The Prevalence and Antibiotic Resistance of Methicillin-Resistant Staphylococcus aureus (MRSA) in Milk and Dairy Products in Balikesir, Turkey

EKTIK NISANUR  
Department of Food Hygiene and Technology, Institute of Health Science, Balikesir University

GÖKMEN MUKADDERAT  
Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Balikesir University

ÇIBIK RECEP  
Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Uludağ University

http://dx.doi.org/10.12681/jhvms.16062

To cite this article:

The Prevalence and Antibiotic Resistance of Methicillin-Resistant Staphylococcus aureus (MRSA) in Milk and Dairy Products in Balikesir, Turkey

Nisanur Ektik¹, Mukadderat Gökmen², Recep Çibik³,

¹ Department of Food Hygiene and Technology, Institute of Health Science, Balikesir University, Balikesir, Turkey
² Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Balikesir University, Balikesir, Turkey
³ Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Uluda University, Bursa, Turkey

ABSTRACT. Methicillin-Resistant Staphylococcus aureus is an important pathogen that causes severe infections in humans and animals. The aim of this study was to determine the prevalence and the antimicrobial profile of methicillin-resistant S. aureus (MRSA) in cow bulk tank milk and dairy products in the region of Balikesir in Turkey. Of 175 milk and dairy products’ samples, 26 were found to be positive for coagulase-positive staphylococci and 3 (2 samples from cow bulk tank milk and 1 sample from tulum cheese) were MRSA phenotypically being resistant against both oxacillin and cefoxitin. Among these, 17 were confirmed as S. aureus by the detection of nuc gene and one as MRSA carrying the mecA gene. All MRSA isolates were found to be also resistant against ampicillin, penicillin and sulfamethoxazole-trimethoprim. Consequently, even though the prevalence of MRSA in cow bulk tank milk and dairy products was relatively low (1.70%), it may pose serious risks in terms of food safety and public health. In order to prevent the prevalence of MRSA in dairy products, hygienic measures, especially in terms of personal hygiene and disinfection of equipment in all stages of dairy production, should be taken, and HACCP and GMP regulations should be implemented.

Keywords: antibiotic resistance, cow bulk tank milk, MRSA, dairy products
INTRODUCTION

Staphylococcal aureus, which is part of the normal flora of the skin and the mucous membranes of humans and animals, is an opportunistic pathogen equipped with numerous virulence factors causing various infections (Ala et al., 2015). Antibiotic resistance is one of these factors involved in nosocomial and community infections (Nada et al., 2018). The improper use of antibiotics both in human and veterinary medicine, in agriculture, and animal husbandry for more than 50 years has led to the development of antibiotic-resistant strains of S. aureus, as well as of other pathogens (Nida et al., 2004). Methicillin resistance among S. aureus strains has especially increased rapidly in last decades (Lee et al., 2015). Methicillin resistance in staphylococci, coded by the name “Celbenin” for the treatment of penicillin-resistant strains has especially increased rapidly in last decades (Palmer et al., 2012). The improper use of antibiotics both in human and veterinary medicine, in agriculture, and animal husbandry for more than 50 years has led to the development of antibiotic-resistant strains of S. aureus, as well as of other pathogens (Nida et al., 2004). Methicillin resistance among S. aureus strains has especially increased rapidly in last decades (Lee et al., 2015). Methicillin resistance in staphylococci, coded by the name “Celbenin” for the treatment of penicillin-resistant strains has especially increased rapidly in last decades (Palmer et al., 2012).

In recent years, MRSA has been identified as an emerging pathogen in livestock (pigs, cattle and poultry), and companion animals (Tomai et al., 2015; Cury et al., 2013). The presence of LA-MRSA in food-producing animals has raised questions regarding the presence of MRSA in food of animal origin (Fellner et al., 2011). The European Food Safety Authority (2008) underlines that products of animal origin represent a potential source of MRSA, and food contaminated by MRSA could be dangerous. The main risk for the development of food poisoning in the presence of S. aureus in foods (Kleitmann, 2010), and MRSA may be involved in food-producing outbreaks (Fors et al., 2002).

The aim of the present study was to determine the prevalence of MRSA, and their antimicrobial growth in core bulk milk collected from farms and dairy products from retail markets in Belokurikha Province, Turkey.

MATERIALS AND METHODS

Sample collection

In total 160 raw bulk milk samples and 125 dairy products samples (15 yoghurt, 40 white cheese, 10 kefir cheese, 15 tetens cheese, 12 industrial cheese, 13 cord cheese, 10 sepet cheese, and 10 butter) were collected from farms and retail markets, respectively in Belokurikha Province and transferred to the Laboratory until refrigeration and analysis in the same day.

Isolation and identification of MRSA

From each sample, 25 ml or less were aseptically weighed and added into stomacher bags containing 225 ml Marshalls-Binet broth (Oxoid CM4045, England) with 0.5% NaCl and homogenized in a stomacher (IU-Interscience, France) for 2 minutes. The homogenates were incubated at 37°C for 16–24 hours for enrichment and then aliquots of 10 µl were spotted onto the CHROMagar MR502, Paris, France) medium plates and incubated at 35±2°C for 16–24 hours. At the end of the incubation, according to the manufacturer’s recommendations, MRSA suspected colonies were transferred onto tryptone soy agar (TSA;BioMerieux, 43011, France) before coagulase test. Puriﬁed colonies without colonies showing a pink to mauve color were left for an additional 24 hours incubation and then re-isolated (EFSA, 2009). Coagulase test was performed by Slipex MRSA (Slipex,DROXBIO, Dussong, UK). In addition, the MRSA Slide latex agglutination test (BioMerieux, Marcy l’Etoile, France) was performed for the detection of protein A, clumping factor and capillary polymorphonuclears. At the completion of the latex test, coagulase-positive staphylococci were incubated in Brain Heart Infusion Broth (BHI; Merck, Germany) at 35±2°C for 24 hours and then were re-isolated in BHI broth with glycerol 20% w/v at –80°C until further analysis.

Detection of the mec gene in S. aureus isolates and the nuc gene in the MRSA isolates

DNA extraction was performed using a commercial Magna Pure LC Total Nucleic Acid Isolation Kit (Roche, Germany), according to the manufacturer’s instructions. A PCR cycling program consisted of an initial denaturation step at 94°C for 10 min, followed by 25 cycles of denaturation at 94°C for 1 min, annealing at 51°C for 1 min, and extension at 72°C for 1 min, and a final extension step of 72°C for 5 min. The mec gene was ampliﬁed using rate 1 (5’-GGGAGTGGATGATAGCTGATT-3’) and rate 2 (5’-AACGAAAGCTTGAAGAGAACGGG-3’) primers. The mecA gene was ampliﬁed using rate 1 (5’-AAAACTTGGAGAATGGTTCG-3’) and rate 2 (5’-GGGAGTGGATGATAGCTGATT-3’) primers (Maes et al., 2002). Erythromycin was prepared using 1.5% agarose gel at 94 V for 75 minutes. Samples were considered positive for S. aureus, and samples at 533 bp were considered MRSA.

Identification of Antibiotic Resistance

The antibiotic susceptibility of the 53 phenotypically MRSA isolates to 16 antibiotics/antibiotic combinations was determined by the disk diffusion method (Bar et al., 1961). Disks containing the following antibiotic were spotted onto the TSA with a 24 mm interval: penicillin G 10 µg, gentamicin 15 µg, streptomycin 5 µg, ampicillin 10 µg, sulfonamide-trimethoprim 25 µg, ciprofloxacin 5 µg, tetracycline 30 µg, chloramphenicol 30 µg, cotrimoxazol 10 µg and oxacillin 1 µg. The results were interpreted according to Clinical and Laboratory Standard Institute (2012; 2014) criteria. Antibiotic discs were purchased from Thermo Scientific (Oxoid, England).

Results

MRSA and mecA gene-positive reference strains were purchased from Microbiologics Inc. (St. Cloud, Minnesota USA).

Conclusions

Coagulase-positive staphylococci were isolated from 26 samples. Of these isolates, 16 were isolated from core bulk tank milk; two from white cheese; three from kefir cheese; two from tetens cheese; and one from sepet cheese. By Slidex MRSA latex agglutination test, three of these isolates found to be MRSA. Of these, two were isolated from bulk tank milk and one from core cheese. The mecA gene was detected in 17 of 26 phenotypically coagulase-positive staphylococci isolates (65.38%), while the remaining 9 isolates were mecA gene-negative (Fig. 1). Table 1. The mecA gene was detected in only one of 17 S. aureus isolates (5.88%).

Discussion

In this study, S. aureus was isolated from 14 of 50 (28%) core’s bulk tank milk samples and two of them (14.28%) were MRSA showing resistance against both oxacillin and cefoxitin (Table 1). The mecA gene was detected in 17 of 26 coagulase-positive staphylococci isolates and 9 isolates were mecA gene-negative (Fig. 1). Consequently, using mecA-specific PCR as the sole molecular method for diagnosing S. aureus might result in the misidentiﬁcation of S. aureus and MRSA on coagulase-negative staphylococci (Hugh et al., 2014).

A few studies have evaluated the prevalence of MRSA in core bulk tank milk samples in Turkey and worldwide. Daha et al. (2012) reported that 78 of the 100 raw milk samples (60.75%) were S. aureus.
Prevalence of genotypically identified S. aureus and MRSA isolates in cow bulk tank milk and dairy products

60.30% of them were phenotypically MRSA. Vyletelova et al. (2011) isolated S. aureus from 326 of 703 tank milk samples (46.30%) and 20 isolates of them (6.10%) were MRSA carrying the mecA gene. Paterson et al. (2012) found MRSA in 32 (3.80%) of the 844 bulk tank milk. This study’s detected in seven (1.83%) of 383 raw milk samples. Kanaan and Al-Shammary (2013) found MRSA phenotypically in 4 (13.40%) from several studies in world (Daka et al., 2012) and results showed a lower prevalence than the results (Kalsoom et al., 2010) reported that 16 of the 93 strains (9.30%) obtained S. aureus from 326 tank milk samples (46.30%) and 20 isolates of them (6.10%) were MRSA carrying the mecA gene. Vyletelova et al. (2011) isolated S. aureus from 326 of 703 tank milk samples (46.30%) and 20 isolates of them (6.10%) were MRSA carrying the mecA gene. Vyletelova et al. (2011) detected the mecA gene in 11 of the 118 S. aureus strains (9.30%) obtained from clinical and subclinical mastitis milks. Two strains (18.10%) were isolated from clinical mastitis milk and nine of them (81.80%) from subclinical mastitis milk. Erdem and Türkyılmaz (2010) reported on the prevalence of MRSA in cheeses. Arefi et al. (2012) identified seven (14%) and 18 (36%) of 100 samples with mastitis (21.20%) were S. aureus and 20 (10.70%) of them were phenotypically MRSA. mecA positive 533 bp) mecA gene was found in 37 isolates (56.90%). Vyletelova et al. (2011) isolated S. aureus from 326 of 703 tank milk samples (46.30%) and 20 isolates of them (6.10%) were MRSA carrying the mecA gene. Two of them were isolated from Feta cheese and six of them were isolated from traditional white cheese. Erdem et al. (2010) reported that they could not detect MRSA in 208 cheese made from raw milk. Yardımcı et al. (2012) reported that 16 from 93 milk samples were phenotypically MRSA and mecA positive isolates and reference strain. 0.46% of the 1500 bulk tank milk sample as mecA positive isolates and reference strain. Figure 1. Electrophoresis image of nuc positive S. aureus isolates. (M: Marker, ATCC 33592 and ATCC 43300: Positive Control Strain, 1: nuc positive 533 bp) mecA positive 533 bp) mecA gene. Two of them were isolated from Feta cheese and six of them were isolated from traditional white cheese. Erdem et al. (2010) reported that they could not detect MRSA in 208 cheese made from raw milk. Yardımcı et al. (2012) reported that 16 from 93 milk samples were phenotypically MRSA and mecA positive isolates and reference strain. 0.46% of the 1500 bulk tank milk sample as mecA positive isolates and reference strain. Table 1. Prevalence of genotypically identified S. aureus and MRSA isolates in cow bulk tank milk and dairy products

<table>
<thead>
<tr>
<th>Samples</th>
<th>Number of S. aureus positive samples(%)</th>
<th>Number of MRSA positive samples(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core Bulk Tank Milk</td>
<td>14 (87.50)</td>
<td>-</td>
</tr>
<tr>
<td>Yogurt</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>White Cheeses</td>
<td>1 (100)</td>
<td>-</td>
</tr>
<tr>
<td>Kultur Cheeses</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tulum Cheeses</td>
<td>1 (100)</td>
<td>-</td>
</tr>
<tr>
<td>Milk Cheeses</td>
<td>1 (100)</td>
<td>-</td>
</tr>
<tr>
<td>Yoghurt</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Butter</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>17 (65.30)</td>
<td>1 (33.30)</td>
</tr>
</tbody>
</table>
61 of the 108 Erzincan Tulum cheese (56%) were MRSA, and 10 of them (16.30%) were genotypically MRSA by PCR. In Mirzaei et al.'s study (2011), the prevalence of MRSA was 4% and the isolates may show phenotypical resistance against cefoxitin and oxacillin, despite the presence of MRSA. In Can and Çelik's study (2011), MRSA was genotypically detected in two (4%) of 50 milk samples that were resistant to aureomycin, tetracycline, and chloramphenicol.

MRSA strains (5 from white cheese samples (100 white cheese and 100 Tulum cheese)) were isolated from Tulum cheese samples. Kanaan and Al-Shammary (2013) found MRSA was genotypically detected in two (4%) of 50 butter samples. Can and Çelik (2011) detected MRSA in 15 soft white raw cheese with whey samples. From 885 traditional cheese samples by PCR. Can and Çelik (2011) detected MRSA in 15 soft white raw cheese with whey samples. From 885 traditional cheese samples by PCR.

CONCLUSIONS

The prevalence of MRSA in raw milk and dairy products, both from pasteurized and unpasteurized milk represents a potential threat for public health. In order to control the presence of MRSA in milk and dairy products, milk must be obtained strictly from hygienic animals, and necessary precautions must be taken to prevent contamination after pasteurization. Regarding the treatment of infections in dairy animals, antibiotic use should be used in a controlled and conscientious manner. Furthermore, the status of antibiotic resistances should be monitored regularly. Studies on the prevalence and antibiotic resistances of MRSA should be conducted regularly, especially in foods of animal origin and the environment food-production practices. Staphylococcus aureus as a public-health threat. Lancet 368:874–885.

REFERENCES


MRSA was genotypically detected in two (4%) of 50 milk samples that were resistant to aureomycin, tetracycline, and chloramphenicol. Despite the presence of MRSA, the isolates may show phenotypical resistance against cefoxitin and oxacillin, despite the presence of MRSA. In Can and Çelik's study (2011), MRSA was genotypically detected in two (4%) of 50 milk samples that were resistant to aureomycin, tetracycline, and chloramphenicol.

MRSA strains (5 from white cheese samples (100 white cheese and 100 Tulum cheese)) were isolated from Tulum cheese samples. Kanaan and Al-Shammary (2013) found MRSA was genotypically detected in two (4%) of 50 butter samples. Can and Çelik (2011) detected MRSA in 15 soft white raw cheese with whey samples. From 885 traditional cheese samples by PCR.


Yuçel N and Anıl Y (2011) Cı süt ve peynir örneklerindeki Staphylococcus aureus ve koagülaz negatif stafilocokların identifikasyonu ve antibiyotik duyarılığı türk hij den biyol