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Identification of Methicillin-Resistant *Staphylococcus aureus* in Bulk Tank Milk

Erhan KEYVAN[1]*, Ozen YURDAKUL[1], Ahu DEMIRTAS[2], Halil YALCIN[1], Nuket BILGEN[3]

Abstract

Staphylococcus aureus is the leading cause of intoxications in humans. Also, methicillin-resistant *S. aureus* (MRSA) is an emerging problem in food-producing animals. The presence of MRSA in milk may create a risk to public health. The aim of this study was to evaluate the presence of MRSA in bulk tank milk samples. One hundred and twenty bulk tank milk samples were analyzed using the convenient sampling method. The isolates were confirmed by real-time polymerase chain reaction (RT-PCR) targeting *nuc* and *mecA* gene in *S. aureus*. Antibiotic resistance profiles of the isolates were tested by disc agar diffusion method. In the current study, *S. aureus* was detected in 44 (36.66%) of the samples. The presence of the *mecA* gene was found to be positive in 40 (75.4%) of the 53 *S. aureus* isolates. As a result of the study, a high incidence of MRSA was detected in bulk tank milk samples. Antibiotic-resistant bacteria are at risk of being transferred to humans via milk. For safe and healthy milk consumption, uncontrolled use of antibiotics in dairy cows should be avoided.

Keywords: antibiotic resistance; bulk tank milk; MRSA.

Practical Application: Monitoring *S. aureus* and MRSA contamination levels in bulk tank milk.

1 Introduction

Foodborne outbreaks caused by milk and dairy products have led to hospitalizations and deaths for human beings (Painter et al., 2013). *Staphylococcus aureus* is an important causative agent of toxin-mediated food poisoning, invasiveness, and antibiotic resistance (Le Loir et al., 2003; Gundogan & Avci, 2014). It can cause skin and wound infections or subclinical mastitis in dairy animals (Kluytmans et al., 1997; Kreausukon et al., 2012; Lee et al., 2014). The presence of biofilm producing ability of *S. aureus* in milk and milking environment is a public health concern for the consumers (Lee et al., 2014; Lee et al., 2016). Also, thermostable enterotoxins prior to pasteurization of raw milk produced by *S. aureus* may cause staphylococcal food poisoning in humans (Hein et al., 2005; Lee et al., 2012).

Antibiotic resistance of bacteria has become a major public health problem all over the world owing to the massive use of antibiotics in feed to promote growth in both agriculture and livestock animals (Normanno et al., 2007; Oniciuc et al., 2017). The antibiotic resistance of methicillin and other beta lactam groups result from a modified penicillin binding protein (PBP 2a) which has a low affinity for the beta lactams. This protein is encoded by *mecA* and *mecC* genes which are localized in a mobile genetic element called Staphylococcal Casette Chromosome *mec* (SCC*mec*) (Paterson et al., 2014). Recent studies reported that the *mecC* gene is required for confirmation of methicillin-resistant *S. aureus* (MRSA) because 70% identity to *mecA* gene (Garcia-Alvarez et al., 2011; Shore et al., 2011; Stegger et al., 2012; Ito et al., 2012). Also, *mecC*-MRSA has been reported in the likelihood of zoonotic transmission (Petersen et al., 2013; Harrison et al., 2013).

MRSA is an emerging pathogen in livestock animals that can infect humans and has become a growing concern for public health. MRSA has been isolated as a mastitis pathogen in bulk tank milk (Moon et al., 2007; Nam et al., 2011; Spohr et al., 2010; Doulgeraki et al., 2017). Hospital-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) infections have been reported in initial studies. Another group called livestock-associated MRSA (LA-MRSA) was first isolated in dairy cattle (Paterson et al., 2012) In recent years, MRSA has become a major concern as an emerging pathogen in livestock that can transfer the methicillin resistance to humans via food or milk (Nemati et al., 2008; Pereira et al., 2009; Wendlandt et al., 2013; Vincze et al., 2014; Wang et al., 2014; Kraushaar & Fetsch 2014; European Food Safety Authority, 2015; Lozano et al., 2016; Asiimwe et al., 2017; Aqib et al., 2017; Can et al., 2017a; Tenhagen et al., 2018).

Even though many researchers have reported the isolation of MRSA from livestock animals and foods of animal origin, the effect of MRSA in food-related problems is very rare; however, there are some concerns about foodborne MRSA infections (EFSA, 2009; Doyle et al., 2011; Herrera et al., 2016). The aims of this study were to evaluate the prevalence of *S. aureus*, antibiotic resistance profiles, and related *mecA* genes among these isolates of *S. aureus* from bulk tank milk samples in Turkey.

2 Materials and methods

2.1 Milk samples

In this study, a total of 120 bulk tank milk samples were obtained between October 2016 and September 2017 in Burdur province, located on the southern side of Turkey. The samples

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[1]*Department of Food Hygiene and Technolgy, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Turkey.*
[2]*Department of Physiology, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Turkey.*
[3]*Department of Genetics, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey.*
*Corresponding author: erhankeyvan@mehmetakif.edu.tr

were taken in sterile plastic collection tubes and transported to the laboratory under refrigeration (4-8 °C), and the samples were directly processed for further analyzes.

2.2 Isolation and identification of S. aureus

Bulk tank milk samples (0.1 mL) were plated on rabbit plasma fibrinogen agar medium (BP-RPF, Oxoid, Italy) and incubated at 37 °C for 24-48 hours. Colonies developing a typical coagulase halo on BP-RPF agar were suspected of *S. aureus*. Two suspected colonies from samples were grown in Brain Heart Infusion broth (BHI, Oxoid, CM1135) at 37 °C for 24 hours. Presumptive colonies of *S. aureus* were confirmed with some properties (Gram staining, catalase reaction, ß hemolysis, DNase, and the ability to coagulate rabbit plasma) (International Organization for Standardization, 1999).

2.3 DNA isolation

Overnight cultures in Brain Heart Infusion broth were used for the DNA isolation. For this purpose, 2 ml of broth cultures were centrifuged at 5 000 g. for 10 minutes and the supernatant were discarded. Bacterial pellets were washed twice with 1 ml of saline solution and centrifuged again. Bacterial pellets were resuspended in 180 µl Tris EDTA buffer (Sigma-Aldrich, 93283) containing 18 µl of lysostaphin (0.5 U/µl, Sigma, L7386) and incubated at 37 °C for 1 hour (Akineden et al., 2008). Genomic DNA was extracted according to GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA) manufacturer's protocol. A nano-drop (NanoDrop2000-Thermoscientific™) technique was used to define the quantification of DNA.

2.4 PCR analysis

In the current study, suspected *S. aureus* isolates were detected by species specific *nuc* gene for confirmation. Then, confirmed *S. aureus* isolates were analyzed for detection of the *mecA* gene. Extracted DNA was subjected to real time polymerase chain reaction (RT-PCR) using the *nuc* and *mecA* gene primers in Table 1. Suspected *S. aureus* isolates were analyzed using the LightCycler® 480 System with a 96-well format (Roche Diagnostics, Tokyo, Japan). The extracted 5 µl DNA was added to 15 µl of LightCycler® 480 SYBR Green I Master (Roche Diagnostics). Thermocycling conditions using the LightCycler system were completed according to the amplification program consisting of an initial denaturation at 95°C for 10 minutes followed by a 45-cycle amplification program heated to 95 °C with a 15-seconds hold, annealing for *nuc* gene at 55 °C, for *mecA* gene at 57 °C with a 10-seconds hold, and extension at 65 °C with a 15-seconds hold.

2.5 Determination of antimicrobial resistance

Confirmed *S. aureus* isolates were tested for antimicrobial susceptibility by the disc agar diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2013). The antibiotic discs were selected in line with the recommendation of CLSI and included penicillin (10 IU/disc), oxacillin (1 µg/disc), tetracycline (30 µg/disc), gentamicin (10 µg/disc), cefoxitin (30 µg/ disc), chloramphenicol (30 µg/disc), erythromycin (15 µg/ disc), ciprofloxacin (5 µg/disc), trimethoprim-sulphamethoxazole (1.25-23.75 µg/disc), and clindamycin (2 µg/disc). The isolates were classified as susceptible, intermediate resistant, and resistant.

2.6 Statistical analysis

The differences between the seasons and total mesophilic aerobic bacteria (TMAB) and *Staphylococcus* spp. levels was determined by one-way analysis of variance (ANOVA) and Tukey's test using the SPSS software package version 15.0 for Windows, $P < 0.05$ was considered statistically significant.

3 Results

3.1 Prevalence of S. aureus

In this study, the prevalence of *nuc* and *mecA* genes in *S. aureus* isolated from bulk tank milk samples are given in tables 2 and 3.

Table 1. *nuc* and *mecA* genes primers.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Reference
nuc	5'-ATA GGG ATG GCT ATC AGT AAT GT -3'	5'-GAC CTG AAT CAG CGT TGT CTT C- 3'	Lem et al. (2001)
mecA	5′-TGG TAT GTG GAA GTT AGA TTG G -3′	5′-GGA TCT GTA CTG GGT TAA TCA G-3′	Mehrotra et al. (2000)

Table 2. Prevalence of *S. aureus* in bulk tank milk.

Total number of *Staphylococcus* spp. isolates	*nuc* gene positive *S. aureus* isolates	*nuc* gene positive *S. aureus* isolates from bulk milk tanks (n=120)
142	53 (37.32%)	44 (36.66%)

Table 3. Prevalence of MRSA in bulk tank milk.

Total number of *S. aureus* isolates	*mecA* gene positive *S. aureus* isolates
53	40 (75.4%)

3.2. Antimicrobial susceptibility

The results of the antibiotic susceptibility test indicated the resistance of the isolates to oxacillin (71.15%), penicillin (69.82%), clindamycin (67.93%) and cefoxitin (67.31%). The most sensitive antibiotics were gentamycin (92.45%), followed by trimethoprim-sulfamethoxazole (86.79%), chloramphenicol (83.01%), ciprofloxacin (79.24%), tetracycline (69.81%) and erythromycin (47.16%).

4 Discussion

Milk contains many bacteria that affect the quality and safety of dairy products (Porcellato et al., 2018). Researches have showed that dairy products such as milk can cause foodborne infections and intoxications. For instance, a study from Malaysia reported that milk samples were contaminated with coliform bacteria (90%), *Eschericia coli* (65%), *S. aureus* (60%), *E. coli* 0157:H7 (33.5%), and *Salmonella* (1.4%) (Chye et al., 2004). Total mesophilic aerobic bacteria in bulk tank milk may indicate the hygienic quality of milk. Milking conditions, seasonal changes, and moisture affect the presence of microorganisms in milk (Elmoslemany et al., 2009). The levels of total bacteria in bulk tank milk may be higher in summer seasons in comparison to winter seasons (Elmoslemany et al., 2010). In our study, TMAB levels of bulk tank milk samples in the winter were higher than those in the summer and the effects of seasonal differences on microorganisms are statistically important. Because of the effective cooling conditions of bulk tank milk in Burdur province, it can be said that seasonal changes have a limited effect on the development of microorganisms because milk hygiene and equipment cleaning are more effective.

Although *S. aureus* is a pathogenic bacterium, it can be found in the normal mucosa of healthy humans and animals, and may cause mastitis in dairy cows. (Kluytmans et al., 1997; Kreausukon et al., 2012). In our study, *S. aureus* was detected in 37.32% of the isolates. The presence of *S. aureus* in bulk milk tank in the current study was reported to be higher by 75%, 70.4% 55.7%, 55.26%, and 39.8%, in comparison to previous study from Norway, Brazil, Czech Republic, Algeria, and Kosova, respectively (Jørgensen et al., 2005; Rall et al., 2008; Zouharova & Rysanek, 2008; Chaalal et al., 2016; Mehmeti et al., 2017). By contrast, researchers from Switzerland, Iran, and Brazil reported lower levels of *S. aureus* than the current study (Muehlherr et al., 2003; Fagundes et al., 2010; Lee et al., 2012; Jamali et al., 2015). This indicates that the milking conditions and the hygienic quality of bulk tank milk may cause differences between levels of *S. aureus* isolates in different countries.

Mastitis is capable of affecting the mammary glands and it changes milk composition (Korhonen & Kaartinen, 1995). It is a disease with high treatment costs which makes mastitis an economical concern for dairy farmers (Duarte et al., 2015). *S. aureus* is one of the main pathogens causing mastitis in dairy animals, which leads to overuse of antimicrobial agents (Roberson et al., 1998; Peles et al., 2007; Olde Riekerink et al., 2006; Barkema et al., 2009; Gomes & Henriques, 2016). The overuse of β-lactam group antibiotics for prophylactic and mastitis treatment in dairy cows may cause MRSA in milk and dairy products (Levy, 1992; Sawant et al., 2005). Previous studies have reported MRSA in mastitis: 48.3% by Guimarães et al. (2017), 15.5% by Wang et al. (2015), 11.6% by Jamali et al. (2014), and 2.5% by Moon et al. (2007). It is also possible for MRSA to contaminate food products without any changes in the milk (Parisi et al., 2016). Transmission of the MRSA to humans through the consumption of milk with mastitis or direct contact with dairy cows may generate serious risks to food safety and public health.

Antibiotic resistant bacteria has become an important public health problem all over the world (Normanno et al., 2007) In this study, according to disk diffusion test results, *S. aureus* isolates were detected to be resistant to a majority of antibiotics, such as oxacillin, penicillin, clindamycin, and cefoxitin at 71.1%, 69.8%, 67.9%, and 67.3%, respectively. Tenhagen et al. (2018) reported that 100% of MRSA isolates are resistant to cefoxitin and penicillin in Germany. In contrast to this study, Jamali et al. (2015) reported the levels of antibiotic resistance were lower for oxacillin, penicillin, clindamycin, and cefoxitin at 13%, 44.4%, 13.6%, and 4.9%, respectively. In Jordan, the levels of oxacillin, penicillin, clindamycin, and gentamicin resistance were observed to be lower than this study (Obaidat et al., 2018). Can et al. (2017b) reported the resistant levels of oxacillin (46.4%), cefoxitin (50%), penicillin (60.7%), and chloramphenicol (5.3%) lower than this study in Turkey. By comparing the results from other studies, it seems that the type of antibiotics used for the treatment of animals may cause different results between countries.

In the current study, a high rate of *mecA* MRSA strains was detected in bulk tank milk samples. Researchers from several countries have reported the rate of MRSA in milk as 50%, 13%, 9.7%, 4.4%, 2.5%, and 0.7% (Kreausukon et al., 2012; Jamali et al., 2015; Parisi et al., 2016; Giacinti et al., 2017; Asiimwe et al., 2017; Tenhagen et al., 2018). Previous studies from Turkey have indicated lower rates than the current study: Ektik et al. (2017) 14.28%, Sayin et al. (2016) 18.6%, Siiriken et al. (2016) 13.3% and Buyukcangaz et al. (2013) 15.8%. By contrast, Can et al. (2017b) reported the rate of MRSA to be much higher at 90%. In comparison with earlier studies, MRSA is still a public health problem for milk and dairy products. Surveillance programs and adopting assurance quality systems are required for controlling *S. aureus*, MRSA and other pathogens in the dairy industry (Silva et al., 2010; Cusato et al., 2014).

In conclusion, the transmission of MRSA to milk and dairy products poses the risk of spreading the antimicrobial resistant bacteria to the general population. The industry needs to apply measures to ensure contamination is minimized. Multi-drug resistant bacteria identified in milk and dairy products should be monitored. Uncontrolled antibiotic use in dairy cattle should be avoided for healthy milk production. The correct application of antibiotics should be evaluated in the udder infections of dairy cows. These are just some of the ways that the spread of MRSA can be alleviated.

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References

Akineden, Ö., Hassan, A. A., Schneider, E., & Usleber, E. (2008). Enterotoxigenic properties of *Staphylococcus aureus* isolated from goats' milk cheese. *International Journal of Food Microbiology*, 124(2), 211-216. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.03.027. PMid:18455257.

Aqib, A. I., Ijaz, M., Anjum, A. A., Malik, M. A. R., Mehmood, K., Farooqi, S. H., & Hussain, K. (2017). Antibiotic susceptibilities and prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from bovine milk in Pakistan. *Acta Tropica*, 176, 168-172. http://dx.doi.org/10.1016/j.actatropica.2017.08.008. PMid:28797802.

Asiimwe, B. B., Baldan, R., Trovato, A., & Cirillo, D. M. (2017). Prevalence and molecular characteristics of *Staphylococcus aureus*, including methicillin resistant strains, isolated from bulk can milk and raw milk products in pastoral communities of South-West Uganda. *BMC Infectious Diseases*, 17(1), 422-429. http://dx.doi.org/10.1186/s12879-017-2524-4. PMid:28610560.

Barkema, H. W., Green, M. J., Bradley, A. J., & Zadoks, R. N. (2009). Invited review: The role of contagious disease in udder health. *Journal of Dairy Science*, 92(10), 4717-4729. http://dx.doi.org/10.3168/jds.2009-2347. PMid:19762787.

Buyukcangaz, E., Kahya, S., Sen, A., Seyrekintas, K., Eyigor, A., Temelli, S., & Carli, K. T. (2013). *MecA* gene prevalence in *Staphylococcus aureus* isolates from dairy cows in Turkey. *Journal of Biodiversity and Environmental Sciences*, 7(21), 183-190.

Can, H. Y., Elmalı, M., & Ergün, Y. (2017b). Methicillin-resistant *Staphylococcus aureus* in milk from dairy cows with chronic mastitis. *Eurasian Journal of Veterinary Sciences*, 33(4), 255-259.

Can, H. Y., Elmalı, M., & Karagöz, A. (2017a). Molecular typing and antimicrobial susceptibility of *Staphylococcus aureus* strains isolated from raw milk, cheese, minced meat, and chicken meat samples. *Korean Journal for Food Science of Animal Resources*, 37(2), 175-180. http://dx.doi.org/10.5851/kosfa.2017.37.2.175. PMid:28515641.

Chaalal, W., Aggad, H., Zidane, K., Saidi, N., & Kihal, M. (2016). Antimicrobial susceptibility profiling of *Staphylococcus aureus* isolates from milk. *British Microbiology Research Journal*, 13(3), 1-7. http://dx.doi.org/10.9734/BMRJ/2016/24064.

Chye, F. Y., Abdullah, A., & Ayob, M. K. (2004). Bacteriological quality and safety of raw milk in Malaysia. *Food Microbiology*, 21(5), 535-541. http://dx.doi.org/10.1016/j.fm.2003.11.007.

Clinical and Laboratory Standards Institute – CLSI. (2013). *M 100-S23: Performance Standards for Antimicrobial Susceptibility Testing*. Wayne: CLSI.

Cusato, S., Gameiro, A. H., Sant'Ana, A. S., Corassin, C. H., Cruz, A. G., & De Oliveira, C. A. F. (2014). Assessing the costs involved in the implementation of GMP and HACCP in a small dairy factory. *Quality Assurance and Safety of Crops & Foods*, 6(2), 135-139. http://dx.doi.org/10.3920/QAS2012.0195.

Doulgeraki, A. I., Di Ciccio, P., Ianieri, A., & Nychas, G. E. (2017). Methicillin-resistant food-related *Staphylococcus aureus*: A review of current knowledge and biofilm formation for future studies and applications. *Research in Microbiology*, 168(1), 1-15. http://dx.doi.org/10.1016/j.resmic.2016.08.001. PMid:27542729.

Doyle, M. E., Hartmann, F. A., & Wong, A. C. L. (2011). *White paper on sources of methicillin-resistant Staphylococcus aureus (MRSA) and other methicillin-resistant staphylococci: Implications for our food supply*. Madison: Food Research Institute, University of Wisconsin.

Duarte, C. M., Freitas, P. P., & Bexiga, R. (2015). Technological advances in bovine mastitis diagnosis: an overview. *Journal of Veterinary Diagnostic Investigation*, 27(6), 665-672. http://dx.doi.org/10.1177/1040638715603087. PMid:26450837.

Ektik, N., Gökmen, M., & Çıbık, R. (2017). The prevalence and antibiotic resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) in milk and dairy products in Balikesir, Turkey. *Journal of the Hellenic Veterinary Medical Society*, 68(4), 613-620. http://dx.doi.org/10.12681/jhvms.16062.

Elmoslemany, A. M., Keefe, G. P., Dohoo, I. R., & Jayarao, B. M. (2009). Risk factors for bacteriological quality of bulk tank milk in Prince Edward Island dairy herds. Part 1. Overall risk factors. *Journal of Dairy Science*, 92(6), 2634-2643. http://dx.doi.org/10.3168/jds.2008-1812. PMid:19447996.

Elmoslemany, A. M., Keefe, G. P., Dohoo, I. R., Wichtel, J. J., Stryhn, H., & Dingwell, R. T. (2010). The association between bulk tank milk analysis for raw milk quality and on-farm management practices. *Preventive Veterinary Medicine*, 95(1-2), 32-40. http://dx.doi.org/10.1016/j.prevetmed.2010.03.007. PMid:20381889.

European Food Safety Authority – EFSA. (2009). Scientific opinion of the panel on biological hazards on a request from the European Commission on assessment of the public health significance of methicillin resistant *Staphylococcus aureus* (MRSA) in animals and foods. *EFSA Journal*, 993, 1-73.

European Food Safety Authority – EFSA. (2015). EU summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2013. *EFSA Journal*, 13(2), 4036.

Fagundes, H., Barchesi, L., Nader, A. Fo, Ferreira, L. M., & Oliveira, C. A. F. (2010). Occurrence of *Staphylococcus aureus* in raw milk produced in dairy farms in São Paulo state, Brazil. *Brazilian Journal of Microbiology*, 41(2), 376-380. http://dx.doi.org/10.1590/S1517-83822010000200018. PMid:24031507.

Garcia-Alvarez, L., Holden, M. T., Lindsay, H., Webb, C. R., Brown, D. F., Curran, M. D., Walpole, E., Brooks, K., Pickard, D. J., Teale, C., Parkhill, J., Bentley, S. D., Edwards, G. F., Girvan, E. K., Kearns, A. M., Pichon, B., Hill, R. L., Larsen, A. R., Skov, R. L., Peacock, S. J., Maskell, D. J., & Holmes, M. A. (2011). Meticillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: A descriptive study. *The Lancet. Infectious Diseases*, 11(8), 595-603. http://dx.doi.org/10.1016/S1473-3099(11)70126-8. PMid:21641281.

Giacinti, G., Carfora, V., Caprioli, A., Sagrafoli, D., Marri, N., Giangolini, G., & Feltrin, F. (2017). Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* carrying *mecA* or *mecC* and methicillin-susceptible *Staphylococcus aureus* in dairy sheep farms in central Italy. *Journal of Dairy Science*, 100(10), 7857-7863. http://dx.doi.org/10.3168/jds.2017-12940. PMid:28780098.

Gomes, F., & Henriques, M. (2016). Control of bovine mastitis: Old and recent therapeutic approaches. *Current Microbiology*, 72(4), 377-382. http://dx.doi.org/10.1007/s00284-015-0958-8. PMid:26687332.

Guimarães, F. F., Manzi, M. P., Joaquim, S. F., Richini-Pereira, V. B., & Langoni, H. (2017). Outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA)-associated mastitis in a closed dairy herd. *Journal of Dairy Science*, 100(1), 726-730. http://dx.doi.org/10.3168/jds.2016-11700. PMid:27837983.

Gundogan, N., & Avci, E. (2014). Occurrence and antibiotic resistance of *Escherichia coli, Staphylococcus aureus* and *Bacillus cereus* in raw milk and dairy products in Turkey. *International Journal of Dairy Technology*, 67(4), 562-569. http://dx.doi.org/10.1111/1471-0307.12149.

Harrison, E. M., Paterson, G. K., Holden, M. T., Larsen, J., Stegger, M., Larsen, A. R., Petersen, A., Skov, R. L., Christensen, J. M., Bak Zeuthen, A., Heltberg, O., Harris, S. R., Zadoks, R. N., Parkhill, J., Peacock, S. J., & Holmes, M. A. (2013). Whole genome sequencing

identifies zoonotic transmission of MRSA isolates with the novel *mecA* homologue *mecC*. *EMBO Molecular Medicine*, 5(4), 509-515. http://dx.doi.org/10.1002/emmm.201202413. PMid:23526809.

Hein, I., Jorgensen, H. J., Loncarevic, S., & Wagner, M. (2005). Quantification of *Staphylococcus aureus* in unpasteurised bovine and caprine milk by real-time PCR. *Research in Microbiology*, 156(4), 554-563. http://dx.doi.org/10.1016/j.resmic.2005.01.003. PMid:15862454.

Herrera, F. C., García-López, M. L., & Santos, J. A. (2016). Characterization of methicillin-resistant *Staphylococcus aureus* isolated from raw milk fresh cheese in Colombia. *Journal of Dairy Science*, 99(10), 7872-7876. http://dx.doi.org/10.3168/jds.2016-11322. PMid:27423957.

International Organization for Standardization – ISO. (1999). *ISO 6888-2: Microbiology of food animal feeding stuffs- Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species). Part 2: Technique using rabbit plasma fibrinogen agar medium. International Organization for Standardization. Geneva: ISO.*

Ito, T., Hiramatsu, K., Tomasz, A., de Lencastre, H., Perreten, V., Holden, M. T., Coleman, D. C., Goering, R., Giffard, P. M., Skov, R. L., Zhang, K., Westh, H., O'Brien, F., Tenover, F. C., Oliveira, D. C., Boyle-Vavra, S., Laurent, F., Kearns, A. M., Kreiswirth, B., Ko, K. S., Grundmann, H., Sollid, J. E., John, J. F., Jr Daum, R., Soderquist, B., & Buist, G. (2012). Guidelines for reporting novel *mecA* gene homologues. *Antimicrobial Agents and Chemotherapy*, 56(10), 4997-4999. http://dx.doi.org/10.1128/AAC.01199-12. PMid:22869575.

Jamali, H., Paydar, M., Radmehr, B., Ismail, S., & Dadrasnia, A. (2015). Prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from raw milk and dairy products. *Food Control*, 54, 383-388. http://dx.doi.org/10.1016/j.foodcont.2015.02.013.

Jamali, H., Radmehr, B., & Ismail, S. (2014). Prevalence and antibiotic resistance of *Staphylococcus aureus* isolated from bovine clinical mastitis. *Journal of Dairy Science*, 97(4), 2226-2230. http://dx.doi.org/10.3168/jds.2013-7509. PMid:24534509.

Jørgensen, H. J., Mørk, T., Høgåsen, H. R., & Rørvik, L. M. (2005). Enterotoxigenic *Staphylococcus aureus* in bulk milk in Norway. *Journal of Applied Microbiology*, 99(1), 158-166. http://dx.doi.org/10.1111/j.1365-2672.2005.02569.x. PMid:15960676.

Kluytmans, J., van Belkum, A., & Verbrugh, H. (1997). Nasal carriage of *Staphylococcus aureus*: Epidemiology, underlying mechanisms, and associated risks. *Clinical Microbiology Reviews*, 10(3), 505-520. http://dx.doi.org/10.1128/CMR.10.3.505. PMid:9227864.

Korhonen, H., & Kaartinen, L. (1995). Changes in the composition of milk induced by mastitis. In M. Sandholm, T. Honkanen-Buzalski, L. Kaartinen, S. Pyörälä (Eds.), *The bovine udder and mastitis* (pp. 76-82). Jyväskylä, Finland: Gummerus.

Kraushaar, B., & Fetsch, A. (2014). First description of PVL-positive methicillin-resistant *Staphylococcus aureus* (MRSA) in wild boar meat. *International Journal of Food Microbiology*, 186, 68-73. http://dx.doi.org/10.1016/j.ijfoodmicro.2014.06.018. PMid:25016468.

Kreausukon, K., Fetsch, A., Kraushaar, B., Alt, K., Müller, K., Krömker, V., Zessin, K. H., Käsbohrer, A., & Tenhagen, B. A. (2012). Prevalence, antimicrobial resistance, and molecular characterization of methicillin-resistant *Staphylococcus aureus* from bulk tank milk of dairy herds. *Journal of Dairy Science*, 95(8), 4382-4388. http://dx.doi.org/10.3168/jds.2011-5198. PMid:22818451.

Le Loir, Y., Baron, F., & Gautier, M. (2003). *Staphylococcus aureus* and food poisoning. *Genetics and Molecular Research*, 2(1), 63-76. PMid:12917803.

Lee, S. H. I., Camargo, C. H., Gonçalves, J. L., Cruz, A. G., Sartori, B. T., Machado, M. B., & Oliveira, C. A. F. (2012). Characterization of *Staphylococcus aureus* isolates in milk and the milking environment from small-scale dairy farms of São Paulo, Brazil, using pulsed-field gel electrophoresis. *Journal of Dairy Science*, 95(12), 7377-7383. http://dx.doi.org/10.3168/jds.2012-5733. PMid:23040016.

Lee, S. H. I., Cappato, L. P., Corassin, C. H., Cruz, A. G. D., & Oliveira, C. A. F. D. (2016). Effect of peracetic acid on biofilms formed by *Staphylococcus aureus* and *Listeria monocytogenes* isolated from dairy plants. *Journal of Dairy Science*, 99(3), 2384-2390. http://dx.doi.org/10.3168/jds.2015-10007. PMid:26723125.

Lee, S. H. I., Mangolin, B. L. C., Gonçalves, J. L., Neeff, D. V., Silva, M. P., Cruz, A. G., & Oliveira, C. A. F. (2014). Biofilm-producing ability of *Staphylococcus aureus* isolates from Brazilian dairy farms. *Journal of Dairy Science*, 97(3), 1812-1816. http://dx.doi.org/10.3168/jds.2013-7387. PMid:24440248.

Lem, P., Spiegelman, J., Toye, B., & Ramotar, K. (2001). Direct detection of *mecA, nuc* and 16S *rRNA* genes in BacT/Alert blood culture bottles. *Diagnostic Microbiology and Infectious Disease*, 41(3), 165-168. http://dx.doi.org/10.1016/S0732-8893(01)00301-7. PMid:11750172.

Levy, S. B. (1992). *The Antibiotic Paradox: How miracle drugs are destroying the miracle*. New York: Plenum Publishing.

Lozano, C., Gharsa, H., Ben Slama, K., Zarazaga, M., & Torres, K. (2016). *Staphylococcus aureus* in animals and food: Methicillin-resistance, prevalence and population structure: A review in the African continent. *Microorganisms*, 4(12), 1-19. http://dx.doi.org/10.3390/microorganisms4010012. PMid:27681906.

Mehmeti, I., Bytyqi, H., Muji, S., Nes, I. F., & Diep, D. B. (2017). The prevalence of *Listeria monocytogenes* and *Staphylococcus aureus* and their virulence genes in bulk tank milk in Kosovo. *Journal of Infection in Developing Countries*, 11(3), 247-254. http://dx.doi.org/10.3855/jidc.8256. PMid:28368859.

Mehrotra, M., Wang, G., & Johnson, W. M. (2000). Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1 and methicillin resistance. *Journal of Clinical Microbiology*, 38(3), 1032-1035. PMid:10698991.

Moon, J. S., Lee, A. R., Kang, H. M., Lee, E. S., Kim, M. N., Paik, Y. H., Park, Y. H., Joo, Y. S., & Koo, H. C. (2007). Phenotypic and genetic antibiogram of methicillin-resistant staphylococci isolated from bovine mastitis in Korea. *Journal of Dairy Science*, 90(3), 1176-1185. http://dx.doi.org/10.3168/jds.S0022-0302(07)71604-1. PMid:17297092.

Muehlherr, J. E., Zweifel, C., Corti, S., Blanco, J. E., & Stephan, R. (2003). Microbiological quality of raw goat's and ewe's bulk-tank milk in Switzerland. *Journal of Dairy Science*, 86(12), 3849-3856. http://dx.doi.org/10.3168/jds.S0022-0302(03)73992-7. PMid:14740819.

Nam, H. M., Lee, A. L., Jung, S. C., Kim, M. N., Jang, G. C., Wee, S. H., & Lim, S. K. (2011). Antimicrobial susceptibility of *Staphylococcus aureus* and characterization of methicillin-resistant *Staphylococcus aureus* isolated from bovine mastitis in Korea. *Foodborne Pathogens and Disease*, 8(2), 231-238. http://dx.doi.org/10.1089/fpd.2010.0661. PMid:21034263.

Nemati, M., Hermans, K., Lipinska, U., Denis, O., Deplano, A., Struelens, M., Devriese, L. A., Pasmans, F., & Haesebrouck, F. (2008). Antimicrobial resistance of old and recent *Staphylococcus aureus* isolates from poultry: First detection of livestock-associated methicillin-resistant strain ST398. *Antimicrobial Agents and Chemotherapy*, 52(10), 3817-3819. http://dx.doi.org/10.1128/AAC.00613-08. PMid:18663024.

Normanno, G., Corrente, M., La Salandra, G., Dambrosio, A., Quaglia, N. C., Parisi, A., Greco, G., Bellacicco, A. L., Virgilio, S., & Celano, G. V. (2007). Methicillin-resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product Italy. *International Journal of Food Microbiology*, 117(2), 219-222. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.04.006. PMid:17533002.

Obaidat, M. M., Salman, A. E. B., & Roess, A. A. (2018). High prevalence and antimicrobial resistance of *mecA Staphylococcus aureus* in dairy cattle, sheep, and goat bulk tank milk in Jordan. *Tropical Animal Health and Production*, 50(2), 405-412. http://dx.doi.org/10.1007/s11250-017-1449-7. PMid:29063353.

Olde Riekerink, R. G. M., Barkema, H. W., Veenstra, S., Poole, D. E., Dingwell, R. T., & Keefe, G. P. (2006). Prevalence of contagious mastitis pathogens in bulk tank milk in Prince Edward Island. *The Canadian Veterinary Journal. La Revue Veterinaire Canadienne*, 47(6), 567-572. PMid:16808229.

Oniciuc, E. A., Nicolau, A. L., Hernández, M., & Rodríguez-Lázaro, D. (2017). Presence of methicillin-resistant *Staphylococcus aureus* in the food chain. *Trends in Food Science & Technology*, 61, 49-59. http://dx.doi.org/10.1016/j.tifs.2016.12.002.

Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V., Braden, C. R., Angulo, F. J., & Griffin, P. M. (2013). Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998-2008. *Emerging Infectious Diseases Journal*, 19(3), 407-415. http://dx.doi.org/10.3201/eid1903.111866. PMid:23622497.

Parisi, A., Caruso, M., Normanno, G., Latorre, R., Sottili, A., Miccolupo, R., Fraccalvieri, G., & Santagada, G. (2016). Prevalence, antimicrobial susceptibility and molecular typing of methicillin-resistant *Staphylococcus aureus* (MRSA) in bulk tank milk from southern Italy. *Food Microbiology*, 58, 36-42. http://dx.doi.org/10.1016/j.fm.2016.03.004. PMid:27217357.

Paterson, G. K., Harrison, E. M., & Holmes, M. A. (2014). The emergence of *mecC* methicillin resistant *Staphylococcus aureus. Trends in Microbiology*, 22(1), 42-47. http://dx.doi.org/10.1016/j.tim.2013.11.003. PMid:24331435.

Paterson, G. K., Larsen, J., Harrison, E. M., Larsen, A. R., Morgan, F. J., Peacock, S. J., Parkhill, J., Zadoks, R. N., & Holmes, M. A. (2012). First detection of livestock-associated meticillin-resistant *Staphylococcus aureus* CC398 in bulk tank milk in the United Kingdom, January to July 2012. *European Communicable Disease Bulletin*, 17(50), 20337. PMid:23241232.

Peles, F. M., Wagner, L., Varga, I., Hein, P., Rieck, K., Gutser, P., Kereszturi, G., Kardos, I., Turcsányi, I., Béri, B., & Szabó, A. (2007). Characterization of *Staphylococcus aureus* strains isolated from bovine milk in Hungary. *International Journal of Food Microbiology*, 118(2), 186-193. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.07.010. PMid:17727995.

Pereira, V., Lopes, C., Castro, A., Silva, J., Gibbs, P., & Teixeira, P. (2009). Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of *Staphylococcus aureus* isolates from various foods in Portugal. *Food Microbiology*, 26(3), 278-282. http://dx.doi.org/10.1016/j.fm.2008.12.008. PMid:19269569.

Petersen, A., Stegger, M., Heltberg, O., Christensen, J., Zeuthen, A., Knudsen, L. K., Urth, T., Sorum, M., Schouls, L., Larsen, J., Skov, R., & Larsen, A. R. (2013). Epidemiology of methicillin-resistant *Staphylococcus aureus* carrying the novel *mecC* gene in Denmark corroborates a zoonotic reservoir with transmission to humans. *Clinical Microbiology and Infection*, 19(1), 16-22. http://dx.doi.org/10.1111/1469-0691.12036. PMid:23078039.

Porcellato, D., Aspholm, M., Skeie, S. B., Monshaugen, M., Brendehaug, J., & Mellegard, H. (2018). Microbial diversity of consumption milk during processing and storage. *International Journal of Food Microbiology*, 266, 21-30. http://dx.doi.org/10.1016/j.ijfoodmicro.2017.11.004. PMid:29161642.

Rall, V. L. M., Vieira, F. P., Rall, R., Vieitis, R. L., Fernandes, A. Jr, Candeias, J. M. G., Cardoso, K. F. G., & Araújo, J. P. Jr (2008). PCR detection of staphylococcal enterotoxin genes in *Staphylococcus aureus* strains isolated from raw and pasteurized milk. *Veterinary Microbiology*, 132(3-4), 408-413. http://dx.doi.org/10.1016/j.vetmic.2008.05.011. PMid:18572331.

Roberson, J. R., Fox, L. K., Hancock, D. D., Gay, J. M., & Besser, T. E. (1998). Sources of intramammary infections from *Staphylococcus aureus* in dairy heifers at first parturition. *Journal of Dairy Science*, 81(3), 687-693. http://dx.doi.org/10.3168/jds.S0022-0302(98)75624-3. PMid:9565871.

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(8), 2991-2999. http://dx.doi.org/10.3168/jds.S0022-0302(05)72979-9. PMid:16027213.

Sayin, Z., Sakmanoglu, A., Ucan, U. S., Pinarkara, Y., Uslu, A., Aras, Z., & Erganis, O. (2016). Detection of methicilline resistant *Staphylococcus aureus* carrying *mecC* gene in mastitic milk samples of cattle in Turkey. *Eurasian Journal of Veterinary Sciences*, 32(3), 182-187. http://dx.doi.org/10.15312/EurasianJVetSci.2016318398.

Shore, A. C., Deasy, E. C., Slickers, P., Brennan, G., O'Connell, B., Monecke, S., Ehricht, R., & Coleman, D. C. (2011). Detection of staphylococcal cassette chromosome mec type XI encoding highly divergent *mecA, mecI, mecR1, blaZ* and *ccr* genes in human clinical clonal complex 130 methicillin-resistant *Staphylococcus aureus. Antimicrobial Agents and Chemotherapy*, 55(8), 3765-3763. http://dx.doi.org/10.1128/AAC.00187-11. PMid:21636525.

Siiriken, B., Yildirim, T., Güney, A. K., Erol, I., & Durupinar, B. (2016). Prevalence and molecular characterization of methicillin-resistant *Staphylococcus aureus* in foods of animal origin, Turkey. *Journal of Food Protection*, 79(11), 1990-1994. http://dx.doi.org/10.4315/0362-028X.JFP-16-161. PMid:28221920.

Silva, R., Cruz, A. G., Faria, J. A., Moura, M. M., Carvalho, L. M., Water, E. H., & Sant'Ana, A. S. (2010). Pasteurized milk: efficiency of pasteurization and its microbiological conditions in Brazil. *Foodborne Pathogens and Disease*, 7(2), 217-219. http://dx.doi.org/10.1089/fpd.2009.0332. PMid:19785537.

Spohr, M., Rau, J., Friedrich, A., Klittich, G., Fetsch, A., Guerra, B., Hammerl, J. A., & Tenhagen, B. A. (2010). Methicillin-resistant *Staphylococcus aureus* (MRSA) in three dairy herds in Southwest Germany. *Zoonoses and Public Health*, 58(4), 252-261. http://dx.doi.org/10.1111/j.1863-2378.2010.01344.x. PMid:20630047.

Stegger, M., Andersen, P. S., Kearns, A., Pichon, B., Holmes, M. A., Edwards, G., Laurent, F., Teale, C., Skov, R., & Larsen, A. R. (2012). Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new *mecA* homologue *mecA(LGA251). Clinical Microbiology and Infection*, 18(4), 395-400. http://dx.doi.org/10.1111/j.1469-0691.2011.03715.x. PMid:22429460.

Tenhagen, B. A., Alt, K., Pfefferkorn, B., Wiehle, L., Käsbohrer, A., & Fetsch, A. (2018). Short communication: Methicillin-resistant *Staphylococcus aureus* in conventional and organic dairy herds in Germany. *Journal of Dairy Science*, 101(4), 3380-3386. http://dx.doi.org/10.3168/jds.2017-12939. PMid:29395146.

Vincze, S., Stamm, I., Kopp, P. A., Hermes, J., Adlhoch, C., Semmler, T., Wieler, L. H., Lübke-Becker, A., & Walther, B. (2014). Alarming proportions of methicillin-resistant *Staphylococcus aureus* (MRSA) in wound samples from companion animals, Germany 2010–2012. *PLoS One*, 9(1), e85656. http://dx.doi.org/10.1371/journal.pone.0085656. PMid:24465637.

Wang, D., Wang, Z., Yan, Z., Wu, J., Ali, T., Li, J., Lv, Y., & Han, B. (2015). Bovine mastitis *Staphylococcus aureus*: antibiotic susceptibility profile, resistance genes and molecular typing of methicillin-resistant

and methicillin-sensitive strains in China. *Infection, Genetics and Evolution*, 31, 9-16. http://dx.doi.org/10.1016/j.meegid.2014.12.039. PMid:25582604.

Wang, G., Li, G., Xia, X., Yang, B., Xi, M., & Meng, J. (2014). Antimicrobial susceptibility and molecular typing of methicillin-resistant *Staphylococcus aureus* in retail foods in Shaanxi, China. *Foodborne Pathogens and Disease*, 11(4), 281-286. http://dx.doi.org/10.1089/fpd.2013.1643. PMid:24404781.

Wendlandt, S., Schwarz, S., & Silley, P. (2013). Methicillin-resistant *Staphylococcus aureus*: A food-borne pathogen? *Annual Review of Food Science and Technology*, 4(1), 117-139. http://dx.doi.org/10.1146/annurev-food-030212-182653. PMid:23190141.

Zouharova, M., & Rysanek, D. (2008). Multiplex PCR and RPLA identification of *Staphylococcus aureus* enterotoxigenic strains from bulk tank milk. *Zoonoses and Public Health*, 55(6), 313-319. http://dx.doi.org/10.1111/j.1863-2378.2008.01134.x. PMid:18489539.