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

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**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

Staphylococcus 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 serious infections (Ito et al., 2003). Antibiotic resistance is one of these factors involved in nosocomial and community infections (Sidla et al., 2008). 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 other pathogens (Riley et al., 2004). Methicillin-resistant S. aureus (MRSA) has especially increased rapidly in the last decades (Io et al., 2003). Methicillin (2,6-dimethoxyphenyl penicillin) was first developed in 1959 by George Rawlinson and Ralph Batchelor in England (Rolinson and Ralph Batchelor, 1990; Dutfield, 2009) and released under the name “Celbenin” for the treatment of penicillin-resistant S. aureus led to the development of antibiotic-resistant strains of S. aureus. Initially, MRSA was found in hospitals and livestock-associated (LA-MRSA) (Kapiris, 2008) and food contaminated by MRSA could be dangerous. The main risk for the development of food poisoning is the presence of S. aureus in foods (Kierzek, 2010), and MRSA may be involved in food-poisoning outbreaks (Ito et al., 2003). The aim of the present study was to determine the prevalence of MRSA, and their antimicrobial pattern in cow bulk milk collected from farms and dairy produce from retail markets in Balikesir Province, Turkey.

MATERIALS AND METHODS

Sample collection

In total 36 cow bulk tank milk samples and 125 dairy products samples (15 yoghurt, 40 white cheese, 10 kefir cheese, 15 feta cheese, 12 inbuh cheese, 15 curd cheese, 10 sheep cheese, and 10 honey) were collected from farms and retail markets respectively in Balikesir Province and transferred to the laboratory under refrigeration and analyzed in the same day.

Isolation and identification of MRSA

From each sample, 25 ml of milk was aseptically weighed and added to stomacher bags containing 225 ml Marzetti-Hinton broth (Oxoid CM0405, England) with 6.50% NaCl and homogenised in a stomacher (IUL, blender, Spain) for 2 minutes. The homogenates were incubated at 35±2 °C for 16–20 hours for enrichment and then aliquots of 100 µl were spread onto the CHROMagar MRSA (CHROMagar MR502, Paris, France) medium with glycerol 20% w/v at –80 °C until further analysis.

Detection of the mec gene in S. aureus isolates and the mec A 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 23 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72°C for 2 min, and a final extension step of 72°C for 5 min. The mecA gene was amplified using reverse (5'-GGATGATGGTGATACGGT-3') and reverse (5'-GGCAGCTTGTTAGAGCGCA-3') primers. The mecA gene was amplified using mecA 1 (5'-AAAATGGTTAAGCGTTTCTACG-3') and mecA 2 (5'-GGTGAGCTGACGAAGCAGAC-3') primers (Mats et al., 2002). Electrophoresis was performed using 1.5% agarose gel at 94 V for 75 minutes. Samples were cut by considered positive for S. aureus, and samples at 535 by were considered MRSA.

Determination of Antibiotic Resistance

The antibiotic susceptibility of the 15 phenotypically MRSA isolates to 10 antibiotics/antibiotic combination was determined by the disk diffusion method (Bauer et al., 1966). Disks containing the following antibiotics were spotted over the TSA, with a 24 mm interval: penicillin G 10 µg, gentamicin 15 µg, streptomycin 5 µg, ampicillin 10 µg, sulfamethoxazole-trimethoprim 25 µg, ciprofloxacin 5 µg, tetracycline 30 µg, lincomycin 30 µg, cefsulfomide 30 µg, cefoxitin 10 µg and nalidixic acid 1 µg. The results were interpreted according to Clinical and Laboratory Standards Institute (2012; 2014) criteria.

RESULTS

Coagulase-positive staphylococci were isolated from 26 samples. Of these isolates, 16 were isolated from cow bulk tank milk, two from white cheese; three from kefir cheese; two from feta cheese; five from the milk curd, and one from sheep cheese. By slides MRSA latex agglutination test, three of these isolates found to be MRSA. Of these, two were isolated from bulk tank milk and one from sheep cheese. The mec 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 out of 17, S. aureus isolate (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 cotrimoxazole (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 (Hough et al., 2014).

A few studies have evaluated the prevalence of MRSA in core bulk tank milk samples in Turkey and worldwide. Duka et al. (2012) reported that 78 of the 166 raw milk samples (46.70%) were S. aureus...
Prevalence of genotypically identified S. aureus and MRSA isolates in cow bulk tank milk and dairy products

Vyletelova et al. (2010) reported that 16 of the 91 (17.20%) S. aureus strains isolated from the mastitis cow milk samples were phylogenetically MRSA and all of them were carrying mecA gene. Palhová et al. and Yardımcı (2011) reported that 65 of the 106 milk samples with mastitis (61.00%) were S. aureus and 20 (18.90%) of them were phylogenetically MRSA. mecA gene was found in 37 isolates (56.90%) and 20 (30.70%) 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. Kanaan and Al-Shammary (2013) found MRSA genotypically in 7 (0.46%) of the 1500 bulk tank milk samples. Ünal (2013) reported that mecA gene was found in two isolates (3.30%) from 60 bulk tank milk samples. Erdem and Türkyılmaz (2010) reported that 16 of the 93 isolates (17.20%) were isolated from clinical mastitis and 9 of them (81.80%) from subclinical mastitis.

Some national and international studies have been reported on the prevalence of MRSA in cheese. Arefi et al. (2012) isolated 100 raw milk samples and reported that 14 (14%) isolates carried the mecA gene. Therefore, the results of our study showed similarity with the results of Arefi et al. (2012).

A few national and international studies have been reported on the prevalence of MRSA in cheeses. Arefi et al. (2012) identified 140 and 10 (10%) of 100 cheese samples (50 Feta cheese and 50 traditional white cheese) as MRSA and S. aureus, respectively. Eight of these 23 isolates (34.78%) were found to carry the mecA gene. Two of them were isolated from Feta cheese and six of them were isolated from traditional white cheese. Höhler et al. (2010) reported that they could not detect MRSA in 159 cheese made from raw milk. Yıldız and Aydın (2013) obtained 16 S. aureus isolates from 90 cheese samples. Also methicillin resistance was detected in 78 (11.59%) carrabin positive isolates which were isolated from cheeses. Özpinar (2011) reported that methicillin resistance was detected in 9 of 100 

| 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>Cow Bulk Tank Milk</td>
<td>14 (67.90)</td>
<td>-</td>
</tr>
<tr>
<td>Yogurt</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>White Cheese</td>
<td>1 (50.00)</td>
<td>-</td>
</tr>
<tr>
<td>Kashar Cheese</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tulum Cheese</td>
<td>1 (50.00)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Mihalic Cheese</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Septet Cheese</td>
<td>1 (100)</td>
<td>-</td>
</tr>
<tr>
<td>Curd Cheese</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cheese</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>17 (65.30)</td>
<td>3 (33.30)</td>
</tr>
</tbody>
</table>

Table 1. Prevalence of genotypically identified S. aureus and MRSA isolates in cow bulk tank milk and dairy products
61 of the 100 Erzincan Tulum cheese (61%) were MRSA. E. coli and 10 of them (10.00%) were genotypically MRSA by PCR. In Mirzadeh’s et al. study (2011), MRSA was genotypically detected in two (2%) of 90 traditional cheese samples by PCR. Can and Celik (2012) found E. coli in 12 of the 200 unpasteurized cheese samples (60 white cheese and 100 Tulum cheese). The use of 0.5% seawater (0.5% seawater cheese and seaweed from Tulum cheese) were found to be MRSA. MRSA positive samples were isolated from Tulum cheese Kafkas and Al-Monitorie (2011) found MRSA phage type 44 in 46% of the 15 soft white cheese with white samples. This study’s results are similar to those of Arab et al. (2010) and others (Ozcan et al., 2011) and higher than other researchers’ results (Ozcan et al., 2011, Yildiz and Alt, 2011; Ozcan et al., 2011, Mirzadeh et al., 2011). Can and Celik, 2012; Kafkas and Al-Monitorie 2013). These differences may be due to variations in cheese-production technology, the number of samples, and the fact that the milk used in production was not pasteurized. They may also be related to different levels of hygiene standards performed during their production and after production, storage and retail sales conditions.

Tulum cheese has a high dry matter and fat content, and production techniques vary from region to region. In the traditional manufacture of Turkish Tulum cheese, the cheese milk is not pasteurized. So, the presence of MRSA in tulum cheese samples is probably linked to the use of raw milk in their production, the use of animal skins as containers during ripening and filling them by hand or with a contaminated thick teat (Celik, 2000). Turkish Food Codex, 2015). Furthermore, the production under unhygienic conditions, mostly in temporary locations during covering and filling, may be linked to the use of raw milk in their production.

MRSA isolates may show phenotypical resistance to clindamycin and oxacillin, despite the absence of the mec A gene. This resistance may be regulated by newly described two mec A homologue mec C genes or other factors such as overproduction of beta-lactamase and mutations that occur in the structure of PBP (Garcia-Alvarez et al., 2013; Paterson, 2001, 2006). Lee (2001) found 12 MRSA strains isolated from bovine milk samples that were resistant to clindamycin, penicillin, and amoxicillin; one of the 12 MRSA isolates was resistant to cloxacillin, three were resistant to ciprofloxacin, eight to erythromycin, 11 to gentamicin, and five to tetracycline. None of the isolates were resistant to trimethoprim- sulfamethoxazole.

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 should be obtained strictly from healthy animals. HACCP (Hazard Analysis and Critical Control Points) and GAP (Good Agricultural Practices) and GHP (Good Hygiene Practices) should be implemented for milk and dairy products throughout the production chain of dairy products from milking to retail sales. In addition, the use of raw milk in cheese production should be avoided; adequate pasteurization should be applied and necessary precautions must be taken to prevent contamination after pasteurization. Regarding the treatment of infections in dairy animals, antibiotics should be used in a controlled and conscientious manner. Furthermore, the status of antibiotic resistance should be monitored regularly. Studies on the prevalence and antibiotic resistance of MRSA should be conducted regularly, especially in foods of animal origin and the environment food-production should be conducted regularly, especially in foods of animal origin and the environment food-production should be conducted regularly, especially in foods of animal origin and the environment food-production should be conducted regularly, especially in foods of animal origin and the environment food-production should be conducted regularly, especially in foods of animal origin and the environment.

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DISCLOSURE STATEMENT

No competing financial interests exist.

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