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

Copyright © 2018 NISANUR EKTIK, MUKADDERAT GÖKMEN, RECEP ÇIBIK

To cite this article:

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

Nisanur Ektik\textsuperscript{1}, Mukadderat Gökmen\textsuperscript{2}, Recep Çibik\textsuperscript{3},

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

\textbf{ABSTRACT.} Methicillin-Resistant \textit{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 \textit{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 \textit{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 \textit{S. aureus} by the detection of \textit{nuc} gene and one as MRSA carrying the \textit{mecA} gene. All MRSA isolates were found to be also resistant against ampicillin, penicillin and sulfmethoxazole-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.

\textbf{Keywords:} antibiotic resistance, cow bulk tank milk, MRSA, dairy products

\textbf{Corresponding Author:}
Mukadderat Gökmen
Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Balikesir University, 10145, Balikesir, Turkey
E-mail: mgokmen@balikesir.edu.tr;

\textbf{Date of initial submission:} 18-10-2016
\textbf{Date of revised submission:} 19-11-2016
\textbf{Date of acceptance:} 11-1-2017
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 various infections (K. alp et al., 2012). Antibiotic resistance is one of these factors involved in nosocomial and community infections (Sadaa 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 (Blády et al., 2004). Methicillin resistance among S. aureus strains has especially increased rapidly in the last decades (Ise et al., 2003). Methicillin (2,6-dimethoxyphenyl penicillin) was first developed in 1959 by George Rawlinson and Ralph Batchelor in England (Rollinson, 1960; Rawlinson, 2004) and released under the name “Celbenin” for the treatment of penicillin-sensitive staphylococcal infections (Ise et al., 2003). Shortly thereafter in 1961, the first isolation of MRSA was reported in England (Armon, 1961; Eitgeht et al., 2002). An altered new form of penicillin-binding protein PBP2a was synthesized by MRSA strains called “PBP2a” (Eitgeht et al., 2002). PBP2a mediates methicillin resistance in Staphylococcus, caused by the mecA gene (Chao et al., 2000).

The increase of infections caused by antibiotic-resistant bacteria continues to be a major problem worldwide (Spallholz et al., 2000; Arroyo et al., 2014). MRSA can cause severe invasive infections such as pneumonia, sepsis, osteomyelitis, and endocarditis in humans (Mitre et al., 2010). MRSA is the most frequently identified antibiotic-resistant pathogen in many parts of the world, and especially in Europe, America and Middle Eastern countries (Grandien et al., 2004). The MRSA strains have been divided into three groups: hospital-associated (HA-MRSA), community-associated (CA-MRSA) and livestock-associated (LA-MRSA) (Karayelkova et al., 2009; Shieh et al., 2010). Initially, MRSA was known to be associated with hospital-acquired infections, and then it began to appear frequently in healthy individuals in the community (White et al., 2008). In recent years, MRSA has been identified as an emerging pathogen in livestock (pigs, cattle and poultry), and companion animals (Holmoe et al., 2011; Cory 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 (Fielder et al., 2011). The European Food Safety Authority (EFSA 2008) undermines that products of animal origin represent a potential source of MRSA, and food contaminated by MRSA could be hazardous. 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-poisoning outbreaks (Fielder et al., 2002).

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

MATERIALS AND METHODS

Sample collection

In total 55 core bulk tank milk samples and 125 dairy products samples (15 yoghurt, 40 white cheese, 10 kefir cheese, 11 tulum cheese, 12 multivit cheese, 11 curd cheese, 10 sheep cheese, and 10 butter) were collected from farms and retail markets respectively in Balikesir Province and transported to the laboratory under refrigeration and analyzed in the same day.

Isolation and identification of MRSA

From each sample, 25 ml of it was aseptically weighed and added into stomacher bags containing 225 ml Marzilli-Binet broth (Oxoid CM0451, England) with 5.50% NaCl and homogenized in a stomacher (Interscience, Marcy l’Etoile, France) for 2 minutes. The homogenates were incubated at 37±2 °C for 16-24 hours for enrichment and then aliquots of 10 µl were spread onto the CHROMagar MRSA (CHROMagar MR502, Paris, France) medium plates and incubated at 35±2 °C for 10-24 hours. At the end of the incubation, according to the manufacturer’s recommendations, MRSA suspected colonies were transferred onto Typase Soy Agar (TSAG, BioMérieux, 43101, France) before confirmative test. Pedi dishes without colonies showing a pink to mauve color were left for an additional 24 hours incubation and then re-inoculated to CHROMagar MRSA (CHROMagar, Marcy l’Etoile, France) for the detection of protein A, clumping factor and capsule polysaccharides. At the completion of the latest test, coagulase-positive staphylococci were incubated in Brucella Heart Infusion Broth (BHIB, Merck, Germany) at 35-5°C for 24 hours and then were stored in BHI broth with glycerol 20% v/v at –80 °C until further analysis.

Detection of the mecA gene in S. aureus isolates and the mecA gene in the MRSA isolates

DNA extraction was performed using a commercial MagNaPure 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 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 primers 1 (5’-GGTATGATTGAGATGACGTT-3’) and one 2 (5’-AGGGAAGTTCGGAAGAAGTC-3’) primers. The mecA gene was amplified using mecA 1 (5’-GAGATGGAGATGACGTTGACGTT-3’) and mecA 2 (5’-AGCAGGATGATTGAGATGACGTT-3’) primers (Maes et al., 2002). Electrophoresis was performed using 1.5% agarose gel at 9 V for 75 minutes. Samples at 279 bp were considered positive for S. aureus, and samples at 533 by were considered MRSA.

Determination of Antibiotic Resistance

The antibiotic susceptibility of the 5 phenotypically MRSA isolates to 10 antibiotics/antibiotic combinations was determined by the disk diffusion method (Bauer et al., 1961). Disks containing the following antibiotics were spread onto the TSA: (1) 15 µg ampicillin, 10 µg, 15 µg, tetracycline, 30 µg, chloramphenicol 30 µg, cefotaxime 10 µg, ceftriaxone 25 µg, ciprofloxacin 5 µg, trimethoprim 50 µg, and cloxacillin 1 µg. The results were interpreted according to Clinical and Laboratory Standard Institute (2012; 2014). Criteria. Antibiotic discs were purchased from Thermo Scientific (Oxford, England).

Reference strains

S. aureus (ATCC 11632) mecA gene and mecA-negative reference strains were purchased from Microbiological Inc. (Sc. Cloud, Minnesota USA).

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 tulum cheese; one from multivit cheese, and one from sheep cheese. By Slidex MRSA latex agglutination test, three of these isolates found to be MRSA. Of these, two were isolated from bulk tank milk milk and one from tulum 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).

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 coagulase and catalase (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. Daba et al. (2012) reported that 78 of the 106 core milk samples (73.08%) were S. aureus

http://epublishing.ekt.gr | e-Publisher: EKT | Downloaded at 26/01/2020 02:46:33 |
and 60.10% 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. Pirzadeh et al. (2012) found MRSA strains in seven (0.46%) of the 1500 bulk tank milk sample as they carry the mecA gene. Kanaan and Al-Shammary (2013) found MRSA phenotypically in 4 (13.40%) of the 30 cheeses. Arefi et al. (2010) reported that 16 of the 93 S. aureus strains isolated from cheeses were phenotypically MRSA and of all of them were carrying mecA gene. Pehlivanoğlu et al. (2013) detected mecA gene in 11 of the 118 S. aureus strains (9.30%) obtained from clinical and subclinical mastitis milks. Two strains (18.18%) were isolated from clinical mastitis milk and nine of them (81.80%) from subclinical mastitis milk. Erdem and Türkyılmaz (2010) reported that 14 (14%) isolates carried the mecA gene. Therefore, the results of our study showed similarity with the results of Arefi et al. (2010).

A few national and international studies have been reported on the prevalence of MRSA in cheeses. Ardil et al. (2013) identified seven (4.60%) and 10 (16.10%) 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. Hisham et al. (2010) reported that they could not detect MRSA in 200 cheese made from raw milk. Yücel and Anıl (2011) obtained 16 S. aureus isolates from 90 cheese samples. Also methicillin resistance was detected in nine of 79 (11.39%) epinephrine positive isolates which were isolated from cheeses. Özpinar (2011) reported that the difference in animal husbandry systems, in national antimicrobial policies and regulations which may contribute to the variety of prevalence estimates (Grave et al., 2010). Mirzaei et al. (2012) examined 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 Mirzaei et al. (2012).

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 (67.50)</td>
<td>-</td>
</tr>
<tr>
<td>Yogurts</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>White Cheeses</td>
<td>1 (50.00)</td>
<td>-</td>
</tr>
<tr>
<td>Kızıltrım 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>Sepet Cheese</td>
<td>1 (100)</td>
<td></td>
</tr>
<tr>
<td>Cevzf Cheese</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>

Table 1. Prevalence of genotypically identified S. aureus and MRSA isolates in cow bulk tank milk and dairy products

http://epublishing.ekt.gr | e-Publisher: EKT | Downloaded at 26/01/2020 02:46:33 |
of the 108 Ertzian Tulum cheese (6%) were S. aureus and 10 from (19.2%) were genetically MRSA by PCR. In a different study (2011) MRSA was genetically identified in 46% of 50 traditional cheese samples by PCR. Can and Celik (2012) found S. aureus in 52 of the 200 unpasturized cheese samples (26% white cheese and 16 Tulum cheese). The use of 2.5% sour cream (5% from white cheese and seven from Tulum cheese) was found to be MMSA. MRSA-positive samples were isolated from Tulum cheese (Karama and Al-Mantawy, 2011) and MRSA genetically identified in 48% of the 15 soft white cheese with white samples. This study's results are similar to those of Arif et al. (2010) and higher than other researchers' results (Stuber et al., 2010; Yücel and Anıl, 2011; Özpınar, 2011; Karaman and Al-Mantawy, 2011). 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 of the milk, storage, and milk sales conditions.

Tulum cheese has a high dry matter and fat content, and production techniques vary from region to region. 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 stick (Tekin İen, 2000; Turkish Production, 2011). In traditional manufacture, 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, antimicrobial 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 and food production facilities.

DISCLOSURE STATEMENT

No competing financial interests exist.

ACKNOWLEDGEMENTS

No external funding was received from the master thesis of Nisanur EKT (2015).

REFERENCES

618 NISANUR EKTİK, MUKADDERAT GÖKMEN, RECEP ÇIBIK

http://epublishing.ekt.gr | e-Publisher: EKT | Downloaded at 26/01/2020 02:46:33 |
Yücel N and Anıl Y (2011) Çi süt ve peynir örneklerinden Staphylococcus aureus ve koagülaz negatif stafylokokların identifikasyonu ve antibiyotik duyarılılı. Türk Hıj Den Biyol