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Short communication

MRSA detection in raw milk, some dairy products and hands of dairy workers in Egypt, a mini-survey

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ABSTRACT

The main goal of this study was to report the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in raw milk and some dairy products sold in the local markets and villages at Dakahlia province, Egypt. Raw milk, kariesh cheese, ice cream and hand swabs of dairy workers were sampled. It was predicted the high prevalence and counts of detected coagulase positive *Staphylococcus aureus* (CP *S. aureus*) throughout all of the examined samples and swabs, which certainly reflect the neglected hygienic practices either in the production of raw milk and dairy products or in the personal hygiene. Molecular detection of *mec*A gene in the CP *S. aureus* isolates was adopted for the identification of MRSA strains, which could be only identified from 5 samples of raw milk and dairy products, and it was not detected in any of the hand swabs. One out of the five MRSA isolates showed resistance against both vancomycin and oxacillin, while other strains showed resistance against oxacillin alone. This study is the first in term of MRSA detection in raw milk and its products in Egypt, and also elucidates a possible way of transmission of community acquired MRSA.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a common member of the natural microflora of human skin and nasal passage (Hanson et al., 2011). In addition, as a potential pathogen, it may adversely affect human and animal health by causing sever necrotic lesions, abscesses (Lowy, 1998) and bacteremia (Reacher et al., 2000). Moreover, besides these pathogenic symptoms, toxigenic foodborne strains of *S. aureus*, if they get multiplied in food to a certain level of about 10^5–10^6 CFU/g, may secret potent, heat stable enterotoxins responsible for food-borne intoxication (Tranter, 1990). *S. aureus* intoxication ranked third of food poisoning cases all over the world (Asao et al., 2003; Zhang, Iandolo, & Stewart, 1998) and had been implicated with different categories of food including raw milk (Jorgensen, Mork, Hogasen, & Rovik, 2005), dairy products (Headrick et al., 1998), chicken, pork, beef and salad dishes (Bryan, 1998). Furthermore, *S. aureus* constitutes a primary cause of mastitis in dairy cattle (Virgin, Van Slyke, Lombard, & Zadoks, 2009).

Among *S. aureus*, Methicillin-resistant strains (MRSA), has recently emerged as a serious life-threaten infective agent which does not respond to a lot of antimicrobial treatments. Previous reports have shown an annual estimate of 94,000 MRSA infections in the United States, with nearly 20% mortality rate (Klevens et al., 2007).

MRSA synthesizes a penicillin binding protein (PBP2a), encoded by the *mec*A gene on a mobile genetic element (Staphylococcal cassette chromosome *mec SCCmec*), which has a role of counteracting the inhibitory effect of Beta-lactam (β-lactam) antibiotics by preventing them from effectively binding to cell wall proteins. Moreover, MRSA may also resist vancomycin (Cui, Murakami, Kuwahara-Arai, Hanaki, & Hiramatsu, 2000).

MRSA transmission has two main forms, hospital-acquired (HA) and community-acquired (CA). Although, HA MRSA infection is thoroughly investigated as the major form, CA MRSA presently represents an imminent hazard and may have severe consequences (Calfee, Durbin, & Germanson, 2003). Whilst, HA MRSA is strictly linked to hospitals and health workers, CA MRSA is more widespread and has no definite spreading vicinity (Wannet, Heck, Pluister, Spalburg, & De Neeling, 2004) and its risk against industrialized nations have been increased (Baggett et al., 2004; Doufour et al., 2002; Frank, Marcinak, Mangat, & Schreckenberg, 1999; Groom et al., 2001; O'Brien, Pearman, Gracey, Piley, & Grubb, 1999; Salmenlinna, Lyytikäinen, & Vuopio-Varkila, 2002; Witte, Cuny, Strommenger, Braulke, & Heuck, 2004).

Since *S. aureus* is highly prevalent in food and food environment, MRSA may have the same pattern of linkage. Many reports (De Boer

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et al., 2009; Kitai et al., 2005; Kwon et al., 2006; Lim et al., 2010; Lozano et al., 2009; Pu, Han, & Ge, 2009; Van Loo et al., 2007; Weese, Avery, & Reid-Smith, 2010; Weese, Reid-Smith, Rousseau, & Avery, 2010) have identified presence of MRSA in different retailed meat products from different regions worldwide with varied prevalence. Normanno et al. (2007) isolated MRSA strains from bovine milk and some cheese varieties in Italy. Moreover, several food-borne acquired MRSA outbreaks have been also reported (Jones, Kellum, Porter, Bell, & Schaffner, 2002; Kluytmans et al., 1995).

Recent reports revealed that MRSA was also associated with cases of bovine and caprine mastitis (Aras, Aydinb, & Kav, 2012; Vanderhaeghen et al., 2010). MRSA strains have been found among the *S. aureus* strains isolated from bovine mastitis milk but they are not more prevalent (Hendriksen et al., 2008; Juhàsz-Kaszanyitzky et al., 2007; Kwon et al., 2005; Lee, 2003; 2006; Moon et al., 2007). In addition, specific MRSA strain CC398 has been linked with different food animals and people in contact, which arise a new MRSA form, livestock-associated (LA-MRSA). LA-MRSA has been isolated from both human and animal infections (Krziwanek, Metz-Gercek, & Mittermayer, 2009) and from bovine mastitis case (Monecke, S., Kuhnert, Hotzel, Slickers, & Ehricht, 2007). Aforementioned reports may elucidate a possible way of transmitting either CA-MRSA or LA-MRSA between food/animal handlers and foods.

To the best of our knowledge, this is the first study to reveal the prevalence of MRSA strains in raw milk, some dairy products and on hands of dairy workers (Milkers and food handlers) in Egypt. Molecular MRSA identification was accomplished by PCR detection of *mecA* gene. Antibiotic susceptibility of isolated MRSA strains was also tested.

2. Materials and methods

2.1. Samples collection

A total of 120 samples (Table 1) were collected from the rural areas (local markets and/or villages) located in Dakahlia province, Egypt during the period of December, 2011 through February, 2012. All samples were kept at 4 °C in insulated ice box and transferred to the dairy microbiology laboratory, Food control department, Zagazig University and analyzed within 4 h of collection.

2.2. Sample preparation

In order to prepare decimal dilutions, raw milk and swab soaking broth were used directly, whereas ice cream samples were initially left at room temperature to be melted, while kariesh cheese samples were homogenized (11 g of cheese sample with 99 ml 0.1% sterile peptone water (Oxoid) for 2 min) using Syclon-04C Stomacher blender (Ningbo Sklon, China).

2.3. Staphylococcus aureus count and isolation

3M™ petrifilm™ Staph Express count plates (STX) (3M Corporate Headquarters; St. Paul, MN, USA) were used to enumerate and isolate *S. aureus* (AOAC, 2003). Briefly, 1 ml of appropriate dilution was inoculated onto petrifilm and the inoculum was evenly distributed with a sterile plastic spreader. After one minute at ambient temperature, the petrifilm was incubated aerobically at 35 °C for 24 h. According to the manufacturer's instructions, red-violet colonies were counted as *S. aureus* and any plates with background colonies were subjected to, 3M™ Petrifilm™ Staph Express Disk testing to differentiate *S. aureus according to the manufactures instructions. S. aureus* colonies appear surrounded with halo zone, which were then counted, picked up and streak plated on Brain Heart infusion Agar (BHA, BD) to establish pure cultures. Pure cultures were maintained on Brain Heart Infusion broth (BHI) with glycerol (50% v/v) at −70 °C.

2.4. MRSA molecular identification

S. aureus isolates were grown in BHI prior to DNA extraction and purification according to the DNA Purification Kit (Qiagen) procedure. *S. aureus* (MRSA) isolates were detected using the following primers set: *mecA*For (AAGCAATAGAATCATCAGAT) and *mecA*Rev (AGTTCTGCAGTACCGGATTTGC) which were generated by Primer-BLAST software using *mec*A gene sequence obtained from Gene Bank® (National Center for Biotechnology Information; NCBI). Amplification was done with the following profile: Initial denaturation at 94 °C for 5 min, 40 cycles as follow: denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, followed by final extension step at 72 °C for 5 min. The PCR product (451 bp) were electrophoreted on 0.5% agarose gel and visualized by ethidium bromide staining. GeneRuler™ 100 bp DNA ladder (Fermentas) was used as a molecular weight standard. PCR was performed on *S. aureus* ATCC 33591 (positive control) and *Staphylococcus epidermidis* (locally isolate, negative control).

2.5. Antibiotic resistance of MRSA

This experiment was designed to determine the minimal inhibitory concentration (MIC) of oxacillin and vancomycin against isolated MRSA strains. Graded-concentration antibiotic strips (M.I.C.E. strips; Oxoid) were used. The strips were used according to the manufacturer's protocol. Simply, Muller-Hinton agar plates (MH) was used and 0.5 McFarland inoculum of the *S. aureus* isolated strains was swab-spread over the plate, then, M.I.C.E. strips were aseptically placed on the dried surface within 15 min. Plates were incubated at 37 °C for 24 h.

Table 1
Numbers of samples, sampling conditions, incidence and count of coagulase positive *S. aureus* mean count ± standard deviation per examined samples.

Samples	Number of samples	Sample condition	Number of positive samples (%)	Mean count ± SD (CFU/ml or gm)
Raw milk	35	50 ml milk samples were collected from individual manually milked cow's milk, farm tank milk, collector's buckets milk and local market milk. Samples transferred immediately into sterile sampling tubes.	33 (94.3)	$5.5 \times 10^5 \pm 3.2 \times 10^4$
Kariesh cheese	30	Acid coagulated skimmed soft cheese, locally manufactured. 250 g of freshly prepared cheese were collected.	28 (93.3)	$6.2 \times 10^5 \pm 7.1 \times 10^4$
Ice cream	30	Produced at small scale; aseptically collected from markets and street vendors.	17 (56.7)	$8.9 \times 10^3 \pm 4.8 \times 10^2$
Workers'/handlers' hand swabs	25	Each swab represented a worker or a handler. Dorsal and palmar surfaces and fingertips were swabbed using moistened cotton sterile swab (using sterile ringer's solution) which was rubbed gently against surfaces.	20 (80)	–

2.6. Statistical analysis

Data were analyzed based on descriptive statistical analysis using SAS software (SAS, 2006), and results were reported as mean value ± standard error of mean (SE).

3. Results and discussion

Counting and isolating *S. aureus* in food depends usually on culturing viable cells on Baird Parker medium (Wehr & Frank, 2004); although, many constraints have been associated with this medium, such as low selectivity and laborious preparation (Schoeller & Ingham, 2001). Another important limitation of Baird parker method, its inability to distinguish between thermonuclease positive and negative *S. aureus* strains. Thus, STX plates were used in this study to overcome all these shortages with Baird Parker medium, since STX plates offer the selective differentiation between the DNase producer and non-producer *S. aureus* strains, in addition to a more convenient applicability. While, regarding the STX's efficiency of isolating and counting coagulase and thermonuclease *S. aureus* in raw milk and dairy products, a trial had been done in this aspect and it was found that STX plates showed better performance more than standard Baird Parker medium (Viçosa, Moraes, Yamazi, & Nero, 2010).

Results of *S. aureus* counts in tested products varied between each other (Table 1). Certainly, many factors are responsible for variations in prevalence and counting of food-borne pathogens, and mostly, the neglected hygienic practices were the main factors in case of *S. aureus*.

Out of 35 raw milk samples tested, nearly 94% (33 samples) were contaminated by CP *S. aureus* with an average count of 5.5×10^5 CFU/ml.; for the kariesh cheese, 28 out of 30 (93%) samples tested were positive for CP *S. aureus*, with an average count of 6.2×10^5 CFU/g. On the other hand, CP *S. aureus* contamination for ice cream was 17 out of 30, (57%) with an average count of 8.9×10^3 CFU/ml. Many surveys and reports have been done regarding the prevalence of CP *S. aureus* in raw milk and dairy products. Makita, Desissa, Teklu, Zewde, and Grace (2012) remarked a high prevalence of *S. aureus* in raw bulk milk (43.5% of examined samples), and this value increased significantly at the raw milk collection centers (72.0% of examined samples), which was attributed to faulty handling. André et al. (2008) tested 24 samples of raw milk and 24 samples of minas frescal cheese (soft cheese) for the presence of CP *S. aureus*, and they reported that these samples were contaminated with CP *S. aureus* with a percentage of 66.7 and 70.8% respectively, which were slightly less than our results. Although, a higher prevalence in raw milk was reported by other researchers (Adesiyun, Webb, & Romain, 1998). However, several reports revealed higher values than ours in case of contaminated soft cheese (Araújo, Pagliares, Queiroz, & Freitas-Almeida, 2002; Rosengren, Fabricius, Guss, Sylven, & Lindqvist, 2010). In a recent report of incidence of CP *S. aureus* in Turkish cheese, 9.5% were contaminated with a mean log of 4.79 CFU/g (Yesim Can and Haluk Çelik, 2012); although, higher incidences were reported in other studies (Kuplulu, Sarımehmetoglu, & Celık, 2004; Normanno et al., 2005). In another survey, 100% of examined soft raw milk cheese samples were contaminated by *S. aureus* strains which were confirmed by PCR for the presence of enterotoxin genes (*sea*, *sed* and *sej*), which were detected in many *S. aureus* isolates (Cremonesi et al., 2007).

On the other hand, reports concerning ice cream contamination with CP *S. aureus* have reported different percentages. Normanno et al. (2005) found that out of 350 examined ice cream samples, only 23 were contaminated by CP *S. aureus*. Warke, Kamat, Kamat, and Thomas (2000) studied the surveillance of CP *S. aureus* in packed and open retailed ice cream samples in India, and they found that 100% of both brands were contaminated with CP *S. aureus* with an average count of 2–3 log CFU/ml.

As a leading causative agent of bovine mastitis all over the world (Buzzola et al., 2001), *S. aureus* usually finds its way to bulk milk and in turn contaminates other raw milk products (André et al., 2008).

Hand Swabs of dairy workers and food handlers revealed high frequency of CP *S. aureus* colonizing their skins. 80% of hand swab sample were positive for CP *S. aureus*; consequently, they may constitute another sustainable source of CP *S. aureus* contamination of dairy products. As an evidence of the seriousness of hand contamination with CP *S. aureus*, toxic shock syndrome toxin 1 (TSST-1) gene was detected in *S. aureus* isolated from food handlers (Rapini, Cerqueira, Carmo, Veras, & Souza, 2005) and from milk of clinical and subclinical mastitis cases and from bulk tank milk (Takeuchi, Ishiguro, Ikegami, Kaidoh, & Hayakawa, 1998). On the controversy of our findings, a lower incidence of *S. aureus* isolated from dairy workers' hands has been mentioned (Sospedra, Mañes, & Soriano, 2012).

From the aforementioned results, it can be clearly concluded that the hygienic qualities of examined raw milk and dairy products in this study were poor, in addition to poor personal hygiene, which might play an important role in transferring *S. aureus* to dairy products. Even though, in this study, the detection of preformed enterotoxin in foods was not addressed, the numbers of recovered *S. aureus* in either of raw milk and dairy products were quite enough for toxin production ($>10^5$ CFU/ml or gm, Tranter, 1990).

Regarding to MRSA identification, two identical CP *S. aureus* colonies from each positive sample were selected for MRSA identification, which was accomplished using *mec*A gene PCR detection. Routine epidemiological surveys usually choose only one colony for molecular identification of MRSA (Gouloumès et al., 1996); however, the selection of two colonies was adopted here to confer more creditability to the experiment. MRSA identification based on *mec*A gene detection is considered the best method for this purpose (Al-Ruaily & Khlail, 2011; Aras et al., 2012; Normanno et al., 2007; Vanderhaeghen et al., 2010) rather than phenotypic methods which have usually many errors (Oliveira & Lencastre, 2002).

Based on PCR amplification results, only 5 samples out of 95 tested yielded positive results for the *mec*A gene. Of the 5 isolates, 3 were from raw milk samples and 1 each from Kariesh cheese and ice cream samples. While, none of the CP *S. aureus* isolates from swabs of workers' hands was positive for the *mec*A gene.

Reports regarding prevalence of MRSA in food and in particularly in milk and dairy products are generally scarce. Eleven MRSA strains were identified among 118 *S. aureus* previously identified as a causative agent of mastitis (Vanderhaeghen et al., 2010). Another report revealed the detection of 2 MRSA strains among 42 *S. aureus* strains isolated from caprine clinical mastitis cases (Aras et al., 2012). Another study was able to isolate MRSA strains from raw goat's milk and associated workers in Czech Republic (Stastkova, Karpiskova, & Karpiskova, 2009).

Considering MRSA identification in other food stuffs, many reports have identified its prevalence (retail chicken meats, Kwon et al., 2006; cage-cultured Tilapia, Atyah, Zamri-Saad, & Siti-Zahrah, 2010; retail meats and meat products, Hanson et al., 2011; Pu et al., 2009; Weese, Avery, et al., 2010).

Bulk tank milk analyzes concerning MRSA detection were yet very few; based mainly on either phenotypic or antibiotic susceptibility differentiation. While, Erskine, Walker, Bolin, Bartlett, and White (2002) and Makovec and Ruegg (2003) were able to detect MRSA in Bulk tank milk (with a limited frequencies), Anderson, Lyman, Bodeis-Jones, and White (2006) reported that out of 357 *S. aureus* strains isolated from raw milk at North Carolina, no strain has any resistance against oxacillin.

To check out the antibiotic resistance of isolated MRSA strains, two antibiotics (vancomycin and oxacillin) were chosen. Both antibiotics have been widely used as the drug of choice for most MRSA infections (Domaracki, Evans, & Venezia, 2000; Labrou et al., 2012). Graded antibiotic strips were used to determine the MIC of each antibiotic, which can be defined as "the lowest antibiotic concentration that will inhibit the visible growth of a microorganism after overnight incubation" (Andrews, 2001). Generally, no statistical differences for the minimum inhibitory concentration of oxacillin were found between the tested 5 isolates and it was clearly demonstrated that all detected MRSA strains in this study showed resistance to oxacillin (MIC were >256 μg/ml). 4 MRSA isolates were susceptible to vancomycin (MIC ranged from 0.06 to 0.12 μg/ml), however, only one MRSA isolate (isolated from raw milk) showed resistance against both oxacillin and vancomycin (MIC was >256 and >2 μg/ml, respectively).

Multidrug resistance of MRSA strains is not uncommon. MRSA strains isolated from Turkish Tulum cheese were found to have resistances to multiple antibiotics (Yesim Can and Haluk Çelik, 2012). Sawant, Sordillo, and Jayarao (2005) reported LA MRSA resistance against β-lactam, aminoglycosides and macrolides. Multidrug resistance by MRSA strains isolated from raw meat was also reported by Hanson et al. (2011). Moreover, different antibiotic resistances by *S. aureus* strains isolated from milk and dairy products were reported repeatedly (Aarestrup, Wegener, & Rosdahl, 1995; Gianneechini, Concha, & Franklin, 2002; Lange, Cardoso, Senczek, & Schwarz, 1999; Tondo, Guimarães, Henriques, & Ayub, 2000).

4. Conclusion

CP *S. aureus* high prevalence among tested raw milk, milk products and hand swabs highlighted the necessity of enforcement of hygienic implementations and practices within dairy facilities. Although, the experimental results showed a relatively low MRSA detection in raw milk and dairy products samples, they possibly represent major threats for transmission of this multidrug resistant pathogen. As a future work, subsequent molecular and ecological characterization of isolated MRSA strains should follow.

References

Aarestrup, F. M., Wegener, H. C., & Rosdahl, V. T. (1995). Evaluation of phenotypic and genotypic methods for epidemiological typing of *Staphylococcus aureus* isolates from bovine mastitis in Denmark. *Veterinary Microbiology, 45*, 139–150.

Adesiyun, A. A., Webb, L. A., & Romain, H. T. (1998). Prevalence and characteristics of *Staphylococcus aureus* strains isolates from bulk and composite milk and cattle handlers. *Journal of Food Protection, 61*, 629–632.

Al-Ruaily, M. A., & Khlail, O. M. (2011). Detection of (mecA) gene in methicillin resistant *Staphylococcus aureus* (MRSA) at Prince a/Rhman Sidery hospital, al-Jouf, Saudi Arabia. *Journal of Medical Genetics and Genomics, 3*(3), 41–45.

Anderson, K. L., Lyman, R. L., Bodeis-Jones, S. M., & White, D. G. (2006). Genetic diversity and antimicrobial susceptibility profiles among mastitis-causing *Staphylococcus auras* isolated from bovine milk samples. *American Journal of Veterinary Research, 67*, 1185–1191.

André, M. D. P. B., Campos, M. R. H., Borges, L. J., Kipnis, A., Pimenta, F. C., & Serafini, A. B. (2008). Comparison of *Staphylococcus aureus* isolates from food handlers, raw bovine milk and Minas Frescal cheese by antibiogram and pulsed-field gel electrophoresis following SmaI digestion. *Food Control, 19*, 200–207.

Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy, 48*, 5–16.

AOAC International. (2003). *Official methods of analysis of AOAC International*. 2nd revision (17th ed). Gaithersburg, MD, USA: Association of Analytical Communities.

Aras, Z., Aydinb, I., & Kav, K. (2012). Isolation of methicillin-resistant *Staphylococcus aureus* from caprine mastitis cases. *Small Ruminant Research, 102*, 68–73.

Araújo, V. S., Pagliares, V. A., Queiroz, M. L., & Freitas-Almeida, A. C. (2002). Occurrence of *Staphylococcus* and *enteropathogens* in soft cheese commercialized in the city of Rio de Janeiro, Brazil. *Journal of Applied Microbiology, 92*, 1172–1177.

Asao, T., Kumeda, T., Kawai, T., Shibata, H., Oda, H., Haruki, K., et al. (2003). An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan: estimation of enterotoxin A in the incriminated milk and powdered skim milk. *Epidemiology and Infection, 130*, 30–40.

Atyah, M. A. S., Zamri-Saad, M., & Siti-Zahrah, A. (2010). First report of methicillin-resistant *Staphylococcus aureus* from cage-cultured tilapia (*Oreochromis niloticus*). *Veterinary Microbiology, 144*, 502–504.

Baggett, H. C., Hennessy, T. W., Rudolph, K., Bruden, D., Reasonover, A., Parkinson, A., et al. (2004). Community-onset-methicillin-resistant *Staphylococcus aureus* associated with antibiotic use and the cytotoxin Panton–Valentine leukocidin during a furunculosis outbreak in rural Alaska. *Journal of Infectious Disease, 189*, 1565–1573.

Bryan, F. L. (1998). Risks associated with vehicles of foodborne pathogens and toxins. *Journal of Food Protection, 51*(6), 498–508.

Buzzola, F. R., Quelle, L., Gomez, M. I., Catalano, M., Steele-Moore, L., Berg, D., et al. (2001). Genotypic analysis of *Staphylococcus aureus* from milk of dairy cows with mastitis in Argentina. *Epidemiology and Infection, 126*, 445–452.

Calfee, D. P., Durbin, L. J., & Germanson, T. P. (2003). Spread of methicillin-resistant *Staphylococcus aureus* (MRSA) among household contacts of individuals with nosocomially acquired MRSA. *Infection Control & Hospital Epidemiology, 24*, 422–426.

Cremonesi, P., Perez, G., Pisoni, G., Moroni, P., Morandi, S., Luzzana, M., et al. (2007). Detection of enterotoxigenic *Staphylococcus aureus* isolates in raw milk cheese. *Letters in Applied Microbiology, 45*, 586–591.

Cui, L., Murakami, H., Kuwahara-Arai, K., Hanaki, H., & Hiramatsu, K. (2000). Contribution of a thickened cell wall and its glutamine nonami-dated component to the vancomycin resistance expressed by *Staphylococcus aureus* Mu50. *Antimicrobial Agents and Chemotherapy, 44*, 2276–2285.

De Boer, E., Zwartkruis, J. T., Wit, B., Huijsdens, X. W., de Neeling, A. J., Bosch, T., et al. (2009). Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. *International Journal of Food Microbiology, 134*, 52–56.

Domaracki, B. E., Evans, A. M., & Venezia, R. A. (2000). Vancomycin and oxacillin Synergy for methicillin-resistant *Staphylococci*. *Antimicrobial Agents and Chemotherapy, 44*(5), 1394–1396.

Doufour, P., Gillet, Y., Bes, M., Lina, G., Vandenesch, F., Floret, D., et al. (2002). Community-acquired methicillin-resistant *Staphylococcus aureus* infections in France: emergence of a single clone that produces Panton–Valentine leukocidin. *Clinical Infectious Disease, 35*, 819–824.

Erskine, R. J., Walker, R. D., Bolin, C. A., Bartlett, P. C., & White, D. G. (2002). Trends in antibacterial susceptibility of mastitis pathogens during a seven-year period. *Journal of Dairy Science, 85*, 1111–1118.

Frank, A. L., Marcinak, J. F., Mangat, P. D., & Schreckenberg, P. C. (1999). Community acquired and clindamycin-susceptible methicillin-resistant *Staphylococcus aureus* in children. *Pediatric Infectious Disease Journal, 18*, 993–1000.

Gianneechini, R. E., Concha, C., & Franklin, A. (2002). Antimicrobial susceptibility of udder pathogens isolated from dairy herds in the west littoral region of Uruguay. *Acta Veterinaria Scandinavica, 43*, 31–41.

Gouloumès, C., Bes, M., Renaud, F., Lina, B., Reverdy, M. E., Brun, Y., et al. (1996). Phenotypic and genotypic (pulsed-field gel eletrophoresis) characteristics of enterotoxin-A-producing *Staphylococcus aureus* strains. *Research in Microbiology, 147*, 263–271.

Groom, A. V., Wolsey, D. H., Naimi, T. S., Smith, K., Johnson, S., Boxrud, D., et al. (2001). Community-acquired methicillin-resistant *Staphylococcus aureus* in a rural America Indian community. *Journal of American Medical Association, 286*, 1201–1205.

Hanson, B. M., Dressler, A. E., Harper, A. L., Scheibel, R. P., Wardyn, S. E., Roberts, L. K., et al. (2011). Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. *Journal of Infection and Public Health, 4*, 169–174.

Headrick, M. L., Korangy, S., Bean, N. H., Angulo, F. G., Altekruse, S. F., Potter, M. E., et al. (1998). The epidemiology of raw milk-associated foodborne disease outbreaks reported in the United States, 1973 through 1992. *American Journal of Public Health, 88*, 1219–1221.

Hendriksen, R. S., Mevius, D. J., Schroeter, A., Teale, C., Meunier, D., Butaye, P., et al. (2008). Prevalence of antimicrobial resistance among bacterial pathogens isolated from cattle in different European countries: 2002–2004. *Acta Veterinaria Scandinavica, 50*, 28.

Jones, T. F., Kellum, M. E., Porter, S. S., Bell, M., & Schaffner, W. (2002). An outbreak of community-acquired foodborne illness caused by methicillin-resistant *Staphylococcus aureus*. *Emerging Infectious Disease, 8*, 82–84.

Jorgensen, H. J., Mork, T., Hogasen, H. R., & Rovik, L. M. (2005). Enterotoxigenic *Staphylococcus aureus* in bulk milk in Norway. *Journal of Applied Microbiology, 99*, 158–166.

Juhàsz-Kaszanyitzky, E., Janosi, S., Somogyi, P., Dan, A., van der Graaf-Van Bloois, L., van Duijkeren, E., et al. (2007). MRSA transmission between cows and humans. *Emerging Infectious Disease Journal, 13*, 630–632.

Kitai, S., Shimizu, A., Kawano, J., Sato, E., Nakano, C., Uji, T., et al. (2005). Characterization of methicillin resistant *Staphylococcus aureus* isolated from retail raw chicken meat in Japan. *Journal of Veterinary Medical Science, 67*, 107–110.

Klevens, R. M., Morrison, M. A., Nadle, J., Petit, S., Gershman, K., Ray, S., et al. (2007). Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *The Journal of the American Medical Association, 298*, 1763–1771.

Kluytmans, J., Van Leeuwen, W., Goessen, W., Hollis, R., Messer, S., Herwaldt, L., et al. (1995). Food-initiated outbreak of methicillin-resistant *Staphylococcus aureus* analyzed by pheno-and genotyping. *Journal of Clinical Microbiology, 33*(5), 1121–1128.

Krziwanek, K., Metz-Gercek, S., & Mittermayer, H. (2009). Methicillin-resistant *Staphylococcus aureus* ST398 from human patients, upper Australia. *Emerging Infectious Disease, 15*, 766–769.

Kuplulu, O., Sarımehmetoglu, B., & Celık, T. H. (2004). Determination of the enterotoxigenicity of coagulase positive *staphylococci* isolated from cheese by ELISA. *Milchwissenschaft-Milk Science International, 59*(1–2), 17–19.

Kwon, N. H., Park, K. T., Jung, W. K., Youn, H. Y., Lee, Y., Kim, S. H., et al. (2006). Characteristics of methicillin resistant *Staphylococcus aureus* isolated from chicken meat and hospitalized dogs in Korea and their epidemiological relatedness. *Veterinary Microbiology, 117*, 304–312.

Kwon, N. H., Park, K. T., Moon, J. S., Jung, W. K., Kim, S. H., Kima, J. M., et al. (2005). 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. *Journal of Antimicrobial Chemotherapy, 56*, 624–632.

Labrou, M., Michail, G., Ntokou, E., Theodore, E., Pournaras, P. S., & Tsakris, A. (2012). Activity of oxacillin versus vancomycin against oxacillin-susceptible mecA-positive *Staphylococcus aureus* clinical isolates by population analyses, time-kill assays and a murine thigh infection model. *Antimicrobial Agents and Chemotherapy, 56*(6), 3388–3391.

Lange, C., Cardoso, M., Senczek, D., & Schwarz, S. (1999). Molecular subtyping of *Staphylococcus aureus* isolates from cases of bovine mastitis in Brazil. *Veterinary Microbiology, 67*, 127–141.

Lee, J. H. (2003). Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. *Applied and Environmental Microbiology, 69*, 6489–6494.

Lee, J. H. (2006). Occurrence of methicillin-resistant *Staphylococcus aureus* strains from cattle and chicken, and analysis of their mecA, mecR1 and mecI genes. *Veterinary Microbiology, 114*, 155–159.

Lim, S. K., Nam, J. M., Park, H. J., Lee, H. S., Choi, M. J., Jung, S. C., et al. (2010). Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* in raw meat in Korea. *Journal of Microbiology and Biotechnology, 20*, 775–778.

Lowy, F. D. (1998). *Staphylococcus aureus* infection. *New England Journal of Medicine, 339*, 520–532.

Lozano, C., Lopez, M., Gomez-Sanz, E., Ruiz-Larrea, F., Torres, C., & Zarazaga, M. (2009). Detection of methicillin-resistant *Staphylococcus aureus* ST398 in food samples of animal origin in Spain. *Journal of Antimicrobial Chemotherapy, 64*, 1325–1326.

Makita, K., Desissa, F., Teklu, A., Zewde, G., & Grace, D. (2012). Risk assessment of staphylococcal poisoning due to consumption of informally-marketed milk and home-made yoghurt in Debre Zeit, Ethiopia. *International Journal of Food Microbiology, 153*, 135–141.

Makovec, J. A., & Ruegg, P. L. (2003). Antimicrobial resistance of bacteria isolated front dairy cow milk samples submitted for bacterial culture: 8905 samples (1994–2001). *Journal of the American Veterinary Medical Association, 222*, 1582–1589.

Monecke, S., Kuhnert, P., Hotzel, H., Slickers, P., & Ehricht, R. (2007). Microarray based study on virulence-associated genes and resistance determinants of *Staphylococcus aureus* isolates from cattle. *Veterinary Microbiology, 125*, 128–140.

Moon, J. S., Lee, A. R., Kang, H. M., Lee, E. S., Kim, M. N., Paik, Y. H., et al. (2007). Phenotypic and genetic antibiogram of methicillin-resistant *staphylococci* isolated from bovine mastitis in Korea. *Journal of Dairy Science, 90*, 1176–1185.

Normanno, G., Corrente, M., La Salandra, G., Dambrosio, A., Quaglia, N. C., Parisi, A., et al. (2007). Methicillin-resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product in Italy. *International Journal of Food Microbiology, 117*, 219–222.

Normanno, G., Firinu, A., Virgilio, S., Mula, G., Dambrosio, A., Poggiu, A., et al. (2005). Coagulase-positive *Staphylococci* and *Staphylococcus aureus* in food products marketed in Italy. *International Journal of Food Microbiology, 98*, 73–79.

O'Brien, F. G., Pearman, J. W., Gracey, M., Piley, T. V., & Grubb, W. E. (1999). Community strain of methicillin-resistant *Staphylococcus aureus* involved in a hospital outbreak. *Journal of Clinical Microbiology, 37*, 2858–2862.

Oliveira, D. C., & Lencastre, H. (2002). Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy, 46*, 2155–2161.

Pu, S., Han, F., & Ge, B. (2009). Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from Louisiana retail meats. *Applied and Environmental Microbiology, 75*, 265–267.

Rapini, L. S., Cerqueira, M. M. O. P., Carmo, L. S., Veras, J. F., & Souza, M. R. (2005). Presence of *Staphylococcus* strains producer of enterotoxins and toxic shock toxin syndrome isolated from goat's cheese handlers. *Brazilian Journal of Veterinary and Animal Sciences, 57*, 825–829.

Reacher, M. H., Shah, A., Livermore, D. M., Wale, M. C., Graham, C., Johnson, A. P., et al. (2000). Bacteraemia and antibiotic resistance of its pathogens reported in England and Wales between 1990 and 1998: trend analysis. *British Medical Journal, 320*, 213–216.

Rosengren, A., Fabricius, A., Guss, B., Sylven, S., & Lindqvist, R. (2010). Occurrence of foodborne pathogens and characterization of *Staphylococcus aureus* in cheese produced on farm-dairies. *International Journal of Food Microbiology, 144*, 263–269.

Salmenlinna, S., Lyytikäinen, O., & Vuopio-Varkila, J. (2002). Community-acquired methicillin-resistant *Staphylococcus aureus* in Finland. *Emerging Infectious Disease, 8*, 602–607.

SAS Institute Inc. (2006). *SAS|STAT user's guide*. Cary, NC: SAS Institute Inc.

Sawant, A. A., Sordillo, L. M., & Jayarao, B. M. (2005). A survey on antibiotic usage in dairy herds in Pennsylvania. *Journal of Dairy Science, 88*, 2991–2999.

Schoeller, N. P., & Ingham, S. C. (2001). Comparison of the Baird-Parker agar and 3M Petrifilm rapid *S. aureus* count plate methods for detection and enumeration of *Staphylococcus aureus*. *Food Microbiology, 18*, 581–587.

Sospedra, I., Mañes, J., & Soriano, J. M. (2012). Report of toxic shock syndrome toxin 1 (TSST-1) from *Staphylococcus aureus* isolated in food handlers and surfaces from foodservice establishments. *Ecotoxicology and Environmental Safety, 80*, 288–290.

Stastkova, Z., Karpiskova, S., & Karpiskova, R. (2009). Occurrence of methicillin-resistant strains of *Staphylococcus aureus* at a goat breeding farm. *Veterinarni Medicina-International Journal for Veterinary and Biomedical Science, 54*, 419–426.

Takeuchi, S., Ishiguro, K., Ikegami, M., Kaidoh, T., & Hayakawa, Y. (1998). Production of toxic shock syndrome toxin by *Staphylococcus aureus* isolated from mastitic cow's milk and farm bulk milk. *Veterinary Microbiology, 59*, 251–258.

Tondo, E. C., Guimaráes, M. C., Henriques, J. A., & Ayub, M. A. (2000). Assessing and analyzing contamination of a dairy products processing plant by *Staphylococcus aureus* using antibiotic resistance and PFGE. *Canadian Journal of Microbiology, 46*, 1108–1114.

Tranter, H. S. (1990). Foodborne staphylococcal illness. *Lancet, 336*(8722), 1044–1046.

Van Loo, I. H., Diederen, B. M., Savelkoul, P. H., Woudenberg, J. H., Roosendaal, R., van Belkum, A., et al. (2007). Methicillin-resistant *Staphylococcus aureus* in meat products, The Netherlands. *Emerging Infectious Diseases, 13*, 1753–1755.

Vanderhaeghen, W., Cerpentier, T., Adriaensen, C., Vicca, J., Hermans, K., & Butaye, P. (2010). Methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 associated with clinical and subclinical mastitis in Belgian cows. *Veterinary Microbiology, 144*, 166–171.

Viçosa, G. N., Moraes, P. M., Yamazi, A. K., & Nero, L. A. (2010). Enumeration of coagulase and thermonuclease-positive *Staphylococcus* spp. in raw milk and fresh soft cheese: an evaluation of Baird-Parker agar, Rabbit Plasma Fibrinogen agar and the Petrifilm Staph Express count system. *Food Microbiology, 27*, 447–452.

Virgin, J. E., Van Slyke, T. M., Lombard, J. E., & Zadoks, R. N. (2009). Methicillin-resistant *Staphylococcus aureus* detection in US bulk tank milk. Short communication. *Journal of Dairy Science, 92*, 4988–4991.

Wannet, W., Heck, M., Pluister, G., Spalburg, E., & De Neeling, A. J. (2004). Panton-valentine leucocidine positive MRSA in 2003: the Dutch situation. *European Surveillance, 9*(11), 28–29.

Warke, R., Kamat, A., Kamat, M., & Thomas, P. (2000). Incidence of pathogenic psychrotrophs in ice creams sold in some retail outlets in Mumbai, India. *Food Control, 11*, 77–83.

Weese, J. S., Avery, B. P., & Reid-Smith, R. J. (2010). Detection and quantification of methicillin-resistant *Staphylococcus aureus* (MRSA) clones in retail meat products. *Letters in Applied Microbiology, 51*, 338–342.

Weese, J. S., Reid-Smith, R., Rousseau, J., & Avery, B. P. (2010). Methicillin-resistant *Staphylococcus aureus* (MRSA) contamination of retail pork. *The Canadian Veterinary Journal, 51*, 749–752.

Wehr, H. M., & Frank, J. F. (2004). *Staphylococcus aureus direct* plate count. In *Standard methods for the examination of dairy products* (17th ed). (pp. 132–135). Washington D.C: American Public Health Press.

Witte, W., Cuny, C., Strommenger, B., Braulke, C., & Heuck, D. (2004). Emergence of community-acquired MRSA in Germany. *European Surveillance, 9*, 16–18.

Yesim, C. H., & Çelik, T. H. (2012). Detection of enterotoxigenic and antimicrobial resistant *S. aureus* in Turkish cheeses. *Food Control, 24*, 100–103.

Zhang, S., Iandolo, J., & Stewart, C. (1998). The enterotoxin D plasmid of *Staphylococcus aureus* encodes a second enterotoxin determinant (sej). *FEMS Microbiology Letters, 168*, 227–233.