Turkish Journal of Agriculture - Food Science and Technology, 11(2): 355-362, 2023
DOI: https://doi.org/10.24925/turjaf.v11i2.355-362.5817

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Antimicrobial Resistance Properties, Biofilm, and *mec*A Gene Presence in *Staphylococcus Aureus* Isolated from Raw Milk Sold in Van, Türkiye

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ARTICLE INFO

Research Article

Received : 01/12/2022
Accepted : 03/01/2023

Keywords:
Biofilm genes
*mec*A
Staphylococcus aureus
Public health
Raw milk

ABSTRACT

Staphylococcus aureus can cause foodborne poisoning and can form biofilms, reducing enterotoxin production and the penetration rate of antibiotics. Therefore, infections and poisonings caused by *S. aureus* can be difficult to treat. The aim of this study was to investigate the antibiotic resistance levels of *S. aureus* isolates obtained from raw milk and the presence of biofilm and mecA gene and to reveal the risk to public health. S. aureus was isolated in 30 (30%) of 100 raw milk samples obtained from Van province. A total of 48 *S. aureus* isolates were obtained from 30 samples. All 48 isolates (100%) obtained were resistant to penicillin G and cefoxitin, 4 (8.33%) to sulfamethoxazole-trimethoprim and chloramphenicol, and 25 (52.08%) to erythromycin. All of the isolates (100%) were found to be susceptible to ceftriaxone. In addition, 26 (54.16%) of the obtained isolates were found to be resistant to at least 3 antibiotics. The strains found to be resistant to penicillin and cefoxitin were also intermediate to at least one of the antibiotics. Biofilm genes were detected in 18 of the *S. aureus* isolates (37.5%). Twelve of the biofilm-forming isolates contain icaA (66.6%), 3 contain icaD (16.6%) and the other 3 contain bap genes (16.6%). Three of the isolates contain icaA and icaD genes and the other three isolates contain icaA and bap genes together. It was determined that only 2 of the isolates contained the mecA gene. The isolates containing the mecA gene also contained the icaA and icaD genes. In conclusion, the fact that *S. aureus* isolates had high antibiotic resistance, biofilm-forming genes, and methicillin resistance genes showed that raw milk may be a serious public health problem.

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Introduction

Staphylococcus aureus is a Gram-positive bacterium that emerged from the Staphylococcaceae family as a common colonizer of warm-blooded animals and human mucocutaneous membranes (Schleifer and Bell, 2009; Marker et al., 2013; Quinn et al., 2014). It causes many diseases in farm animals, especially mastitis and intramammary infections, and poses a serious problem in the milk and dairy sector (Marshall and Levy, 2011; Montville, 2012).

Other factors that can affect the risk of food contamination with *S. aureus* include personnel working in the food processing department, tools and equipment on the production line, livestock, water, milking equipment, and the environment. In addition, *S. aureus* increases in inappropriate food storage and storage conditions. For all these reasons, food poisoning diseases caused by *S. aureus* pose a public health risk. It is also one of the worldwide food-borne economic problems (Bergonier et al., 2003; Jørgensen et al., 2005; El-Jakee et al., 2013)

The pathogenicity of *S. aureus* is influenced by the fact that it is a common inhabitant of a large part of the population and has the ability to produce several virulence factors under selective pressure with the disease when given the appropriate and right opportunity. Virulence factors of *S. aureus* include the enzymes they produce, the toxins they secrete, protein A, and biofilm. Biofilm formation increases the virulence of bacterial species, including *S. aureus*, and provides protection from host defenses (Paharik and Horswill, 2016; Mulcahy and McLoughlin, 2016; Gazi, 2021)

Some surface proteins, extracellular proteins, capsular polysaccharides, adhesins, and autolysin encoded by the atIE gene play a role in the regulation of biofilm production. The *ica* gene encodes intracellular adhesion (*ica*) and is essential for biofilm production (Vuong et al., 2003; Ryder et al., 2012). In addition, the *bap* gene increases biofilm formation and renders antibiotic treatment ineffective against biofilm-forming bacteria. Co-expression of the *ica*A and *ica*D genes facilitates slime

production, and *ica*C acts as a receptor for polysaccharides. The role of *ica*B has not been fully elucidated (Cucarella et al., 2001; Atshan et al., 2012)

Factors that increase antibiotic resistance in microorganisms include selective pressure for antimicrobial use, increased and unconscious use of antibiotics, microbial characteristics, host susceptibility, and errors in infection control programs. In addition, biofilm formation in *S. aureus* may also lead to an increase in antibiotic resistance. The emergence of antibiotic-resistant bacterial pathogens poses a public health threat (Asadi et al., 2014; Bhattacharya et al., 2015; Osman et al., 2017).

S. aureus shows high resistance to antibiotics such as tetracycline, methicillin, kanamycin, gentamicin, and streptomycin. This indicates that *S. aureus* has a high potential to develop resistance to antimicrobial agents (Jamali et al., 2015). Methicillin-resistant *S. aureus* (MRSA) is one of the most common pathogens, especially in hospitals and community infections. MRSA strains are divided into "community-acquired MRSA" strains acquired from community settings and "nosocomial strains", which are resistant to many antibiotics and originate from nosocomial infections. In addition, foodstuffs are recognized as a source of MRSA strains and are reported to be frequently encountered in foodborne disease outbreaks (Yamamoto et al., 2013; Zhang et al., 2020).

The aim of this study was to associate biofilm, antimicrobial resistance, and genes in *S. aureus* isolates obtained from raw milk sold in Van province and to reveal the public health risk.

Material and Methods

Bacterial Strains

S. aureus (ATCC® 25923) were procured from the Food Hygiene and Technology Department of the Veterinary Faculty of Van Yüzüncü Yıl University were used as the reference strains.

Sample Collection

A total of 100 raw milk samples were used as the study material. The samples were obtained from the sales points under aseptic conditions, placed in sterile sample containers of at least 500 ml, brought to the laboratory at +4°C, and analyzed immediately.

Isolation and Identification of S. Aureus Isolates

The isolation of *S. aureus* strains from raw milk was performed according to TS EN ISO 6888-1. Typical and atypical colonies on Baird-Parker Agar (Oxoid CM275) were subcultured and identified by gram staining, catalase test, coagulase test, DNase activity, and mannitol fermentation (Bennett et al., 2013; TS, 2021) The isolates that were identified to be *S. aureus* were confirmed using PCR.

A commercial kit (GeneAll, Exgene™ Cell SV, South Korea) and master mix (Abm® 2X PCR Taq Plus Mastermix, G014, Canada) was used for DNA extraction of the S. aureus colonies that were isolated from the raw milk samples. The specific primer pair (5'-GGACGACATTAGACGAATCA-3'; 5'-CGGGCACCTATTTTCTATCT-3', 1318 bp) that was developed by Riffon et al. (2001) used for the PCR confirmation of the *S. aureus* isolates. For the preparation of the PCR mixture, 10 µL of master mix, 1.5 µL (10 µM) of each primer, and 5 µL of genomic DNA were added and the total volume was brought to 25 µL using PCR water. After keeping the mixture at 94°C for 10 min for predenaturation, a 35-cycle amplification procedure was employed comprising denaturation at 94°C for 60 s, annealing at 51°C for 60 s, extension at 72°C for 60 s, and final extension at 72°C for 10 min. The gel electrophoresis of the amplicons was carried out using 1.5% agarose gel (Bioshop, Canada) in a horizontal tank (Major Science, multiSUB Midi, England) at 70-V electric current for 120 min.

Antibiotic Susceptibility Testing

Antibiotic Resistance tests were tested by the standard disk diffusion method of Kirby-Bauer (Bauer et al., 1966) on Mueller Hinton Agar (Oxoid CM0337, UK). Penicillin G (P, 10 U, Liofilchem®, Italy), cefoxitin (FOX, 30 µg, Liofilchem®, Italy), trimethoprim-sulfamethoxazole (SXT, 25 µg, Liofilchem®, Italy), ceftriaxone (CRO, 30 µg, Liofilchem®, Italy), erythromycin (E, 15 µg, Liofilchem®, Italy), chloramphenicol (C, 30 µg, Liofilchem®, Italy) were used to determine the antibiotic resistance of the *S. aureus* isolates. The results were evaluated according to the disk diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI, 2020).

PCR Detection of Biofilm and Antimicrobial Resistance Genes

The primer pairs and their properties used in the determination of biofilm (*ica*A, *ica*D, *bap*) and methicillin resistance genes (*mec*A) in *S. aureus* isolates confirmed by PCR method are given in Table 1. For the PCR mix of each gene, 10 µl of master mix, 5 µl of genomic DNA, and 1.5 µl (10 µM) of each primer were added and the total volume was completed to 25 µl with PCR water. After keeping the mixture at 94°C for 10 min for predenaturation, a 35-cycle amplification procedure was employed comprising denaturation at 94°C for 30 s, annealing temperatures given in table 1 for 45 s, extension at 72°C for 30 s, and final extension at 72°C for 7 min. Amplicons obtained as a result of PCR were electrophoresed as described previously and positive bands were observed.

Statistical Analysis

In the study, SPSS 13.0 package program was used to calculate the analysis results as a percentage (SPSS, 2006).

Results

Isolation of S. Aureus

S. aureus was isolated in 30 (30%) of 100 raw milk samples obtained from Van province. A total of 48 *S. aureus* isolates were obtained from 30 samples.

Biofilm and mecA Genes in S. Aureus Isolates

Biofilm and *mec*A gene presence in *S. aureus* isolates are given in Table 2. (Figure 1).

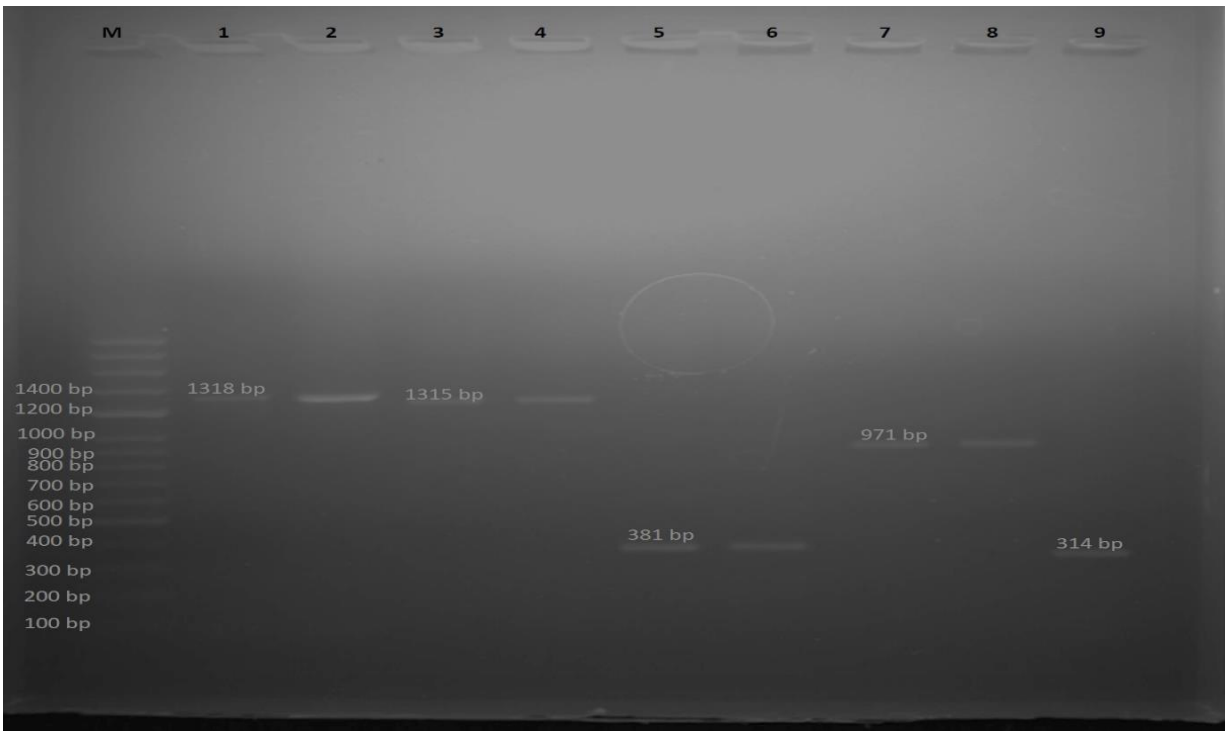

Figure 1. Agragose gel image (1.5%) [M:100 bp Marker; 1,2: *S. aureus* amplicons (1318 bp); 3,4: *ica*A amplicons (1315 bp); 5,6: *ica*D amplicons (381 bp); 7,8: *bap* amplicons (971 bp); 9: *mec*A amplicons (314 bp)]

Table 1. Biofilm and antimicrobial resistance genes used in *S. aureus* isolates

Genes		Oligonucleotide	bp	Annealing ℃	Reference
Biofilm	*ica*A	F: CCTAACTAACGAAAGGTAG R: AAGATATAGCGATAAGTGC	1315	50	Darwish and Asfour, 2013
	*ica*D	F: AAACGTAAGAGAGGTGG R: GGCAATATGATCAAGATAC	381	50	
	bap	F: CCCTATATCGAAGGTGTAGAATTGCAC R: GCTGTTGAAGTTAATACTGTACCTGC	971	60	Cucarella et al., 2004
Methicillin	*mec*A-1 *mec*A-2	5'-CCTAGTAAAGCTCCGGAA-3' 5'-CTAGTCCATTCGGTCCA-3'	314	48	Choi et al., 2003

Table 2. Distribution of biofilm and *mec*A genes in *S.aureus* isolates

İzolat	icaA	icaD	Bap	mecA	İzolat	icaA	icaD	Bap	mecA
3	+				27	+			
4	+	+			30	+		+	
19	+	+		+	32	+			
20	+	+		+	37	+			
22	+		+		42	+			
23	+		+		45	+			

Table 3. Resistance status in *S. aureus* isolates

	P	FOX	SXT	E	C		P	FOX	STX	E	C		P	FOX	STX	E	C	P	FOX	STX	E	C	
1	R	R	I	I	I	13	R	R	I	R	I	25	R	R	I	S	S	37	R	R	I	I	I
2	R	R	I	I	I	14	R	R	R	R	S	26	R	R	I	I	I	38	R	R	I	R	S
3	R	R	I	I	I	15	R	R	I	S	S	27	R	R	I	R	S	39	R	R	I	R	I
4	R	R	I	R	I	16	R	R	I	R	R	28	R	R	I	R	S	40	R	R	I	R	R
5	R	R	I	S	S	17	R	R	R	R	I	29	R	R	I	S	I	41	R	R	I	R	I
6	R	R	I	S	S	18	R	R	I	R	I	30	R	R	I	S	I	42	R	R	I	I	R
7	R	R	I	R	I	19	R	R	I	R	S	31	R	R	I	S	I	43	R	R	I	S	I
8	R	R	S	R	I	20	R	R	I	S	I	32	R	R	I	I	S	44	R	R	I	S	S
9	R	R	I	S	S	21	R	R	I	S	I	33	R	R	I	R	I	45	R	R	R	R	S
10	R	R	I	R	I	22	R	R	I	R	S	34	R	R	I	R	I	46	R	R	S	S	S
11	R	R	I	R	R	23	R	R	I	I	S	35	R	R	I	I	I	47	R	R	S	R	S
12	R	R	I	R	I	24	R	R	S	I	I	36	R	R	I	R	S	48	R	R	R	R	S

P: Penicillin, FOX: Cefoxitin, SXT: Trimethoprim-sulfamethoxazole, E: Erythromycin, C: Chloramphenicol, R: Resistance, I: Intermediate, S: Susceptible

Antibiotic Susceptibility Testing

The antibiotic resistance of *S. aureus* isolates is given in Table 3. All isolates (100%) were susceptible to ceftriaxone.

Discussion and Conclusion

S. aureus is an important pathogen causing foodborne illness and poisoning in humans and animals. *S. aureus* is one of the main causes of eczema, especially from food. In addition, if a food contains *S. aureus*, the consumption of that food can cause serious gastrointestinal diseases. Staphylococcal food poisoning is caused by the ingestion of sufficient amounts of staphylococcal enterotoxins present in contaminated food. *S. aureus*, a leading cause of foodborne illness worldwide, colonization in dairy cattle, and subsequent contamination of raw milk by pathogenic *S. aureus* remains a major problem for both dairy producers and public health (Cosgrove, 2006; Argudín et al., 2010; Spanu et al., 2012; Robinson et al., 2016; Tsilochristou et al., 2019).

In the study, *S. aureus* was detected in 30 (30%) out of 100 raw milk samples. The findings of this study were higher than the findings of Jamali et al. (2015) (15.7%), Girardini et al., (2016) (12.8%), Liu et al., (2017) (27.7%), and Johler et al., (2018) (19%), lower than the findings of Ateba et al., (2010) (100%), Aydin et al. (2011) (41.6%), Akindolire et al. (2015) (75%), Duyuk, (2015) (52.08%), Keyvan (2019) (51.6%), and Kou et al. (2021) (43.1%), and similar to the study of Bissong and Ateba, (2020) (32.8%). These differences between the studies are thought to be due to the differences in hygienic measures taken during milking, storage, and sale.

The potential pathogenicity of *S. aureus* is based on its ability to produce various virulence factors. The biggest factor that increases the virulence of *S. aureus* is its ability to form biofilms (Cosgrove, 2006; Neopane et al., 2018). The presence of potentially biofilm-producing and antibiotic-resistant *S. aureus* in milk intended for human consumption can lead to serious health problems (Corrente et al., 2007). Biofilm-forming *S. aureus* strains are resistant to antibiotics, disinfectants, and environmental factors (de la Fuente-Nú˜nez et al., 2013; Wang et al., 2018; Chang et al., 2019; Sedarat and Taylor-Robinson, 2022).

In this study, a total of 48 *S. aureus* isolates were obtained from 30 samples. *S. aureus* isolates were investigated for genes involved in biofilm formation (*ica*A, *ica*D, *bap*) and biofilm genes were detected in 18 isolates (37.5%). Among the detected genes, the most common *ica*A gene was detected in 66.6% of the isolates, while *ica*D and *bap* genes were detected in 16.66%. In some studies, biofilm genes were examined in mastitis milk, raw milk, and *S. aureus* obtained from various foods and animals. Darwish and Asfour (2013) found *ica*A in 15%, *ica*D in 62.5% and *bap* gene in 25% of the isolates, Khoramrooz et al. (2016) found *ica*A in 56.25%, *ica*D in 87.5% and *bap* gene in 5%, Salimena et al. (2016) found *ica*A+D in all isolates and *bap* gene in 95.6%. Gowrishankar et al., (2016) detected ica genes in 84.12% of the isolates in their study. Felipe et al. (2017) in their study conducted in Argentine dairy farms, reported that all *S. aureus* isolates (100%) had the *ica*A+D gene and 11% had the bap gene. Marques et al. (2017) identified *ica*A, *ica*D, and *bap* genes in 85%, 95%, and 5% of isolates, respectively. Sharma et al. (2017) in northwest India and Wang et al. (2018) in Beijing found the *ica*A/D gene in all isolates (100%). Avila-Nova et al. (2018) reported that they detected the *ica*A+D gene in 25% of the *S. aureus* isolates in their study. Bissong and Ateba (2020) reported that they detected the *ica*A gene in 63.6%, the *ica*D gene in 55.8%, and the *bap* gene in 15.6% of *S. aureus* isolates from milk in South Africa. Biofilm genes detected in our study were found to be lower than in other studies. The differences between the studies are thought to be due to the sources of the isolates (such as mastitis milk, and raw milk), geographical differences, and different biofilm-forming abilities of the isolates.

In our study, as in some other studies (Duyuk, 2015; Marques et al., 2017; Bissong and Ateba, 2020), the incidence of the *bap* gene was found to be lower than that of the *ica* genes. This is in line with the finding of Bissong and Ateba (2020) that although not all genes responsible for biofilm formation were tested, the *ica*-dependent mechanism may be primarily responsible for adhesion and biofilm formation in these isolates. Furthermore, in contrast to our study, Cucarella et al. (2001) reported that bap is not only involved in the primary binding step but also in cell-to-cell aggregation with PIA and thus in biofilm maturation, while Lasa and Penades (2006) reported that staphylococcal isolates harboring the *bap* gene are strong biofilm producers even in the absence of the *ica*ADBC operon. Salimena et al. (2016) confirmed the importance of this gene in biofilm formation by detecting the *bap* gene in 95.6% of the isolates.

In the veterinary field, the use of antibiotics in farm animals is increasing and their misuse and/or misuse leads to the emergence of resistant strains, resulting in the widespread presence of pathogenic resistant organisms in food products that pose a potential threat to human health (Landers et al., 2012). Therefore, animal foods should be screened for antibiotic-resistant pathogens (Bissong and Ateba, 2020).

In this study, the resistance of *S. aureus* isolated from raw milk to seven antibiotics was investigated phenotypically and resistance was observed against all antibiotics except ceftriaxone. However, the highest resistance was detected against penicillin G and cefoxitin. Klein (2007) and Şahin (2017), in their study, revealed the high level of resistance of *S. aureus* to B-lactam group antibiotics and emphasized the importance of personnel-borne contamination in the food production process, which confirms our study. The widespread use of penicillins in the treatment of farm animals and the prevention of diseases in Türkiye is effective in the formation of high resistance to this drug (Budak, 2008). In addition, a high rate of resistance was observed against trimethoprim-sulfamethoxazole, erythromycin, and chloramphenicol. In this study, 83.33% of the isolates were found to be intermediate and 8.33% resistant to trimethoprim-sulfamethoxazole. 79.17% of the isolates were intermediate and 8.33% were resistant to chloramphenicol. The results of the analysis of high resistance to penicillin and ceftriaxone in some previous studies are similar to our study (Parisi et al., 2016; Bissong and ateba, 2020). Begum et al. (2007) found 82.86% of the isolates obtained from mastitis milk were resistant to penicillin. Pereira et al.

(2009) reported that *S. aureus* isolates isolated from raw milk and various foods were 73% resistant to penicillin, 60% intermediate and 5% resistant to erythromycin, and 1% intermediate and 1.4% resistant to chloramphenicol. In some studies, penicillin and cefoxitin resistance was found to be lower Jamali et al., 2015; Girardini et al., 2016). Peles et al. (2007) found that 30.5% of *S. aureus* isolated from cattle milk in Hungary were penicillin-resistant. They stated that all of their isolates were sensitive to cefoxitin, erythromycin, and trimethoprim-sulfamethoxazole. Sharma et al. (2017) in their study on raw bovine milk in North-West India, found that 90% of the isolates were penicillin-resistant, 6.66% chloramphenicol-resistant, and 6.66% intermediate. Wang et al. (2018) reported that 31.3% of the isolates were resistant to penicillin, 5.2% were resistant to erythromycin and 2.1% were intermediate, 1% were resistant to chloramphenicol and 2.1% were intermediate in their study on mastitic milk in China. Kou et al. (2021) found 72.6% of S. aureus isolates resistant to penicillin, 19.4% to trimethoprim-sulfamethoxazole, and 32.3% to erythromycin of the *S. aureus* isolates obtained from their study on raw milk of various animals. Plasmidic penicillin resistance spreads rapidly between strains and is very common in foodborne *S. aureus* (Aydin et al., 2011). The ratio of trimethoprim-sulfamethoxazole and chloramphenicol varies in some studies (Aydin et al., 2011; Parisi et al., 2016). Aydin et al. (2011) in their study in Turkey, determined that of the isolates obtained from raw milk were 4.67% intermediate and 18.75% resistant to trimethoprim-sulfamethoxazole, 4.67% were intermediate and 10.94% resistant to chloramphenicol. Jamali et al. (2015) found all *S. aureus* isolates (100%) susceptible to trimethoprim-sulfamethoxazole, 3.7% resistant to chloramphenicol, and 7.9% resistant to erythromycin. Parisi et al. (2016) found all isolates resistant to chloramphenicol. Keyvan (2019), in his study on raw milk, found that 9.67% of the isolates were intermediate and 6.46% resistant to trimethoprim-sulfamethoxazole, 16.13% resistant to chloramphenicol, and 41.93% intermediate and 6.46% resistant to erythromycin. Bissong and Ateba (2020) determined that 14.3% of the isolates were resistant to trimethoprim-sulfamethoxazole, 20.8% to erythromycin, and 14.3% to chloramphenicol. In our study, all isolates (100%) were susceptible to ceftriaxone. The rate determined in our study is similar to the rate determined in the study by Sharma et al. (2017). Bissong and Ateba (2020) found 87% of the isolates were resistant to ceftriaxone in their study. In this study, 20.83% and 52.08% of the isolates obtained from raw milk were found to be intermediate and resistant to erythromycin, respectively. The differences between our study and other studies may be due to the different *S. aureus* isolates and the use of preferred antibiotics at different levels. In addition, *S. aureus* isolates detected as intermediate in the study may develop complete resistance to these antibiotics. Therefore, it is important to monitor antibiotics that develop intermediate in isolates.

It has been reported that *S. aureus* isolates with multiple antibiotic-resistant properties adversely affect the treatment of staphylococcal infections, especially in immunocompromised individuals, the elderly, and young children (Ito et al., 2003).

In our study, 26 of 48 isolates (54.16%) were resistant to at least three antibiotics. In addition, strains resistant to at least two antibiotics were found to be intermediate to at least one antibiotic (Table 3). Normanno et al. (2007) reported that 9.6% of *S. aureus* strains showed resistance to three and 4% to four antibiotics. Furthermore, Chao et al. (2007) found a high level (79%) of multidrug resistance among the isolates. Aydin et al. (2011) reported that 25.3% of *S. aureus* strains showed multidrug resistance against penicillin G, erythromycin, and sulfamethoxazole-trimethoprim. Jamali et al. (2015), in their study on milk and dairy products, determined that 46.6% of the isolates were resistant to two antibiotics and 12.8% to more than two antibiotics. Bissong and Ateba (2020) found resistance to at least three antibiotics (32.5%). In their study, Kou et al. (2021) found multidrug resistance in 46.8% of isolates. The development of multiple antibiotic resistance among most of these isolates can be attributed to the acquisition of plasmid-mediated resistance (R-factor) (Yamamoto et al., 2013). Only methicillin-resistant staphylococci have the chromosomal *mec*A gene that provides methicillin resistance (Peacock and Paterson, 2015; Lee et al., 2018). The most important feature of most MRSA isolates is heterotypic or heterogeneous resistance to β-lactams. Homogeneous resistance refers to a population of cells in which all cells are resistant to high concentrations of methicillin (>128 mg/l), whereas heterogeneous resistance refers to a population of cells in which only a small minority of cells exhibit high levels of methicillin resistance. Since the *mec*A gene is not expressed in heterogeneous resistant *S. aureus* strains, it should be resistant in routine susceptibility tests, but detection as susceptible leads to incorrect treatment. Therefore, investigating the presence of the *mec*A gene by PCR gives the most reliable result. Among the methods for reliable identification of MRSA of *S. aureus* strains, detection of the *mec*A gene is often considered the "gold standard" due to its high sensitivity and speed (Corrente et al., 2007; Lee et al., 2018; Stapleto and Taylor, 2002; Çiftçi et al., 2019). In addition, the absence of the *mec*A gene in phenotypically resistant isolates may be due to characteristics such as point mutations, biofilm formation, or antibiotic tolerance (Bissong and Ateba (2020).

The CLSI (2020) standard recommends the use of cefoxitin to identify methicillin-resistant *S. aureus* strains. In our study, all isolates were phenotypically resistant to cefoxitin. However, the *mec*A gene was detected in only two isolates. This is similar to the situation reported by Aydin et al. (2019), Bissong and Ateba (2020), and Girardini et al. (2016). Detection of *S. aureus* strains that are phenotypically resistant to methicillin but do not carry the *mec*A gene poses serious health problems and is of great clinical importance (Swenson et al., 2007). Parisi et al. (2016), in their study, found that the rate of resistant isolates of cefoxitin was 2.5%, and they found *mec*A gene presence in all of them. Johler et al. (2018) did not detect *mec*A gene in any of the isolates obtained in their study. Omwenga et al. (2021), in their study on various milks, detected *mec*A gene in 38.8% of the isolates. The absence of *mec*A gene in phenotypically resistant isolates in our study may be due to characteristics such as point mutations, biofilm formation, or antibiotic tolerance.

According to the statistical results of the study, a significant correlation was found between *ica*A gene and icaD and bap at P<0.05, between *ica*A gene and *mec*A gene at P<0.01, and between *ica*D and *mec*A gene at P<0.05 level.

As a result, although it showed that the rate of *S.aureus* in raw milk was low, the high antibiotic resistance of the *S. aureus* isolates obtained, biofilm-forming genes, and methicillin resistance genes showed that raw milk may be a serious problem for public health. In addition, while bacteria are phenotypically sensitive to antibiotics, the formation of biofilms that make the bacteria resistant to antibiotics may pose a risk to public health by causing incorrect treatment in cases of food poisoning. As raw milk is one of the main sources of complementary nutrition for consumers, it is important that it is healthy and safe. Therefore, regular inspection and management of milk collection, transportation, and sales points are required to reduce the risk of *S. aureus* caused by raw milk. In addition, veterinarians and animal breeders should pay attention to unnecessary and incorrect use of antibiotics to protect the health and safety of consumers, and antibiotic use in animals should be strictly controlled.

Acknowledgments

We would like to thank Koray TUNCAY for his support.

References

Akindolire MA, Babalola OO, Ateba CN. 2015. Detection of Antibiotic Resistant *Staphylococcus aureus* from Milk: A Public Health Implication. IJERPH, 12(9): 10254-10275. https://doi.org/10.3390/ijerph120910254

Argudín AM, Mendoza MC, Rodicio MR. 2010. Food Poisoning and *Staphylococcus aureus* Enterotoxins. Toxins, 2: 1751-1773. https://doi.org/10.3390/toxins2071751

Asadi S, Kargar M, Solhjoo K, Najafi A, Ghorbani-Dalini S. 2014. The association of virulence determinants of uropathogenic Escherichia coli with antibiotic resistance. Jundishapur J Microbiol. 7: e9936. https://doi.org/10.5812/jjm.9936

Ateba CN, Mbewe M, Moneoang M S, Bezuidenhout CC. 2010. Antibiotic-resistant *Staphylococcus aureus* isolated from milk in the Mafikeng Area, North West province, South Africa: Research article. S Afr J Sci, 106(11): 1-6. https://doi.org/10.10520/EJC96992

Atshan SS, Nor Shamsudin M, Sekawi Z, Lung LTT, Hamat RA, Karunanidhi A, Ali AM, Ghaznavi-Rad E, Ghasemzadeh-Moghaddam H, Seng JSC, Nathan JJ, Pei Pei C. 2012. Prevalence of adhesion and regulation of biofilm-related genes in different clones of *Staphylococcus aureus*. J BioMed Biotechnol, https://doi.org/10.1155/2012/976972

Avila-Novoa MG, Iñíguez-Moreno M, Solís-Velázquez OA González-Gómez JP, Guerrero-Medina PJ, Gutiérrez-Lomelí M. 2018. Biofilm Formation by *Staphylococcus aureus* Isolated from Food Contact Surfaces in the Dairy Industry of Jalisco, Mexico. J Food Qual, e1746139. https://doi.org/10.1155/2018/1746139

Aydin A, Muratoglu K, Sudagidan M, Bostan K, Okuklu B, Harsa S. 2011. Prevalence and antibiotic resistance of foodborne *Staphylococcus aureus* isolates in Turkey. Foodborne Path Dis, 8: 63-69. https://doi.org/10.1089/fpd.2010.0613

Bauer RW, Kirby MDK, Sherris JC, Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol, 45:493-496.

Begum HA, Uddin MS, Islam MJ, Nazir KH, Islam MA, Rahman MT. 2007. Detection of biofilm producing coagulase positive *Staphylococcus aureus* from bovine mastitis, their pigment production, hemolytic activity and antibiotic sensitivity pattern. J Bangladesh Soc Agric Sci Technol, 4: 97-100.

Bennett RW, Hait JM, Tallent SM. 2013. *Staphylococcus aureus*. In: Labbé RG, García S (editors). Guide to Foodborne Pathogens, 2nd ed, Wiley-Blackwell: India.

Bergonier D, De Cremoux R, Rupp R, Lagriffoul G, Berthelot X. 2003. Mastitis of dairy small ruminants. Veterinary Research, 34: 689e716.

Bhattacharya M Wozniak DJ, Stoodley P, Hall-Stoodley L. 2015. Prevention and treatment of *Staphylococcus aureus* biofilms. Expert Rev. Anti-Infect Ther, 13: 1499-1516. https://doi.org/10.1586/14787210.2015.1100533.

Bissong MEA, Ateba CN. 2020. Genotypic and phenotypic evaluation of biofilm production and antimicrobial resistance in *Staphylococcus aureus* isolated from milk, North West Province, South Africa. Antibiotics, 9(4): 156. https://doi.org/10.3390/antibiotics9040156

Budak O. 2008. Isolation and Identification of *S. aureus*, Detect These Bacteria Enterotoxins and Determine Number of somatic Cells and Antibiotic from Mastitic Cows' Milk in Kocaeli Region and Enviromental Effects in the Milk. MSc Thesis, Gebze Institute of Technology, Institute of Science and Engineering.

Chang Y, Wang PC, Ma HM, Chen SY, Fu YH, Liu YY, Wang X, Yu GC, Huang T, Hibbs DE, Zhoua H, Chena W, Lina J, Wange C, Zhengd J, Suna P. 2019. Design, synthesis and evaluation of halogenated furanone derivatives as quorum sensing inhibitors in Pseudomonas aeruginosa. Eur J. Pharm Sci, 140: 105058. https://doi.org/10.1016/j.ejps.2019.105058

Chao G, Zhou X, Jiao X, Qian X, Xu L. 2007. Prevalance and antimicrobial re- sistance of foodborne pathogens isolated from food products in China. Foodborne Pathog Dis, 4: 277-284. https://doi.org/10.1089/fpd.2007.0088

Choi SM, Kim SH, Kim HJ, Lee DG, Choi JH, Yoo JH, Kang JH, Shin WS, Kang MW. 2003. Multiplex PCR for the detection of genes encoding aminoglycoside modifying enzymes and methicillin resistance among *Staphylococcus* species. J Korean Med Sci, 18: 631-636. https://doi.org/10.3346/jkms.2003.18.5.631

CLSI, 2020. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, USA.

Corrente M, Normanno G, Martella V Bellacicco AL, Quaglia NC, D'Abramo M, Buonavoglia C. 2007. Comparison of methods for the detection of methicillin resistance in *Staphylococcus aureus* isolates from food products. Lett Appl Microbiol, 45: 535-539. https://doi.org/10.1111/j.1472-765X.2007.02226.x

Cosgrove SE. 2006. The relationship between antimicrobial resistance and patient outcomes: Mortality, length of hospital stays, and health care costs. Genet Mol Res, 42: S82-S89. https://doi.org/10.1086/499406

Cucarella C, Solano C, Valle J, Amorena B, Lasa Í, Penadés JR. 2001. *Bap*, a *Staphylococcus aureus* Surface Protein Involved in Biofilm Formation. J Bacteriol, 183(9): 2888-2896. https://doi.org/10.1128/JB.183.9.2888-2896.2001

Cucarella C, Tormo MA, Ubeda C, Trotonda MP, Monzón M, Peris C, Amorena B, Lasa I, Penadés JR. 2004. Role of biofilm-Associated protein *bap* in the pathogenesis of bovine *Staphylococcus aureus*. Infect Immun, 72: 2177–2185. https://doi.org/10.1128/IAI.72.4.2177-2185.2004

Çiftçi İH, Altindiş M, Çetinkaya Z, Aşık G, Aktepe OC. 2009. Investigation of mecA genes in Staphylococcus strains isolated from clinical samples. The Medical Journal of Kocatepe, 10(1): 17-20.

Darwish SF, Asfour HA. 2013. Investigation of biofilm forming ability in Staphylococci causing bovine mastitis using phenotypic and genotypic assays. Sci World J, https://doi.org/10.1155/2013/378492

De la Fuente-Núˆnez C, Reffuveille F, Fernandez L, Honcock REW. 2013. Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. Curr Opi Microbiol, 16:580e589. https://doi.org/10.1016/j.mib.2013.06.013

Duyuk G. 2015. Detection of *Staphylococcus aureus* and Stapylococcal Enterotoxins in Raw Milk Samples, MSc Thesis, Adnan Menderes Unıversity, Aydın, Türkiye, 2015.

El-Jakee JK, Aref NE, Gomaa A, El-Hariri MD, Galal HM, Omar SA, Ahmed S. 2013. Emerging of coagulase negative staphylococci as a cause of mastitis in dairy animals: An environmental hazard. International Journal of Veterinary Science and Medicine. 1-5. https://doi.org/10.1016/j.ijvsm.2013.05.006

Felipe V, Morgante CA, Somale PS, Varroni F, Zingaretti ML, Bachetti RA, Correa SG, Porporatto C. 2017. Evaluation of the biofilm forming ability and its associated genes in Staphylococcus species isolates from bovine mastitis in Argentinean dairy farms. Microbial Pathogenesis, 104: 278-286. https://doi.org/10.1016/j.micpath.2017.01.047

Gazi DU. 2021. Biofilm Assessment of *Staphylococcus aureus* From Different Clinical Specimens. Ph.D. Thesis, Near East University, Cyprus.

Girardini LK, Paim DS, Ausani TC, Lopes GV, Pellegrini DCP, Brito MAV, Cardoso M. 2016. Antimicrobial resistance profiles of *Staphylococcus aureus* clusters on small dairy farms in southern Brazil. Pesquisa Veterinária Brasileira, 36: 951-956. https://doi.org/10.1590/S0100-736X2016001000006

Gowrishankar S, Kamaladevi A, Balamurugan K, Pandian SK. 2016. In Vitro and In Vivo Biofilm Characterization of Methicillin-Resistant *Staphylococcus aureus* from Patients Associated with Pharyngitis Infection. BioMed Res Int, e1289157. https://doi.org/10.1155/2016/1289157

Ito T, Okuma K, Ma XX, Yuzawa H, Hiramatsu K. 2003. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: Genomic Island SCC. Drug Resist Updat, 6: 41-52. https://doi.org/10.1016/S1368-7646(03)00003-7

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. https://doi.org/10.1016/j.foodcont.2015.02.013

Johler S, Macori G, Bellio A, Acutis PL, Gallina S, Decastelli, L. (2018). Short communication: Characterization of *Staphylococcus aureus* isolated along the raw milk cheese production process in artisan dairies in Italy. J Dairy Sci, 101(4): 2915-2920. https://doi.org/10.3168/jds.2017-13815

Jørgensen HJ, Mørk T, Høgåsen HR, Rørvik LM Enterotoxigenic *Staphylococcus aureus* in bulk milk in Norway. J Appl Microbiol, 99(1): 158-166. https://doi.org/10.1111/j.1365-2672.2005.02569.x

Keyvan E. 2019. Detection of antibiotic resistance properties of *Staphylococcus aureus* isolated from raw milk samples. Journal of the Turkish Veterinary Medical Society, 90(1): 9-14. https://doi.org/10.33188/vetheder.475938

Khoramrooz SS, Mansouri F, Marashifard M, Malek Hosseini SAA, Akbarian-Chenarestane-Olia F, Ganavehei B, Gharibpour F, Shahbazi A, Mirzaii M, Darban-Sarokhalil D. 2016. Detection of biofilm related genes, classical enterotoxin genes and agr typing among *Staphylococcus aureus* isolated from bovine with subclinical mastitis in southwest of Iran. Microbial Pathogenesis, 97: 45–51. https://doi.org/10.1016/j.micpath.2016.05.022

Klein G. 2007. Antibiotika-Resistenzen Bei Lebensmittelinfectionserregern. Handbuch Lebensmittelhygiene. B. Behr's Verlag GmbH & Co. KG, Hamburg. pp. 88-97.

Kou X, Cai H, Huang S, Ni Y, Luo B, Qian H, Ji H, Wang X. 2021. Prevalence and Characteristics of Staphylococcus aureus Isolated from Retail Raw Milk in Northern Xinjiang, China. Front Microbiol, 12. https://www.frontiersin.org/articles/10.3389/fmicb.2021.705947

Landers TF, Cohen B, Wittum TE, Larson EL. 2012. A review of antibiotic use in food animals: perspective, policy, and potential. Public Health Rep, 127(1): 4-22. https://doi.org/10.1177/003335491212700103

Lasa I, Penades JR. 2006. *Bap*: a family of surface proteins involved in biofilm formation. Res Microbiol, 157: 99-107. https://doi.org/10.1016/j.resmic.2005.11.003

Lee AS, de Lencastre H, Garau J, Kluytmans J, Malhotra-Kumar S, Peschel A, Harbarth S. 2018. Methicillin-resistant *Staphylococcus aureus*. Nature Reviews Disease Primers, 4(1): 18033. https://doi.org/10.1038/nrdp.2018.33

Liu H, Li S, Meng L, Dong L, Zhao S, Lan X, Wang J, Zheng N. 2017. Prevalence, antimicrobial susceptibility, and molecular characterization of *Staphylococcus aureus* isolated from dairy herds in northern China. J Dairy Sci, 100(11): 8796-8803. https://doi.org/10.3168/jds.2017-13370

Markey B, Leonard F, Archambault M, Cullinane A, Maguire D. Section 2: Bacteriology. In Clinical Veterinary Microbiology, 2nd ed.; China: Mosby Elsevier, pp. 105-121. ISBN 9780723432371

Marques VF, Motta CCD, Soares BDS, Melo DAD, Coelho SDMDO, Coelho IDS Barbosa HS, Souza MMSD. 2017. Biofilm production and beta-lactamic resistance in Brazilian *Staphylococcus aureus* isolates from bovine mastitis. Braz J Microbiol, 48(1): 118-124. https://doi.org/10.1016/j.bjm.2016.10.001

Marshall BM, Levy SB. 2011. Food animals and antimicrobials: Impacts on human health. *Clin* Microbio Rev, 24: 718–733. https://doi.org/10.1128/CMR.00002-11

Montville TJ. 2012. Biosafety challenges for the food microbiology laboratory. J Food Safety, 32: 184-188. https://doi.org/10.1111/j.1745-4565.2012.00366.x

Mulcahy ME, McLoughlin RM. 2016. *Staphylococcus aureus* and influenza a virus: partners in coinfection. *MBio*, 7(6): e02068-16. https://doi.org/10.1128/mBio.02068-16

Neopane P, Nepal HP, Shrestha R, Uehara O, Abiko Y. 2018. In vitro biofilm formation by *Staphylococcus aureus* isolated from wounds of hospital-admitted patients and their association with antimicrobial resistance. Int J General Med, 11: 25–32. https://doi.org/10.2147/IJGM.S153268

Normanno G, La Salandra G, Dambrosio A, Quaglian C, Corrente M, Parisi A, Santagada G, Firinu A, Crisetti E, Celano GV. 2007. Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. Int J Food Microbiol, 115290-115296. https://doi.org/10.1016/j.ijfoodmicro.2006.10.049 Get rights and content

Omwenga I, Aboge GO, Mitema ES Obiero G, Ngaywa C, Ngwili N, Wamwere G, Wainaina M, Bett B. 2021. Antimicrobial Usage and Detection of Multidrug-Resistant *Staphylococcus aureus*, Including Methicillin-Resistant Strains in Raw Milk of Livestock from Northern Kenya. Microb Drug Resist, 27(6): 843-854. https://doi.org/10.1089/mdr.2020.0252

Osman K, Alvarez-Ordonez A, Ruiz L, Badr J, ElHofy F, Al-Maary KS, Moussa IMI, Hessain AM, Orabi A, Saad A, Elhadidy M. 2017. Antimicrobial resistance and virulence characterization of *Staphylococcus aureus* and coagulase-negative staphylococci from imported beef meat. Ann Clin Microb Anti, 16(1): 1-10. 10.1186/s12941-017-0210-4

Paharik AE, Horswill AR. 2016. The staphylococcal biofilm: adhesins, regulation, and host response. Virulence mechanisms of bacterial pathogens, 529-566. https://doi.org/10.1128/9781555819286.ch19

Parisi A, Caruso M, Normanno G, Latorre L, Sottili R, Miccolupo A, Fraccalvieri R, 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. https://doi.org/10.1016/j.fm.2016.03.004

Peacock SJ, Paterson GK. 2015. Mechanisms of Methicillin Resistance in *Staphylococcus aureus*. Ann Rev Biochem, 84(1): 577-601. https://doi.org/10.1146/annurev-biochem-060614-034516

Peles F, Wagner M, Varga L, Hein I, Rieck P, Gutser K, Keresztúri P, Kardos G, Turcsányi I, Béri B, Szabó A. 2007. Characterization of *Staphylococcus aureus* strains isolated from bovine milk in Hungary. Int J Food Microbiol. 118(2): 186-193. https://doi.org/10.1016/j.ijfoodmicro.2007.07.010

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. https://doi.org/10.1016/j.fm.2008.12.008

Quinn RA, Lim YW, Maughan H, Conrad D, Rohwer F, Whiteson KL. 2014. Biogeochemical forces shape the composition and physiology of polymicrobial communities in the cystic fibrosis lung. MBio, 5(2). https://doi.org/10.1128/mBio.00956-13

Riffon R, Sayasith K, Khalil H, Dubreuil P, Drolet M Lagacé, J. 2001. Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. J Clin Microbiol, 39(7): 2584-2589. https://doi.org/10.1128/JCM.39.7.2584-2589.2001

Robinson TP, Bu DP, Carrique-Mas J, Fevre EM, Gilbert M, Grace D, Hay S I, Jiwakanon J, Kakkar M, Kariuki S, Laxminarayan R, Lubroth J, Magnusson U, Thi Ngoc P, Van Boeckel T P, Woolhouse MEJ. 2016. Antibiotic resistance is the quintessential One Health issue. Trans R Soc Trop Med Hyg, 110: 377-380. https://doi.org/10.1093/trstmh/trw048

Ryder VJ, Chopra I, O'Neill AJ. 2012. Increased mutability of Staphylococci in biofilms as a consequence of oxidative stress. PloS one. 7(10): e47695. https://doi.org/10.1371/journal.pone.0047695

Salimena AP, Lange CC, Camussone C, Signorini M, Calvinho L F, Brito MA, Borges CAV, Guimarães AS, Ribeiro JB, Mendonça LC, Piccoli RH. 2016. Genotypic and phenotypic detection of capsular polysaccharide and biofilm formation in *Staphylococcus aureus* isolated from bovine milk collected from Brazilian dairy farms. Vet Res Commun, 40(3): 97-106.

Schleifer KH, Bell JA. 2009. Staphylococcaceae. In: Vos PD, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH, Whitman WB (editors). Bergey's Manual of Systematic Bacteriolog, 2nd ed. USA: Springer.

Sedarat Z, Taylor-Robinson AW. 2022. Biofilm formation by pathogenic bacteria: applying a *Staphylococcus aureus* model to appraise potential targets for therapeutic intervention. Pathogens, 11(4): 388. https://doi.org/10.3390/pathogens11040388

Sharma V, Sharma S, Dahiya DK, Khan A, Mathur M, Sharma A. 2017. Coagulase gene polymorphism, enterotoxigenecity, biofilm production, and antibiotic resistance in *Staphylococcus aureus* isolated from bovine raw milk in North West India. Ann Clin Microb Ant. 16(1): 65. https://doi.org/10.1186/s12941-017-0242-9

Spanu V, Spanu C, Virdis S, Cossu F, Scarano C, de Santis EPL. 2012. Virulence factors and genetic variability of *Staphylococcus aureus* strains isolated from raw sheep's milk cheese. Int J Food Microbiol, 153: 53–57. https://doi.org/10.1016/j.ijfoodmicro.2011.10.015

SPSS, 2006. IBM SPSS statistics version 13.0 for Windows. New York: IBM.

Stapleton PD, Taylor PW. 2002. Methicillin resistance in *Staphylococcus aureus*: mechanisms and modulation. Science Progress, 85(1): 57-72. https://doi.org/10.3184/003685002783238870

Swenson JM, Lonsway D, McAllister S, Thompson A, Jevitt L, Zhu W, Patel JB. 2007. Detection of mecA-mediated resistance using reference and commercial testing methods in a collection of *Staphylococcus aureus* expressing borderline oxacillin MICs. Diagn Micr Infec Dis, 58(1): 33-39. https://doi.org/10.1016/j.diagmicrobio.2006.10.022

Şahin K. 2017. TÜBA-İnsan ve Hayvan Sağlığında Akılcı Antibiyotik Kullanımı ve Antibiyotik Dirençlilik Raporu. TÜBA-Gıda ve Beslenme Çalışma Grubu. Türkiye Bilimler Akademisi Yayınları.

TS, 2021. Turkish Standard. Microbiology of the food chain - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Method using Baird-Parker agar medium. (TS EN ISO 6888-1), Turkish Standards Institution, Ankara, Türkiye.

Tsilochristou O, du Toit G, Sayre PH, Roberts G, Lawson K, Sever ML, Bahnson HT, Radulovic S, Basting M, Plaut M, Lack G, Chan S, Fox A, Fisher H, Abraham M Adam M, Coverdale L, Duncan C, Nixon A, Mason T. 2019. Association of *Staphylococcus aureus* colonization with food allergy occurs independently of eczema severity. J Allergy Clin Immun, 144(2): 494–503. https://doi.org/10.1016/j.jaci.2019.04.025

Vuong C, Gerke C, Somerville GA, Fischer ER, Otto M. 2003. Quorum sensing control of biofilm factors in Staphylococcus epidermidis. J Infec Dis, 188: 706-718. https://doi.org/10.1086/377239

Wang W, Lin X, Jiang T, Peng Z, Xu J, Yi L, Li F, Fanning S, Baloch Z. 2018. Prevalence and characterization of *Staphylococcus aureus* cultured from raw milk taken from dairy cows with mastitis in Beijing, China. Front Microbiol, 9: 11-23. https://doi.org/10.3389/fmicb.2018.01123.

Yamamoto T, Hung WC, Takano T, Nishiyama A. 2013. Genetic nature and virulence of community-associated methicillin-resistant *Staphylococcus aureus*. BioMedicine, 3(1): 2-18. https://doi.org/10.1016/j.biomed.2012.12.001

Zhang P, Miao X, Zhou L, Cui B, Zhang J, Xu X, Wu C, Peng X, Wang X. 2020. Characterization of Oxacillin-Susceptible mecA-Positive *Staphylococcus aureus* from Food Poisoning Outbreaks and Retail Foods in China. Foodborne Pathog Disease, 17(11): 728-734. https://doi.org/10.1089/fpd.2019.2774