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RESEARCH ARTICLE

Investigation of Methicillin and Panton-Valentine Leukocidin Genes in *Staphylococcus aureus* Strains Isolated from Clotted Creams Sold in Afyonkarahisar

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ABSTRACT

This study aimed to investigate the methicillin and Panton-Valentine leukocidin genes in *Staphylococcus aureus* strains isolated from clotted cream samples produced and sold in Afyonkarahisar. A total of 110 clotted cream samples sold in public bazaars of Afyonkarahisar were collected between November 2019 and December 2020. Conventional cultural methods achieved the isolation of *S. aureus* from clotted cream samples. For the confirmation of *S. aureus* strains isolated from samples and determination of *mecA* and *pvl* genes in the strains, PCR was used. In this study, while *S. aureus* was isolated from 14 of 110 clotted cream samples by standard cultural methods, 13 (11.8%) of 110 samples were typed to be *S. aureus* by PCR. The *mecA* and *pvl* genes were found in none of the 13 *S. aureus* strains. In this study, in which the *pvl* gene was investigated for the first time in the *S. aureus* strains isolated from clotted creams in Turkey, it was thought that more research should be done to determine *mecA* and *pvl* genes in this traditional product and other dairy products.
Keywords: Clotted cream, *mecA, pvl, Staphylococcus aureus*

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Afyonkarahisar'da Satışa Sunulan Kaymaklardan İzole Edilen *Staphylococcus aureus* Suşlarında Metisilin ve Panton-Valentine Lökosidin Genlerinin Araştırılması

ÖZ

Bu çalışmada, Afyonkarahisar'da üretilen ve satılan kaymak örneklerinden izole edilen *Staphylococcus aureus* suşlarında metisilin ve Panton-Valentine lökosidin genlerinin araştırılması amaçlandı. Kasım 2019 ile Aralık 2020 tarihleri arasında Afyonkarahisar halk pazarlarında satışa sunulan toplam 110 kaymak örneği toplandı. Kaymak örneklerinden *S. aureus* izolasyonu konvansiyonel kültür yöntemleri kullanılarak gerçekleştirildi. Örneklerden izole edilen *S. aureus* suşlarının doğrulanması ve suşlarda *mecA* ve *pvl* genlerinin belirlenmesi amacıyla PZR kullanıldı. Çalışmada 110 kaymak örneğinin 14'ünden standart kültürel yöntemlerle *S. aureus* izole edilirken, 110 örneğin 13'ü (%11,8) PZR ile *S. aureus* olarak tiplendirildi. On üç *S. aureus* suşunun hiçbirinde *mecA* ve *pvl* genleri bulunmadı. Türkiye'de kaymaklardan izole edilen *S. aureus* suşlarında ilk kez *pvl* geninin araştırıldığı bu çalışmada, bu geleneksel ürün ve diğer süt ürünlerinde *mecA* ve *pvl* genlerinin belirlenmesi için daha fazla araştırma yapılması gerektiği düşünüldü.
Anahtar kelimeler: Kaymak, *mecA, pvl, Staphylococcus aureus*

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INTRODUCTION

Staphylococcus aureus is among the leading causes of foodborne infections resulting from the consumption of contaminated food (Thaker et al. 2013). The isolation of *S. aureus* has been reported from raw and pasteurized milk, cheese, ice cream, clotted cream and butter, as well as other foods of animal origin in different countries (Pamuk et al. 2012, Rahimi 2013, Basanisi et al. 2017, Dai et al. 2019, Keyvan et al. 2020). Generally, dairy animals with mastitis, mammary glands, milking equipment, animal skin, inappropriate food handling, and unhygienic environment are recognized as the contamination sources of milk and milk products (Thaker et al. 2013, Gezgen and Seker 2016, Dittmann et al. 2017).

Recently, unnecessary, prolonged, and erroneous use of antibiotics has caused *S. aureus* strains of human and animal origin to develop resistance to drugs used for therapeutic purposes. One of these resistance mechanisms is methicillin resistance encoded by the *mecA* gene (Algammal et al. 2020). The number of studies on the isolation of methicillin-resistant *S. aureus* (MRSA) from foodstuffs of animal origin has increased after these foods are identified as a potential mediator of transmission of MRSA strains to humans (Normanno et al. 2007, Basanisi et al. 2017, Saka ve Terzi Gulel 2018, Keyvan et al. 2020, Abdeen et al. 2021). Similarly, the presence of Panton-Valentine leukocidin (PVL) toxin, which is thought to be an important virulence factor in the pathogenesis of MRSA infections in humans, is being investigated, especially in the strains isolated from milk with mastitis (Zecconi et al. 2006, Türkyılmaz et al. 2010, Gezgen and Seker 2016, Şeker et al. 2019). However, the studies on the *pvl* gene prevalence in *S. aureus* or MRSA strains obtained from dairy products are limited (Papadopoulos et al. 2018).

In Turkey, Afyonkarahisar has a significant market share in the traditional dairy products production and consumption such as clotted cream. Although there are various researches on clotted cream's microbiological and chemical quality, most of these studies focused on the total bacterial load in the clotted cream. While the number of studies investigating the presence of *mecA* gene in *S. aureus* strains isolated from clotted creams is limited, there is no research on the prevalence of *pvl* gene in *S. aureus* strains obtained from these products. Therefore, we aimed to investigate the presence of *mecA* and *pvl* genes in *S. aureus* strains isolated from this traditional Turkish dairy product sold in Afyonkarahisar in the present study.

MATERIALS and METHODS

Clotted Cream Samples

In this study, a total of 110 homemade clotted cream samples (200 g each) sold in the public bazaars located in the center districts and villages of Afyonkarahisar between November 2019 and December 2020 were used. The clotted cream samples taken were immediately transported to the microbiology laboratory in aseptic conditions and in a cool box on ice on the same day. The samples were originated from cow milk (n=85), water buffalo milk (n=14) and cow and water buffalo milk (n=11).

Phenotypic Isolation and Identification of *S. aureus*

After each sample was aseptically homogenized by mixing in its container, 10 g were taken from each sample and transferred into 90 mL Brain Heart Infusion Broth (BHIB) for pre-enrichment. The broths were vortexed and then aerobically incubated at 37°C for 24-48 hours. After the incubation broths were vortexed again and 10 µL from each broth were inoculated onto Baird-Parker agar (BPA) containing egg yolk tellurite supplement added according to manufacturer's recommendation. BPA petri dishes were aerobically incubated at 37°C for 24-48 hours. Following the incubation, samples found to have grown at least five gray-black colored colonies surrounded by a dull zone on agar were considered suspicious for *S. aureus* (Singh and Prakash 2008). Gram staining, oxidase, slide and tube catalase, slide and tube coagulase, aerobic and anaerobic fermentation of glucose and mannitol tests were applied to suspicious colonies (Quinn et al. 2004, Garipcin and Seker 2015, Gezgen and Seker 2016). Strains identified to be *S. aureus* following the standard biochemical tests were stored at -20°C in BHIB containing 15% glycerol until DNA extraction.

Detection of 16S rDNA, *mecA* and *pvl* genes by PCR

DNA extraction from all strains was achieved using boiling method (Gezgen and Seker 2016). For detection of the 16S rDNA, *mecA* and *pvl* genes, the primer sets recommended by Strommenger et al. (2003), Choi et al. (2003) and Lina et al. (1999), respectively and the PCR protocols previously described by Gezgen and Seker (2016) were used in the present study. PCR amplifications were carried out in final volumes of 25 µL. All products were analyzed by 1.5% agarose gel electrophoresis and visualized using ethidium bromide under U.V. light. Molecular size markers (100-bp DNA ladder) were included in each agarose gel (Gezgen and Seker 2016).

RESULTS and DISCUSSION

In this study, after the conventional culture methods and biochemical tests were applied to the colonies for *S. aureus* identification, *S. aureus* isolation, and identification was performed from 14 (12.7%) of 110 homemade clotted cream samples. Of the 14 strains identified from clotted cream samples, seven, five, and two were obtained from clotted creams made with cow milk, water buffalo milk, and a mixture of cow and buffalo milk, respectively.

According to duplex PCR results, while 13 of 14 *S. aureus* strains were confirmed in terms of 16S rDNA gene, none of the strains harboured the *mecA* gene (Figure 1). Thus, *S. aureus* isolation rate from 110 clotted cream samples was found to be 11.8% (n=13) in this study. One of the strains isolated from cow's milk origin samples by classical methods was not confirmed as *S. aureus* by PCR. Also, *pvl* toxin gene was determined in none of 13 strains typed by PCR.

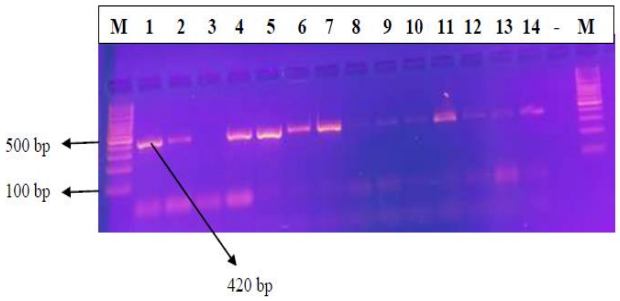

Figure 1. Duplex PCR findings for 16S rDNA ve *mecA* genes. M: DNA ladder (100 bp); lanes 1,2,4-14: 16S rDNA positive *S. aureus* test strains (420 bp); lane 3: 16S rDNA negative test strain; lanes 1-14: *mecA* negative *S. aureus* test strains; -: sterile distilled water.

Clotted cream, a traditional Turkish dairy product, is a sought-after product that benefits both the region where it is produced and the country's economy and has a high consumer share. Therefore, the various studies related to this product's microbiological and chemical quality have been reported in Turkey (Sağun et al. 2001, Pamuk et al. 2012, Sağlam and Şeker 2018, Saka ve Terzi Gulel 2018). However, most of the researches have generally focused on the total bacterial load of clotted cream. In contrast, the bacterial identification studies at the species level are limited (Sağun et al. 2001, Pamuk et al. 2012, Saka ve Terzi Gulel 2018). In a study conducted to determine the microbiological and chemical quality of dairy products offered for consumption in breakfast saloons in Van, it was reported that *S. aureus* was isolated from two (20%) of the 10 clotted cream samples (Sağun et al. 2001). Pamuk et al. (2012) from Afyonkarahisar determined the *S. aureus* was isolated from 26 (21.6%) of the 120 buffalo clotted creams samples, while in Samsun the isolation rate of *S. aureus* from 50 buffalo clotted cream samples was reported to be 18% (n=9) (Saka and Terzi Gulel 2018). In our study, a total of 110 homemade clotted cream samples sold in the public bazaars located in center districts and villages of Afyonkarahisar were examined for the presence of *S. aureus*. While *S. aureus* isolation was achieved from 14 of 110 samples by conventional culture methods, 13 of 14 isolates was confirmed to be *S. aureus* after the PCR identification. Thus, the isolation rate of *S. aureus* from 110 samples was found to be 11.8%. Of the 13 strains identified by PCR, six, five and two were obtained from clotted creams made from cow milk, water buffalo milk and the mixture of cow and buffalo milk, respectively. The isolation rate obtained in our study rate was lower than the other researcher's findings (Sağun et al. 2001, Pamuk et al. 2012, Saka and Terzi Gulel 2018). It was thought that the reason of this result may be related to the geographical region where the sampling was made, the number of samples, seasonal differences in the sampling process and the differences in the isolation and identification methods used. Although the isolation rate of 11.8% obtained in the presented study is low, the presence of this bacterium in foodstuffs or food businesses is accepted to be an indicator of inadequacy in personal hygiene practices (Tunail 2000).

The determination that foods of animal origin mediate the spread of MRSA strains has caused research to focus on this area (Wang et al. 2015, Gezgen and Seker 2016, Basanisi et al. 2017, Şeker et al. 2019). However, it seems that studies generally focus on mastitic milk samples or meat and meat products (Normanno et al. 2007, Basanisi et al. 2017, Yilmaz 2019), and there are limited studies on MRSA isolation from clotted creams (Pamuk et al. 2012, Saka and Terzi Gulel 2018). In Italy, it was emphasized that six (3.7%) of 160 *S. aureus* strains isolated from 1634 food samples containing milk and dairy products and meat and meat products carried the *mecA* gene and all MRSA strains were obtained from raw milk and cheese samples (Normanno et al. 2007). Basanisi et al. (2017) found that 40 (8.3%) of 484 *S. aureus* isolates obtained from 3760 milk and dairy product samples were genotypically MRSA. In a study carried out in Afyonkarahisar, it was reported that *mecA* positivity was found none of 23 *S. aureus* strains isolated from 602 mastitic mammary quarter milk samples (Yilmaz 2019). In one of the two studies on buffalo clotted cream samples, 9 out of 26 *S. aureus* strains isolated from 120 samples had *mecA* positivity (Pamuk et al. 2012), while this gene was found in none of 9 S. aureus strains obtained from 50 buffalo clotted creams in other one (Saka and Terzi Gulel 2018). Similar to Saka and Terzi Gulel (2018) finding, none of 13 *S. aureus* strains isolated from 110 clotted cream samples harboured the *mecA* gene in our study. Some researchers emphasized that the

prevalence of MRSA strains of animal food origin is very low compared to hospital-acquired (HA) and community-acquired (CA) MRSA strains (Normanno et al. 2007, Saka and Terzi Gulel 2018; Yilmaz 2019). In addition, it was thought that the antibiotic use histories of the animals from which the samples were obtained, the origin of the strains, the number of tested strains, and geographical differences might also be adequate on this result.

Panton-Valentine leukocidin toxin is a virulence factor thought to be important especially in the pathogenesis of MRSA infections in humans (Lina et al. 1999, Boyle-Vavra and Daum 2007). Although the role of this toxin in human infections is clear, its role in infections caused by *S. aureus* in animals has not been fully elucidated (Rainard et al. 2003, Lo and Wang 2011). Most of the studies on the presence of the *pvl* gene encoding this toxin in *S. aureus* strains in animals have focused on the strains isolated from ruminant milk with mastitis and in these researches the *pvl* gene has been reported in 0-56% of *S. aureus* strains (Zecconi et al. 2006, Türkyılmaz et al. 2010, Gezgen and Seker 2016, Şeker et al. 2019, Yilmaz 2019). However, no study investigated the presence of this gene in *S. aureus* strains obtained from clotted cream samples. In our study investigating the presence of *pvl* toxin gene in *S. aureus* strains isolated from clotted creams produced in Afyonkarahisar for the first time in Turkey the *pvl* gene was not found in any of 13 *S. aureus* strains. Several authors emphasized that the large majority of CA-MRSA more commonly harbours the *pvl* gene compared to HA-MRSA or foodborne MRSA strains and this gene has been considered the principal virulence marker (Lina et al. 1999, Boyle-Vavra and Daum 2007) or a simple determinant of CA-MRSA strains (Voyich et al. 2006). Also, the low number of strains isolated and the inability to identify MRSA strains in our study may be effective on this result.

CONCLUSION

Consequently, the isolation rate of *S. aureus* from 110 clotted cream samples was found to be 11.8% in the present study. Although this rate is not very high, it should be noted that the determination of this bacterium, especially in foods and in the food industry, may be due to inadequate personal hygiene. In addition, *S. aureus* can be found in various products made from milk animals with mastitis, which may pose a potential danger to public health. For this reason, it is important to improve the production and storage conditions of clotted creams offered for human consumption. In this study, *mecA* and *pvl* genes were not detected in any of 13 strains. However, the fact that *mecA* genes can be transferred to humans via *Staphylococcus* strains isolated from foods of animal origin is an issue that should not be ignored. Therefore, it was thought that it may be necessary to focus on studies investigating the presence of this gene in this traditional product. In our study the presence of *pvl* toxin gene was investigated in *S. aureus* strains isolated from clotted cream samples for the first time in Turkey. Although none of the strains have this toxin gene, it was thought that the acceleration of studies on the determination of this toxin gene in clotted cream and other dairy products may contribute to the detection of *pvl* gene prevalence in these products.

Conflict of Interest: The authors declare that there is no conflict of interest.

Ethical Approval: This study is not subject to the permission of HADYEK in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees".

Description: This study was summarized from the master thesis of first author.

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