensen et al., 2005; Ikeda et al., 2005), whereas SEG and SEI (Omoe et al., 2002) and SER, SES, and SET (Ono et al., 2008) were proved to be more or less emetic, with a possible incidence in food safety. TSST-1, the toxic shock staphylococcal toxin, initially designated as SEF, lacks emetic activity (Argudín et al., 2010). TSST-1 causes toxic shock syndrome (TSS), a potentially fatal condition. The symptoms include high fever, rash, desquamation

of skin one to two weeks after onset, hypotension and failure of multiple organs (Shands et al., 1980; Dinges et al., 2000).

S. aureus can also cause severe animal diseases, such as suppurative disease, arthritis and urinary tract infections (Lowy, 1998). The pathogen is also a frequent causative agent of clinical or subclinical mastitis in small ruminants (Contreras et al., 2007; Petridis et al., 2012) and cattles (Asperger and Zangerl, 2003). Presence of *S. aureus* on the skin and mucosae of food-producing animals, such as ruminants, and the frequent association of the pathogen with mastitis, often leads to contamination of milk (Jablonski and Bohach, 1997). Contamination of milk can also occur from environmental sources during handling and processing (Peles et al., 2007). Milk is a good substrate for *S. aureus* growth and dairy products are common sources of staphylococcal food-poisoning (Scherrer et al., 2004; Morandi et al., 2007).

In recent years, there has been increased concern about antibiotic resistant strains of *S. aureus*. Development of resistance has been attributed to the extensive therapeutic use of antimicrobials or to their administration as growth promoters in food animal production (Normanno et al., 2007). Isolates of *S. aureus* are frequently resistant to methicillin and essentially all other β -lactam antibiotics. An organism with this type of resistance is referred to as methicillin-resistant *S. aureus* (MRSA) (Lee, 2003). MRSA infections are more difficult to treat with standard antibiotics and thus more dangerous. MRSA strains were first isolated in 1961, the year in which methicillin was licenced (Jevons, 1961) and has since emerged as a serious concern in human medicine. MRSA was initially reported as a nosocomial pathogen in human hospitals (hospital-associated MRSA) and was isolated from patients with compromised immune systems undergoing medical procedures. MRSA accounts for 30 to 40% of all hospital-acquired infections and for 40% to 70% of *S. aureus* infections in intensive care units (Stefani and Varaldo, 2003; Gordon and Lowy, 2008).

In the 1990s, a major change in the epidemiology of MRSA has been observed, with the appearance of cases affecting people with no epidemiological connection to hospitals; strains that cause such infections are referred to as community-acquired or community-associated MRSA (EFSA, 2009). Until recently, such strains were susceptible to many antibiotics other than β -lactams; however, resistance seems to be increasing,

and multiple antibiotic resistant strains have started to emerge (Tenover and Goering, 2009; Boucher et al., 2010; Otter and French, 2010).

In 1972, the first reported MRSA infection was observed in a cow with mastitis in Belgium (Devriese et al., 1972). Since then, pigs (Voss et al., 2005; Huijsdens et al., 2006; de Neeling et al., 2009; Broek van den et al., 2008; Khanna et al., 2008), horses (Witte et al., 2007; Cuny et al., 2008), poultry (Persoons et al., 2009) and calves (Mooij et al., 2007) have been identified as new reservoirs for MRSA.

There is now increasing concern about the public health impact of MRSA associated with food-producing animals, because MRSA and, consequently, their resistance genes can spread from animals to humans by direct contact or through the food chain (Kluytmans, 2010). MRSA have been isolated from most food-producing animals and foods of animal origin (EFSA, 2009). MRSA strains have been isolated in many countries from cows' or small ruminants' milk and various dairy products (Vyletřlova et al., 2001, Juhasz-Kaszanyitzky et al., 2007; Normanno et al., 2007; Turutoglu et al. 2006, Ateba et al, 2010; Hata et al., 2010; Spanu et al., 2010; Nam et al., 2011; Ünal et al., 2012).

Objective of this review article is to provide an overview of the published data on the prevalence of MRSA in milk and dairy products worldwide.

CHARACTERISTICS OF MRSA

Antibiotic resistance

The term 'MRSA' is used to describe strains of *S. aureus* resistant to semi-synthetic, penicillinase-resistant, β -lactams such as methicillin, oxacillin or cloxacillin. MRSA strains are resistant to all cephalosporins, cephems and other β -lactams, such as ampicillin-sulbactam, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, piperacillin-tazobactam and the carbapenems. This group of organisms is also frequently resistant to most of the commonly used antimicrobial agents, including the aminoglycosides, macrolides, chloramphenicol, tetracycline and fluoroquinolones (Lee, 2003).

β -lactam antibiotics damage bacteria by inactivating penicillin-binding proteins, enzymes that are essential in the assembly of the bacterial cell wall (Pinho et al., 2001). These antibiotics inactivate the four native penicillin-binding proteins found in staphylococci. As a result of the weakened cell wall, treated bacteria

become osmotically fragile and are easily lysed. The staphylococcal β -lactamase protein, which cleaves the β -lactam ring structure, confers resistance to penicillin, but not to semi-synthetic penicillins. In MRSA, resistance to all β -lactam antibiotics, including the semi-synthetic penicillins, is conferred by the penicillin-binding protein PBP2' (or PBP2a) that has a very low affinity for β -lactam antibiotics and is thought to aid cell wall assembly when normal penicillin-binding proteins are inactivated (Hartman and Tomasz, 1984). PBP2a is encoded by the *mecA* gene, which is located in the staphylococcal cassette chromosome (*SCCmec*) (Katayama et al., 2000). The confirmation of the presence of the *mecA* gene, has until recently been the 'golden standard' for detection of MRSA worldwide. However, a novel *mecA* homologue (with approximately 70% similarity to the *mecA* gene) that also confers methicillin resistance was identified in *S. aureus* isolates from dairy cattle and humans. This gene, previously denoted as *mecALGA251*, has been designated *mecC* (García-Álvarez et al., 2011; EFSA, 2012; Laurent et al., 2012; Petersen et al., 2012). Additional genes, which are also found in susceptible isolates, can affect the methicillin resistance phenotype in *S. aureus*, resulting in heterogeneity of resistance and making detection of resistance difficult (Montanari et al., 1990; de Lencastre and Tomasz 1994). Some strains of *S. aureus* possess an alternative resistance mechanism, attributable to the hyper-production of the *S. aureus* β -lactamase enzyme, which inactivates the antibiotic agents by hydrolysing the β -lactam ring of penicillin and cephalosporin compounds (Brown et al., 2005). Vancomycin was the only antibiotic available for treating MRSA infections. However, vancomycin-resistant MRSA strains, including some community acquired-MRSA strains, have increasingly been reported, thereby causing public health concern (Wenzel, 1982; CLSI, 2006; Tenover and Goering, 2009).

Identification and typing of MRSA

MRSA can be identified using phenotypic (antimicrobial susceptibility testing) or genotypic methods. In general, the phenotypic methods are easier to perform, easier to interpret, cost-effective and widely available; however they are less discriminatory. The genotypic methods are more discriminatory, but are expensive and technically demanding (Mehndiratta

and Bhalla, 2012).

Measurement of the Minimum Inhibitory Concentration (MIC) by using the dilution method has traditionally been the reference method for primary diagnosis of methicillin resistance. This method, performed on broth or agar, aims to measure the lowest concentration of the assayed antimicrobial agent (oxacillin) that, under defined test conditions, results in visible growth inhibition of the bacterium (Wiegand et al., 2008). Another method commonly used for the detection of MRSA is the disk diffusion test. This test is performed by applying the bacterial inoculum onto the surface of Mueller-Hinton agar plates. Commercially-prepared, fixed-concentration, antibiotic-impregnated paper disks are placed on the inoculated agar surface. After appropriate incubation, the zones of growth inhibition around the antibiotic disks are recorded and resistance is evaluated according to the Clinical Laboratory Standards Institute (2009). The results of the disk diffusion test are influenced by a range of factors, including the growth medium, the NaCl concentration and temperature (Brown, 2001; Reller et al., 2009). Commercial MIC tests and automated antimicrobial susceptibility testing systems are widely used for MRSA detection (Reller et al., 2009). A commercial agglutination test based on the detection of PBP2a is also available for screening of methicillin resistance (Kluytmans et al., 2002). Definitive identification of MRSA is achieved upon detection of the *mecA* gene by Polymerase Chain Reaction (PCR).

The definition of MRSA relying only on susceptibility tests can overestimate methicillin resistance; isolates that do not carry *mecA* can appear to be phenotypically resistant to methicillin (Lee et al., 2004; CFSPH, 2006). In order to harmonize monitoring of MRSA in animals and foods in the EU, EFSA proposed that the MRSA definition should be made by the examination for the presence of *mecA* or the recently described *mecC* using multiplex PCR or, in isolates negative for these genes, by phenotypical tests for resistance to ceftiofuran (EFSA, 2012).

The *S. aureus* population, including MRSA, consists of different clonal lineages, also called clonal complexes. Clones or strains of MRSA are differentiated using genetic typing tests, such as *spa* typing, Multi Locus Sequence Typing, Pulsed-Field Gel Electrophoresis, *SCCmec* typing and other tests (Leonard and Markey, 2008; Catry et al., 2010). These techniques are mainly useful for epidemiological

studies and more than one methods may be necessary to identify a given strain.

At present, the best single method for determining the MRSA lineage is *spa* typing, which involves DNA sequencing of short nucleotide repeats in the polymorphic X region of the *S. aureus* protein A gene (*spa*). Different *spa* repeats are assigned an α -numerical code (r01, r02, etc.) and the repeat succession determines the *spa* type (e.g., t001, t002, etc.) by submission of the results to the RIDOM StaphType Database (www.spaserver.ridom.de) (EFSA, 2012). In general, isolates with a similar succession of *spa* sequences belong to closely related sequence types, which can be assigned to the same CC. *spa* typing can help distinguish isolates that are indistinguishable by Multi Locus Sequence Typing or Pulsed-Field Gel Electrophoresis.

Multi Locus Sequence Typing involves the sequencing of defined regions of seven genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tqi* and *yqi*). A web-based database for Multi Locus Sequence Typing typing is available online (www.mlst.net) (Enright et al., 2000). Multi Locus Sequence Typing enables the assignment of sequence types to MRSA isolates. However, this technique is not suitable for routine surveillance of MRSA, because of the high cost involved and the requirement for high-throughput DNA sequencing (Harmsen et al., 2003).

SCC*mec* typing classifies SCC*mec* elements into types and subtypes on the basis of their structural differences. Most of the used methods rely on PCR mapping of cassette elements, such as the *mec* complex, the *ccr* complex and the J region (Mehndiratta and Bhalla, 2012). Until recently, eight main types of SCC*mec* (type I to type VIII) along with many subtypes had been distinguished among MRSA isolates. Some of these types were more common than others. In the last few years, new types of SCC*mec* (IV to XI) were identified and additional subtypes and different variants of already existing ones were discovered (Turlej et al., 2011). SCC*mec* type XI carries the recently described *mecC* gene (EFSA, 2012). Pulsed-Field Gel Electrophoresis of DNA fragments restricted with the *Sma*I enzyme is considered to be the gold standard for typing MRSA isolates (Smith et al., 2008; Bosch et al., 2010; Moussa et al., 2011). However, it is important to follow uniform standard protocols to achieve types of SCC*mec* internationally comparable results.

There is no consensus regarding the best method

for typing MRSA strains. Application of any typing method requires careful assessment of its suitability and an individual approach depending upon the purpose of the study (Mehndiratta and Bhalla, 2012).

Transmission

MRSA can be transmitted from person to person, as well as from animals to humans and *vice-versa*. Transmission usually occurs by direct contact, often via the hands, with colonized or infected people or animals (Lee, 2003; Ferreira et al., 2011).

MRSA carriage rates in the general human population usually vary between geographic regions from <1% to 5% (Leonard and Markey, 2008). In human hospitals, colonized and infected patients are the main reservoirs of MRSA, which is typically spread from patient to patient via hands of staff (EFSA, 2009). Transmission routes of MRSA are probably similar to those of other *S. aureus* strains, but there are likely to be differences in efficiency of host colonization following exposure (Kawada et al., 2003). Whether a person becomes a persistent nasal carrier or not depends on various factors that are still poorly understood (Peacock et al., 2001).

Carrier animals serve as reservoirs of MRSA and they may transmit the pathogens to other animals or humans (Duquette and Nuttall, 2004; Cantry et al., 2010; Cuny et al., 2010). Some MRSA lineages tend to predominate in specific geographical regions and show host specificities; therefore, they tend to be associated with animals more than with humans and *vice-versa* (Sung et al., 2008). CC398 is the MRSA lineage most often associated with asymptomatic carriage in intensively reared food-producing animals, primarily in pigs, but also in cattle and perhaps in poultry (EFSA, 2009). Although this strain is mainly found to colonize animals without causing clinical diseases, in a few isolated cases, it caused clinical infections in animals. Colonization with livestock-associated MRSA, especially CC398, has been reported frequently in people who work with such animals, i.e. farmers, veterinarians and their family members (Leonard and Markey, 2008; van Rijen et al., 2008; Cuny et al., 2009).

MRSA isolates can be also shared between personnel and animals, including dogs, cats and horses, in veterinary hospitals and between companion animals and their owners in households (Cefai et al., 1994; Manian, 2003; Baptiste et al., 2005; van

Duijkeren et al, 2005; Weese et al, 2006). Indeed, in a few cases companion animals have been implicated as sources of human infections (Faires et al., 2009; Ferreira et al., 2011).

Food may be contaminated with MRSA; handling or eating contaminated food is also a potential means of transmission. In hospital outbreaks, contaminated food can disseminate the organism to patients as well as to healthcare workers (EFSA, 2009). The first food-associated MRSA outbreak, which affected 27 patients and 14 hospital workers and resulted in five deaths, was described in The Netherlands. One food handler was found to be colonized with the same MRSA strain that was recovered from a food sample (peeled banana) and the infected patients. Contaminated food likely caused the first case of MRSA septicaemia, which was subsequently transmitted to other patients in a surgical unit by a colonized nurse. Airborne transmission was thought to play an important role in the subsequent spread of the outbreak (Kluytmans et al., 1995).

Considering the increasing evidence of MRSA presence in food-producing animals, the concerns regarding MRSA contamination of food of animal origin, may be reasonable (Weese, 2010). However, and despite the reported increases in both the MRSA food contamination and in the incidence of human community acquired-MRSA infections, there are no reports of a direct link between them (EFSA, 2009). Further investigations are needed to determine the true role of food of animal origin in transmission of MRSA from animals to humans.

Another troubling aspect of food-associated MRSA is that MRSA frequently contain staphylococcal enterotoxin genes, including genes encoding for enterotoxins most often associated with food poisoning (SEA, SEB, SEC, SED) (EFSA, 2008). Different combinations of staphylococcal enterotoxin genes are associated with different MRSA clones, but the reasons of this association remain unclear (Ferry et al., 2006; Tristan et al., 2007). Clinically, food poisoning caused by MRSA should be no different than that caused by other *S. aureus* strains (Weese, 2010). To date, only a small staphylococcal food poisoning outbreak due to MRSA has been reported in Tennessee, USA. Three family members who consumed a meal of shredded pork barbeque and coleslaw salad became ill with nausea, vomiting and stomach cramps. The same strain of MRSA was isolated from the three family members, the coleslaw salad and a food handler at the convenience market where the food was purchased.

This outbreak strain was most likely of human origin (Jones et al., 2002). Increased prevalence of MRSA amongst *S. aureus* strains could lead to a higher prevalence of toxinogenic *S. aureus* (EFSA, 2008).

MRSA PREVALENCE IN MILK AND DAIRY PRODUCTS

It is well known that pasteurization of milk eliminates *S. aureus*. Raw milk and raw-milk cheese contaminated with MRSA have been incriminated in the transmission of the pathogen to humans (Normanno et al., 2007; Haran et al., 2012). Handling or consumption of raw milk and dairy products may lead to the spread of antimicrobial resistance genes of *S. aureus* to humans (McKay, 2008).

Significant differences in MRSA prevalence in milk have been recorded among different countries or even regions within the same country. These differences may be due to differing animal production systems among the various countries. Different national antimicrobial policies and regulations may have also contributed to the different prevalence estimates (Grave et al., 2010). Other factors, such as presence of multiple animal species in the same area that facilitate transfer of genetic material between *S. aureus* strains, may affect the MRSA prevalence (Spohr et al., 2011). Published data revealed various prevalence estimates of MRSA in milk and dairy products in different parts of the world.

Europe

According to a survey conducted in Belgium between 2006 and 2007, 11 of 118 *S. aureus* isolates from bovine subclinical and clinical mastitis were MRSA (Vanderhaeghen et al., 2010).

In the Czech Republic between 2006 and 2009, Stastkova et al. (2009a) examined 299 *S. aureus* isolates and found 18 and four MRSA strains from cows' and goats' bulk tank milk, respectively, as well as one MRSA strain from an individual goat milk sample. The MRSA isolates from one goat farm were also positive for the genes encoding SEs (Stastkova et al., 2009a). Moreover, between 2006 and 2008, Stastkova et al. (2009b) examined 153 milk samples from a goat farm and isolated 34 *S. aureus* strains, among them five MRSA strains (prevalence 15%). The same authors also observed that all MRSA isolates carried the *seb* gene. Recently, a lower prevalence (3%) was reported by Vyletřlova et al. (2011) in the

Czech Republic. In this study, individual goat (n=60), sheep (n=60) and cow (n=120) milk samples were examined and only two MRSA strains were detected, from two dairy cows with subclinical mastitis from one herd.

In France, no MRSA were detected among 119 *S. aureus* isolates from cows' milk with clinical mastitis between 1998 and 2000 (Guerin-Faubleee et al., 2003). In a similar study examining cows' mastitis milk in France, only one isolate was classified as MRSA among 139 *S. aureus* isolates between 2007 and 2008 (Haenni et al. 2011).

In Germany, surveys conducted in bulk tank milk samples in 2009 and 2010 revealed MRSA prevalence estimates of 1% to 2% (Friedrich et al., 2011) and 5% (Kreausukon et al. 2012), respectively. According to a study conducted in three dairy herds located at the southwest of Germany, a substantial prevalence of MRSA was revealed (5%-17% of milk samples and 1%-10% of herds) (Spohr et al., 2011).

In Hungary, from January 2002 to December 2004, 595 milk samples were collected from cows with subclinical mastitis and 27 MRSA (out of 375 *S. aureus* strains) were isolated (Juhász-Kaszanyitzky et al., 2007).

In Ireland, McKay (2008) did not identify any *mecA*-positive *S. aureus* by examining 70 unpasteurized milk samples.

In Italy, Normanno et al. (2007) isolated four MRSA SEs-positive strains from bovine milk and two from dairy products (pecorino cheese and mozzarella cheese), whereas Spanu et al. (2010) examined 36 *S. aureus* strains isolated from cheese made from raw sheep milk, and none of them were identified as MRSA.

In The Netherlands, Fessler et al. (2012) examined 1839 cow milk samples from 26 dairy farms and detected MRSA in 62 of those (prevalence 4%).

In Portugal, among 30 isolates of *S. aureus* responsible for subclinical bovine mastitis, no MRSA were found (Nunes et al., 2007).

In Slovakia, none of 79 *S. aureus* isolates from raw sheep milk and sheep-milk cheese samples could be classified as MRSA (Mašlanková et al., 2009).

In Switzerland, Huber et al. (2010) detected only two MRSA strains among 142 *S. aureus* isolates from bovine mastitis milk.

Reported prevalence of MRSA in milk and dairy products in European countries is summarized in Table 1.

Asia and Africa

In India, Kumar et al. (2010) reported MRSA prevalence of 10% by examining 128 *S. aureus* isolates from 280 animals of Karan Fries (Taurus × Zebu) with mastitis during a survey conducted between 2007 and 2008. The same research team (Kumar et al., 2011) also reported MRSA prevalence of 13% among 107 *S. aureus* strains from cows with mastitis in a herd located in northwest India.

In Iran, Mirzaei et al. (2012) examined 300 samples (100 raw milk, 100 pasteurized milk and 100 ice cream) in 2010 and reported that 20 isolates (14 from raw milk, 6 from ice cream) carried the *mecA* gene, whereas Alian et al. (2012) examined 348 raw milk samples from randomly selected herds between 2010 and 2011 and found a MRSA prevalence of 28%.

In Japan, between 1998 and 2005, Hata et al. (2010) identified four MRSA strains among 363 *S. aureus* isolates from bovine milk originating from 260 dairy farms located in 21 prefectures.

In Korea, Lee et al. (2003) found MRSA prevalence of 1.5% (12 strains) when 894 milk samples of dairy cattle were examined from May 2001 to April 2003, whereas in a survey conducted by Kwon et al. (2005), MRSA prevalence of <0.5% was observed after examination of 9,055 milk samples between 1999 and 2003. The authors reported that all MRSA strains harbored SEs genes (*sed*, *sei*, *sej*). Moreover, MRSA prevalence estimates of 2.5% and 6% were reported after examination of 835 and 402 *S. aureus* strains from milk samples from animals with mastitis according to two surveys conducted between 1997 and 2004 (Moon et al., 2007) and between 2003 and 2009 (Nam et al., 2011), respectively.

In Pakistan, Farzana et al. (2004) reported MRSA prevalence of 10% (8 positive strains among 77 isolates) from 50 raw milk samples.

In a survey conducted in Thailand in 2010, Intrakamhaeng et al. (2012) found 74 MRSA strains by examining 375 *S. aureus* isolates collected from 598 mastitis cases, 376 bulk tank milk samples and 46 pasteurized milk samples.

In Turkey, 18 (17.5%) MRSA strains were identified among 103 *S. aureus* isolates from mastitic milk samples (Turutoglu et al., 2006). In a more recent survey Ünal et al. (2012) did not find any MRSA when testing 21 *S. aureus* isolates from milk samples of 857 ewes and 33 goats, collected from 13 different farms in the Kirikkale province.

In Ethiopia, Daka et al. (2012) reported a MRSA

prevalence of 60% in a total of 78 *S. aureus* isolates from cows' milk.

In Nigeria, Suleiman et al. (2012) examined 339 milk samples from dairy cows and observed that among 73 *S. aureus* isolates, 26 (36%) were oxacillin resistant and two isolates harbored the *mecA* gene (8%).

In South Africa, Ateba et al. (2010) found an MRSA prevalence of 6% in cows' milk produced in two commercial farms.

The MRSA prevalence estimates recorded in milk produced in African and Asian countries are presented in Table 2.

Americas

In a study conducted in Brazil a high prevalence (25%) of MRSA was recorded, when milk samples from 98 cows with subclinical mastitis were tested (Coelho et al., 2009).

In Canada, Saini et al. (2012) detected only one MRSA in 1,802 *S. aureus* isolates from milk samples collected from 79 dairy farms.

Studies have reported zero to low occurrence of MRSA among *S. aureus* isolates from bovine milk in the USA (Table 3). Erskine et al. (2002) reported MRSA prevalence of 1% after examining 846 *S. aureus* isolates from milk samples collected from cows with mastitis in Michigan. Makovec and Ruegg (2003) observed MRSA prevalence of

2% when testing 2132 *S. aureus* isolates from milk samples collected from 1994 to 2001 in Wisconsin. No MRSA was found among 357 *S. aureus* isolates recovered from milk samples from 24 dairy herds in North Carolina and Virginia (Anderson et al., 2006). In a survey conducted by Virgin et al. (2009), none of the tested 542 bulk milk samples were found to be positive for MRSA. D'amico and Donnelly (2011) found two MRSA among 90 *S. aureus* isolates from raw milk used for the production of artisan cheese in Vermont. Haran et al. (2012) identified two MRSA out of 154 strains isolated from 150 bovine bulk milk samples in Minnesota. They also reported that the two MRSA strains were able to produce SEB, SEC, SED and SEE.

CONCLUSIONS

MRSA is an important pathogen, which may be also found in milk and dairy products worldwide. Prevalence of MRSA in milk and dairy products varies among countries, among regions within countries and even among herds within the same region. According to several surveys conducted in European countries, the USA and Canada, MRSA prevalence in milk and dairy products has been found to be low. In contrast, countries in Asia and Africa have a higher MRSA prevalence in milk and dairy products.

CONFLICT OF INTEREST

None to declare. ■

Table 1. Prevalence of MRSA in milk and dairy products in European countries

Year	Country	Samples	MRSA prevalence	Method	Comments	Reference
2006-2007	Belgium	<i>S. aureus</i> isolates from cases of bovine clinical or subclinical mastitis	11/118 (9.3%) (118 isolates from 118 different farms)	Disk diffusion method, detection of <i>mecA</i> gene by PCR	ST398, <i>spa</i> -types t011 or t567, SCC <i>mec</i> -type IVa or V	Vanderhaeghen et al.(2010)
2006-2009	Czech Republic	Bulk tank milk samples & individual samples from 95 farms (89 cow farms, 2 goat farms, 4 sheep farms)	23/299 strains (7.7%) (18 from cow bulk tank milk, 4 from goat bulk tank milk, 1 from individual goat milk sample)	2-step enrichment and sampling in selective chromogenic medium, detection of <i>mecA</i> gene by PCR, <i>mecA</i> positive strains: disk diffusion method	The MRSA isolates from one goat farm were also SEs gene positive	Stastkova et al. (2009a)
2006-2008	Czech Republic	153 goat milk samples (34 bulk tank milk, 119 individual milk samples) at a goat breeding farm	5/34 strains (14.7%) (4 from bulk tank milk samples and 1 from an individual milk sample)	2-step enrichment and sampling in MRSA selective chromogenic medium, detection of <i>mecA</i> gene by PCR, <i>mecA</i> positive strains: disk diffusion method and MIC (E-test)	All obtained MRSA isolates were <i>seb</i> positive, SCC <i>mec</i> type IV, <i>spa</i> type t064	Stastkova et al. (2009b)
Not reported	Czech Republic	240 individual milk samples (goats: 60, sheep: 60, cows:120)	2/79 strains (2.5%)	Disc diffusion method, detection of <i>mecA</i> gene by PCR	The 2 MRSA strains were from two cows with subclinical mastitis	Vyletělova et al. (2011)
1998-2000	France	Individual mammary gland samples from cows with clinical mastitis	0/119 strains	MIC by agar dilution method, detection of <i>mecA</i> gene by PCR		Guerin-Faubleee et al. (2003)

2007-2008	France	<i>S. aureus</i> isolates from cases of bovine mastitis	1/139 strains (0.7%)	Disc diffusion method, detection of <i>mecA</i> gene by PCR	SCC <i>mec</i> -type I, ST5, <i>spa</i> -type t002, SEs genes: <i>sec</i> , <i>sed</i> , <i>sel</i> , <i>sem</i> , <i>seo</i>	Haenni et al. (2011)
2008	Germany	321 individual mammary gland samples from cows with mastitis, 3 bulk tank milk samples from 3 farms	5.1-16.7% of milk samples, 1.4-10.0% of herds	Disc diffusion method, detection of <i>mecA</i> gene by PCR and MRSA-DNA using melting-curve analysis	All MRSA isolates from milk: SCC <i>mec</i> type V, <i>spa</i> type t011	Spohr et al. (2011)
2009-2010	Germany	635 cow bulk tank milk samples	28/635 samples (4.4%)	2-step enrichment and sampling in a MRSA selective chromogenic agar, detection of <i>mecA</i> gene by PCR	36 MRSA: <i>spa</i> types t011 (22 isolates, 61%), t034 (14 isolates, 39%), both associated with CC398	Kreausukon et al. (2012)
Not reported	Germany	180 dairy farms	1-2.2%			Friedrich et al. (2011)
2002-2004	Hungary	595 milk samples from cows with subclinical mastitis on a farm	27/375 strains (7.2%)	Disk diffusion method & MIC (E-test), detection of <i>mecA</i> gene by PCR	<i>Spa</i> type t127, MLST & SCC <i>mec</i> typing on 1 strain: ST1, SCC <i>mec</i> type Iva	Juhasz-Kaszanyitzky et al. (2007)
Not reported	Ireland	70 unpasteurized bovine milk samples	No <i>mecA</i> -positive <i>S. aureus</i> were isolated	Detection of <i>mecA</i> gene by PCR		McKay (2008)
2003-2005	Italy	160 <i>S. aureus</i> strains isolated from 1634 foodstuff samples of animal origin	4 MRSA from bovine milk, 2 from cheese samples (pecorino and mozzarella cheese)	Detection of <i>mecA</i> gene by PCR	All the MRSA strains were enterotoxigenic; 2 SEA/SED, 2 SED, 1SEC	Normanno et al. (2007)
Not reported	Italy	Samples from cheese produced from raw sheep milk from six flocks	0/36 strains	MIC by a broth microdilution method, detection of <i>mecA</i> gene by PCR		Spanu et al. (2010)

2007-2009	The Netherlands	1839 bovine milk samples from 26 farms	62/1839 samples (3.4%), MRSA isolated from all farms in the study	MIC by broth microdilution method	All isolates belonged to CC398	Fessler et al. (2012)
Not reported	Portugal	<i>S. aureus</i> isolates for cases of subclinical mastitis in cows	0/30 strains	MIC by agar dilution method, detection of <i>mecA</i> gene by PCR		Nunes et al. (2007)
2004-2007	Slovakia	Raw sheep milk, cheese samples	0/79 strains (49 from milk, 44 from sheep-milk cheese, 6 from Bryndza cheese)	Disc diffusion method, detection of <i>mecA</i> gene by PCR		Mašlanková et al. (2009)
2009	Switzerland	Bovine mastitis milk	2/142 (1.4%)	Sampling in MRSA selective medium, detection of <i>mecA</i> gene by PCR, phenotypic properties: disk diffusion method and MIC method (E-test)	Both strains: ST398, <i>spa</i> type t011, SCC <i>mec</i> type V, SEs genes negative	Huber et al. (2010)

Table 2. Prevalence of MRSA in milk and dairy products in Asian and African countries

Year	Country	Samples	MRSA prevalence	Method	Comments	Reference
2007-2008	India	Milk samples from 280 animals of Karan Fries (Taurus × Zebu) with mastitis	13/128 strains (10.2%)	Disc diffusion method, detection of <i>mecA</i> gene by PCR		Kumar et al. (2010)
Not reported	India	195 milk samples from cows with mastitis	14/107 strains (13.1%) (10 <i>mecA</i> positive).	Disk diffusion method, detection of <i>mecA</i> gene by PCR	MRSA strains harbored, staphylococcal enterotoxin genes	Kumar et al. (2011)
2010	Iran	300 samples (100 raw milk, 100 pasteurized milk, 100 ice cream)	20/69 strains (29.0%) had the <i>mecA</i> gene (14 from raw milk, 6 from ice cream)	Disk diffusion method, detection of <i>mecA</i> gene by PCR		Mirzaei et al. (2012)
2010-2011	Iran	348 raw milk samples from cows, ewes and goats	13/38 strains (28.3%)	Disk diffusion method		Alian et al. (2012)
1998-2005	Japan	Bovine milk from 260 farms	4/363 strains (1.1%)	Disc diffusion method, detection of <i>mecA</i> gene by PCR	3/4 MRSA strains: ST5, SCC <i>mec</i> type II, <i>spa</i> type t002 or t375, 1 strain: ST89, SCC <i>mec</i> IIIa, t5266	Hata et al. (2010)
1997-2004	Korea	3047 bovine milk samples from cases of mastitis from 153 farms	21/835 strains (2.5%) resistance to methicillin, 13/835 strains (1.6%) were <i>mecA</i> -positive	Oxacillin MIC method, detection of <i>mecA</i> gene by PCR		Moon et al. (2007)
1999, 2000, 2003	Korea	9055 milk samples with >500,000 somatic cells/mL	14 MRSA & 1 silent <i>mecA</i> -carrying methicillin-susceptible <i>S. aureus</i> (smMSSA) (0.2%)	Oxacillin MIC method, detection of <i>mecA</i> gene by PCR	14 MRSA SCC <i>mec</i> new subtype IVg (1 smMSSA strain was not classified), all strains harbored staphylococcal enterotoxin genes (<i>sed</i> , <i>sei</i> , <i>sej</i>)	Kwon et al. (2005)
2001-2003	Korea	894 cow milk samples	12 strains (1.3%)	Phenotypic oxacillin resistance by agar screen test, detection of <i>mecA</i> gene by PCR, <i>mecA</i> -positive tested by disk agar and oxacillin MIC methods	9/12 MRSA from milk were from cows with mastitis	Lee (2003)

2003-2009	Korea	402 <i>S. aureus</i> isolates from milk samples from cases of mastitis	6.2%		11/ 17 strains: SCCmec IVa-t324-ST72, 3 strains: SCCmec IVa-t286-ST1, 3 strains: SCCmec IV-spa untypable-ST72, PFGE: A-C	Nam et al. (2011)
1992	Pakistan	50 raw milk samples	8/77 strains (10.4%)	Disc diffusion method		Farzana et al. (2004)
Not reported	Thailand	598 mastitis cases, 376 bulk tank milk, 46 pasteurized milk samples	74/375 strains (19.7%)	Disk diffusion method	61 isolates harbored at least one classical staphylococcal enterotoxin gene	Intrakamhaeng et al. (2012)
2002-2004	Turkey	Milk samples from cases of mastitis	18/103 strains (7.5%)	Disk diffusion method		Turutoglu et al. (2006)
2009	Turkey	1,604 and 66 individual milk samples from 857 ewes and 33 goats, respectively in 13 farms	0/21 strains (all <i>S. aureus</i> isolates were collected from ewes' milk)	Disk diffusion method, detection of <i>mecA</i> gene by PCR		Ünal et al. (2012)
2011-2012	Ethiopia	160 cows' milk samples (78 <i>S. aureus</i> isolates)	60.3%	Disc diffusion method		Daka et al. (2012)
2008-2009	Nigeria	339 individual milk from 85 cows (30.9% of these, from animals with mastitis)	26/73 strains found to be resistant to oxacillin (35.6%), 2/73 strains were <i>mecA</i> -positive (7.6%)	Disk diffusion method, screening in chromogenic media, MIC method, detection of <i>mecA</i> gene by PCR		Suleiman et al. (2012)
Not reported	South Africa	28 milk samples from 4 farms	64.4% to 100% in milk from 2 communal farms, 6.4% from 2 commercial farms	Disk diffusion method		Ateba et al. (2010)

Table 3. Prevalence of MRSA in milk and dairy products in American countries

Year	Country	Samples	MRSA prevalence	Method	Comments	Reference
Not reported	Brazil	Milk samples from 98 cows with subclinical mastitis	25%	Phenotypic oxacillin resistance by an agar screen test, detection of the <i>mecA</i> gene by PCR		Coelho et al. (2009)
2007-2008	Canada	Milk samples from 79 dairy farms	1/1802 strains (0.05%).	Screening with chromogenic MRSA agar, detection of <i>mecA</i> gene by PCR		Saini et al. (2012)
2007	USA	542 cow bulk tank milk samples	0/190 milk samples	Screening with MRSA selective chromogenic agar, detection of <i>mecA</i> gene by PCR		Virgin et al. (2009)
1994-2000	USA (MI)	Milk samples from cows with possible mastitis	5/846 strains (0.6%)	Disk diffusion method		Erskine et al. (2002)
2009	USA (MN)	150 bovine bulk tank milk samples from 50 farms	MRSA herd prevalence was 4% (2/50), MRSA prevalence in bulk tank milk was 1.3% (2/154 strains)	2-step enrichment and sampling in MRSA selective agar, detection of the <i>mecA</i> gene by PCR	1 st strain <i>spa</i> type t529, MLST: ST5, PFGE: USA100-unknown, 2 nd strain <i>spa</i> type t034, MLST: ST8, PFGE: USA300- <i>spa</i> type t121 genotype, both MRSA strains were able to produce SEB, SEC, SED, SEE	Haran et al. (2012)
Not reported	USA (NC, VA)	Milk samples from 24 dairy herds	0/357 strains	MIC method		Anderson et al. (2006)
2006, 2008, 2009	USA (VT)	90 isolates from raw milk used for production of cheese	2 MRSA isolates	Phenotypic oxacillin resistance by an agar screen test		D'amico and Donnelly (2011)
1994-2001	USA (WI)	Cow milk samples	38/2132 strains (1.8%)	Disk diffusion method		Makovec and Ruegg (2003)

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