Sakarya University Journal of Science

ISSN : 2147-835X
Publisher : Sakarya University

Vol. 28, No. 3, 505-516, 2024
DOI: https://doi.org/10.16984/saufenbilder.1376028

Research Article

Investigation of Biofilm Production and Determination of Antibiotic Resistance Profile of *Staphylococcus aureus* Isolated from Ground Beef and Meatballs

Tuğba Sarı[1], Ceren Başkan[2*]

[1] Amasya University, Sciences Institute, Department of Biology, Amasya, Türkiye, tubiyus.6836@gmail.com
[2]Amasya University, Sabuncuoğlu Şerefeddin Health Services Vocational School, Amasya, Türkiye, ceren.yavuz@amasya.edu.tr
[*] Corresponding Author

ARTICLE INFO

Keywords:
Antibiotic resistance
Biofilm
Ground beef
Meatballs
S. aureus

Article History:
Received: 15.10.2023
Accepted: 13.03.2024
Online Available: 06.06.2024

ABSTRACT

Staphylococcus aureus (*S. aureus*) is bacterium that threatens public health because it causes infections and food intoxication. For this reason, within the scope of this study, it was aimed to determine the presence of *S. aureus*, antibiotic resistance profiles, and biofilm formation in ground beef and meatball samples consumed in Amasya. In the study, 60 meat samples purchased from Amasya were used as material. First, conventional culture technique and PCR testing were used for the isolation of *S. aureus*. Secondly, antibiotic resistance profiles of the *S. aureus* were analyzed by disc diffusion. Finally, biofilm production of the *S. aureus* was analyzed phenotypically by the microtiter plate method and genotypically by PCR. Through the study, we identified 58 *S. aureus* isolates that were confirmed phenotypically and genotypically. Disc diffusion results showed that all *S. aureus* were sensitive to imipenem and piperacillin-tazobactam, but resistant to methicillin 43.10% (25/58), erythromycin 41.37 % (24/58), penicillin 58.62% (34/58), gentamicin 10.34% (6/58), chloramphenicol 17.24% (10/58), tobramycin 6.89% (4/58), and levofloxacin 1.72% (1/58). Biofilm production was determined as 58.62% (34/58) in the microtiter plate. In the PCR analysis, the *icaA* or *icaD* gene of a total of 4 (6.89%) different isolates was evaluated as positive. As a result, the presence of antibiotic-resistant *S. aureus* in ground beef and meatballs and the production of biofilm by the bacteria pose a potential risk. Therefore, it is important for human health to take the necessary precautions to reduce the risk of *S. aureus* contamination during the processing, and transportation.

1. Introduction

Foodborne diseases, which the World Health Organization (WHO) defines as a disease caused by infectious pathogens or toxins thought to be caused by the consumption of contaminated food or water, are an important public health concern around the world [1]. Another important public health problem is that pathogens that gain resistance to antibiotics in foodborne infections transfer these resistance genes through horizontal gene transfer [2, 3]. Infections caused by antibiotic-resistant bacteria through foods spread rapidly in society, causing serious economic losses, and infections with high morbidity and mortality [4, 5]. Antibiotic resistance (AMR) is increasing rapidly around the world through food. According to the latest data from underdeveloped and developed countries, it has been determined that approximately 10% of the population suffers from foodborne diseases [6-8].

S. aureus is a pathogen that plays a role in foodborne infections and intoxications. Staphylococcal food poisoning from food contaminated with enterotoxins of *S. aureus* is a common cause of gastroenteritis. Staphylococcal foodborne poisoning has a short incubation period and is characterized by symptoms such as weakness, vomiting, and diarrhea [9-12]. The human nose is the largest ecological reservoir of

Cite as: T. Sarı, C. Başkan (2024). Investigation of Biofilm Production and Determination of Antibiotic Resistance Profile of Staphylococcus aureus Isolated from Ground Beef and Meatballs, *Sakarya University Journal of Science*, 28(3), 505-516. https://doi.org/10.16984/saufenbilder.1376028

S. aureus, an opportunistic pathogen involved in many life-threatening disease processes such as skin and soft tissue infections, toxic shock syndrome, sepsis, pneumonia, etc. [13, 14]. Most of the food contamination from *S. aureus* is through contact with food handlers who are carriers. Today, the presence of methicillin-resistant *S. aureus* in addition to many antibiotics reduces the treatment rate of food-borne intoxications. Products such as milk and meat, which we frequently consume in terms of health, are among the foods most frequently contaminated with *S. aureus* [15]. Contamination with raw meat can occur at many stages, from the cutting stage to distribution, storage, etc. the vegetative form *S. aureus* can die with proper cooking techniques. However, Staphylococcal enterotoxins are thermostable and therefore cannot be destroyed by thermal processes [16, 17].

The surface of foods with high protein content such as meat are environments where pathogenic microorganisms such as *S. aureus* can come together and form a biofilm by surrounding them with extracellular polymeric substance (EPS). This leads to cross-contamination, reduced shelf life of foods and the potential for foodborne illness. Bacterial biofilms cause problems in food sanitation because they are resistant to environmental stresses. In addition, biofilms that will form in various equipment used in the food industry cause surface corrosion and financial problems. Therefore, elucidating the structure for the removal and inhibition of *S. aureus* biofilms is an area of public health interest [18].

As a result of the literature review, the focus was on determining the antibiotic resistance profile and biofilm production of *S. aureus* isolates, which are mostly responsible for hospital-acquired infections [19-21]. In foods, there are studies to determine the prevalence and antibiotic resistance of *S. aureus*, which is generally isolated from dairy products, especially traditional cheese [22-24]. However, studies conducted to determine the prevalence and antibiotic resistance of *S. aureus* in meat samples in Turkey are limited. Among these, no studies were found to determine the biofilm production genotypically [15, 25]. For this reason, it is important to carry out studies on these issues to prevent food infections caused by *S. aureus*. In the present study, the aim of this study is to determine the antibiotic resistance profiles of *S. aureus* isolates isolated from ground beef and meatball samples and to analyze their biofilm production phenotypically and genotypically.

2. Materials and Methods

2.1.Food samples

In this study, a total of 60 samples (30 ground beef, and 30 meatballs) were purchased from different butchers and supermarkets in Amasya between February and July 2022. The samples were brought to the biological activity laboratory of Amasya University by keeping the cold chain in line with aseptic techniques.

2.2.Isolation of *S. aureus*

The methods used in the isolation of *S. aureus* in the samples previously for the isolation of bacteria from foods were taken as reference [15, 26]. For this purpose, firstly, decimal dilutions (10^{-1}, 10^{-2}, 10^{-3}) of the samples were made with buffered peptone water (0.1%, Biolife, Milano, Italya-4122592), and then spread on Baird Parker Agar (BPA, Biolife, Milano, Italya-NL6209) containing with 5% egg yolk tellurite (Merck, Darmstadt, Germany-K95798185) using the spread plating technique. The plates incubated at 37 °C for 24-48 hours. Then, three or five colonies were selected from lecithinase positive, black, and bright colonies and inoculated on tryptic soy agar (TSA, Merck, Darmstadt, Germany-105458) for phenotypic (Gram stain, catalase test, growth on mannitol salt agar, and coagulase test) and genotypic identification.

2.3.Genotypic identification of isolates and PCR analysis of biofilm-encoding genes

DNA was isolated by boiling from overnight cultures formed by inoculating phenotypically confirmed isolates as *S. aureus* on TSA agar. A colony growing on TSA was taken and homogenized in 500 µl sterile distilled water. Then it was kept in a dry block heater at 95 °C for 15 minutes. Finally, it was centrifuged at 10,000 g for 5 minutes and the supernatant containing genomic DNA was taken into a clean

tube and kept at -20 °C for further studies [27]. For genotypic validation of isolated isolates, PCR was performed using primers of the genus specific (*Staphylococcus* spp.) 16S rRNA gene (756F:5′ AACTCTGTTATTAGGGAAGAACA3′ and 756R:5′ CCACCTTCCTCCGGTTTGTCACC 3′) and the *nuc* gene (F: 5′ GCGATTGATGGTGATACGGTT3′ and R: 5′ AGC CAA GCC TTG ACG AAC TAA AGC3′) encoding the *S. aureus* specific thermonuclease enzyme [28, 29]. The *icaA* (F: 5′ CCTAACTAACGAAAGGTAG3′ and R: 5′ AAGATATAGCGATAAGTGC3′) and *icaD* (F: 5′AAACGTAAGAGAGGTGG3′ and R: 5′ GGCAATATGATCAAGATAC3′) genes responsible for biofilm production of isolates, was determined by PCR [30]. After PCR, Amplicons were separated by molecular size by electrophoresis using 1% Agarose (biomax). After electrophoresis, it was visualized with a UV transilluminator.

2.4.Disc diffusion testing

The antibiotic resistance profile of isolates was performed by disc diffusion test according to CLSI criteria [31]. Overnight cultures of *S. aureus* isolates were set to 0.5 McFarland in sterile 0.9% NaCl and cultured on a Mueller-Hinton agar (MHA, Biolife, Milano, Italy-4017402500) surface. Commercially purchased antibiotic discs [levofloxacin (LEV; 5 µg, ASD04800), tobramycin (TOB; 10 µg, ASD09201), gentamicin (CN; 10 µg, ASD04405), imipenem (IPM; 10 µg, ASD04500), piperacillin-tazobactam (PTZ; 100/10) µg, ASD07620), penicillin (P; 10 µg, ASD07400), methicillin (ME, 5 µg, ASD05430), erythromycin (E; 15 µg, ASD03700) Bioanalyse Ltd., Turkey] were placed on MHA medium with a sterile forceps at appropriate intervals and incubated at 35 °C for 18 hours. The results were evaluated as susceptible, intermediate, and resistant. *S. aureus* ATCC 25923 was used as a reference.

2.5.Quantification of the biofilm production test

Biofilm production of isolates was determined quantitatively by the flat bottomed 96-well microtiter plate method. For this purpose, overnight *S. aureus* cultures were brought to 0.5 McFarland in sterile 0.9% NaCl solution and diluted 1/50 rate. 150 µl of the prepared bacterial suspensions were inoculated into the wells of 96-well microtiter plates and incubated at 37°C for 24 hours. At the end of the incubation time, the broth was drained from the wells and washed three times with phosphate buffer. For fixation purposes, 99% methanol was added to the wells and kept at 25 °C for 15 minutes.

After this time, methanol was drained from the wells and dried. To stain the bacterial growth in the biofilm, 150 µL of 0.1% crystal violet was added to the wells and kept at 25 °C for 15 minutes. After incubation, the dye was drained from the wells and washed three times with distilled water so that the biofilm layer was not damaged. The wells were dried by inverting the plate. To dissolve the formed biofilm layer, 150 µL of ethanol-acetone (80:20) was added to the wells and allowed to stand for 15 minutes. Measurement was made in the microplate reader by adjusting the wavelength to 570 nm [22, 32].

3. Result

A total of 60 meat samples (30 meatballs, 30 ground beef) were analyzed in the study. Approximately 4 or 5 colonies with typical *Staphylococcus* sp. morphology (2-3 mm in diameter, black or gray in color, mostly opaque in appearance, convex, lecithinase positive) were selected on the agar.

From the isolates examined, 297 *Staphylococcus* spp. were isolated and 58 (19.52%, 32 meatball, 26 ground beef) of these isolates were determined to be *S. aureus* phenotypically (Gram staining, catalase, growth on mannitol salt agar, and coagulase test) and genotypically (16S rRNA, *nuc* genes). Photographs of the data from the phenotypic tests are shown in Figure 1. Lecithinase positive, black and shiny colonies were selected on Baird Parker agar, and as a result of phenotypic tests, Gram positive, coagulase and catalase positive, yellow colonies on mannitol salt agar were identified as *S. aureus*.

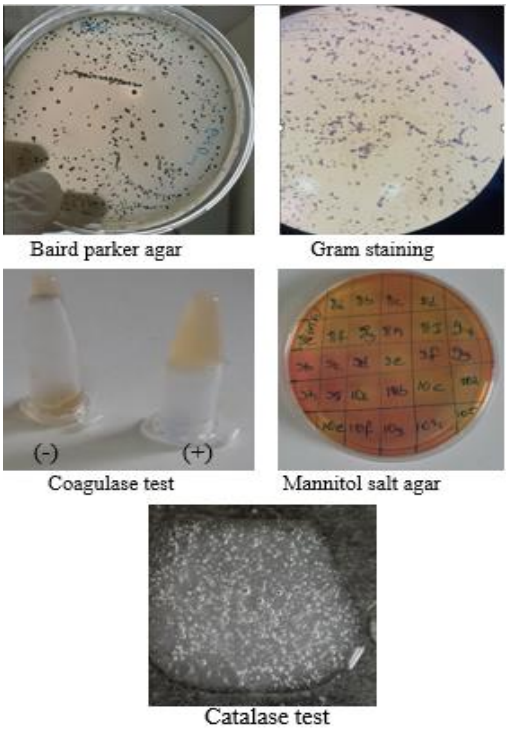

Figure 1. Phentotypic test results of *S. aureus*

From genotypic tests, the presence of the genus-specific 16S rRNA gene region and the species-specific *nuc* gene encoding the thermonuclease enzyme specific to these isolates was determined in 58 isolates. *S. aureus* strain was detected in all 58 isolates examined (Figure 2).

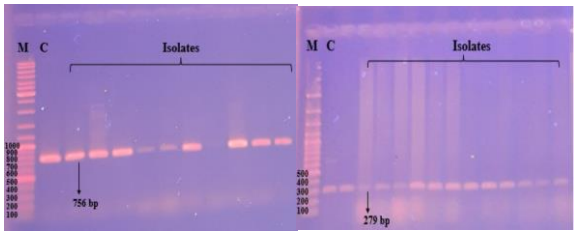

Figure 2. PCR results to confirm genotypic identification of *S. aureus* isolates. 16S rRNA (756 bp), *nuc* gene (279 bp), M: Marker (100 bp) C: Control, *S. aureus* ATCC 25923

As a result of the disc diffusion test, it was determined that at least one of the 58 isolates was resistant to the others except imipenem and piperacillin tazobactam antibiotics. Additionally, of the isolates, 34 (58.62%), 25 (43.10%), 24 (41.37%), 10 (17.24%), 6 (10.34%), 4 (6.89%), and 1 (1.72%) showed resistance (intermediate resistance or resistance) to penicillin, methicillin, erythromycin, chloramphenicol, gentamicin, tobramycin, and levofloxacin, respectively (Table 1).

Biofilm production of a total of 58 isolates was determined by 2 different methods. It was determined that 34 (58.62%) and 4 (6.89%) isolates formed biofilm using microtiter plate and genotypic (determination of *icaA* and *icaD* genes) methods, respectively. The biofilm production value of the isolate used as a reference in the microtiter plate test was read with a multiplate reader and the OD 0.7593 value was obtained. Afterwards, the OD values of the isolates were analyzed and the biofilm production of 34 (58.62%) isolates with a value higher than the positive control (>0.7593) was accepted as positive (Figure 3).

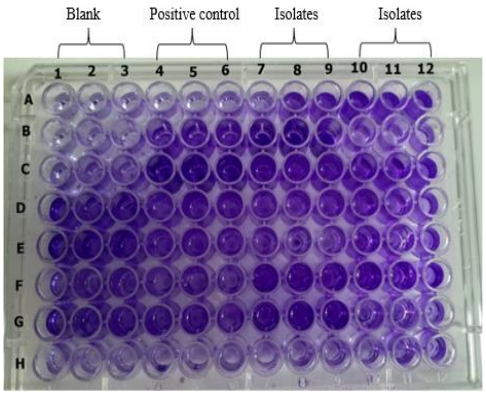

Figure 3. Biofilm formation of *S. aureus* in microtitration plate. It was repeated at least three times for each isolate.

Genotypically, it was determined that 2 isolates had the *icaA* gene and 3 isolates had the *icaD* gene. It was determined that there are 4 (6.89%) different isolates with at least one gene (*icaA* or *icaD*) (Figure 4).

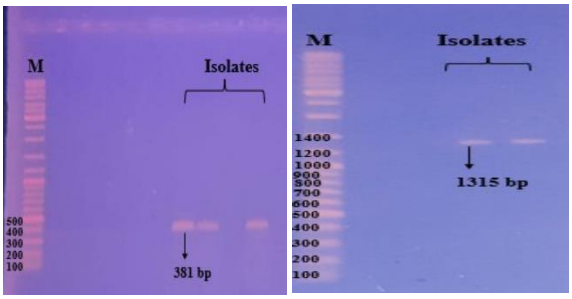

Figure 4. Biofilm production tests of *S. aureus* isolates. *icaD* gene (381 bp), *icaA* gene (1315 bp), M: Marker (100 bp)

Table 1. Antibiotic resistance profiles of *S. aureus* isolates

	S. aureus isolates (N=58)					
	R		**I**		**S**	
	n	**%**	**n**	**%**	**n**	**%**
C	10	17.24	-		48	82.75
CN	5	8.62	1	1.72	52	89.65
E	21	36.20	3	5.17	34	58.62
IPM	-	-	-	-	100	100
LEV	1	1.72	-	-	57	98.27
ME	3	5.17	22	37.93	33	56.89
P	34	58.62	-	-	24	41.37
TPZ	-	-	-	-	100	100
TOB	3	5.17	1	1.72	54	93.10

C: Chloramphenicol, CN: Gentamicin, E: Erythromycin, IPM: Imipenem, LEV: Levofloxacin, ME: Methicillin, P: Penicillin, TOB: Tobramycin, TPZ: Piperacillin-Tazobactam R: Resistant, I: Intermediate resistant, S: Sensitive.

4. Discussion

Meat, consumed throughout the world, is a rich source of many nutrients, especially protein and fat, which are essential for the human body [33]. However, its rich protein content provides a culture medium for the growth of microorganisms. Microbial contamination in meat can occur not only due to poor production practices, but also from food-producing animals, as well as food handlers who are carriers [34, 35]. Pathogens such as *Bacillus* spp., *Escherichia coli*, and *S. aureus* can cause contamination of meat. Among these pathogens, *S. aureus* is one of the pathogens that play a particularly important role in food-borne poisoning. However, its prevalence varies among different foods, animals, and countries [26, 35, 36].

In this study, 58/297 (19.52%) of the bacteria isolated from the ground beef and meatball samples examined bacteriologically were determined to be phenotypically and genotypically *S. aureus*. Previous studies conducted in different countries reported the percentage of positive *S. aureus* isolates in ground beef in Greece to be 26.31% [37], 36% in Iran [38], 26.22% in Iran [26], 16% in Egypt [7], 33.08% in Africa [35] 12.5% in Turkey [39], 21.23% in the Turkey [15]. Interestingly, in another study conducted in Turkey, they determined the prevalence of *S. aureus* obtained from meat and meatball to be as high as 96.2% and 89.1%, respectively [40]. When the study results obtained from different countries or the same countries are examined, differences in the prevalence of *S. aureus* may be caused by people working in the food industry being carriers, not paying attention to hygiene rules during meat slaughter, and contamination during the transportation and storage of meat [35].

Today, the intensive and unconscious use of antibiotics has become a worldwide problem. Intensive use of antibiotics, especially on animals, causes antibiotic residues to form in animal foods and the proliferation of resistant bacteria. Antibiotic resistance genes found in antibiotic-resistant bacteria in foods can be transferred through the food chain. The spread of antibiotic-resistant bacteria causes foodborne infections that are difficult to treat [38, 41].

Intensive use of the antibiotic methicillin, especially in infections caused by *S. aureus*, has led to an increase in the number of methicillin-resistant *S. aureus*. This could lead to an antibiotic resistance crisis that could cause fatal infections worldwide. The presence of livestock-associated MRSA is a concern in cases of staphylococcal food poisoning [36]. Therefore, it is important to investigate the prevalence of *S. aureus* in foods and determine antibiotic resistance profiles to prevent foodborne infections. Studies in the literature have focused on certain groups of antibiotics to determine the antibiotic resistance of *S. aureus* isolates [36, 42, 43].

Unlike studies in the literature, in this study, the resistance profile of *S. aureus* against 9 different antibiotics (chloramphenicol, gentamicin, erythromycin, imipenem, levofloxacin, methicillin, penicillin, tobramycin, piperacillin-tazobactam) was determined. In this study, all *S. aureus* were sensitive to imipenem, a carbapenem antibiotic, and piperacillin tazobactam, a combination beta-lactamase inhibitor. In contrast, it was determined that the isolates showed the highest resistance (resistant or moderately resistant) to erythromycin, methicillin, and penicillin among the antibiotics used. High penicillin G resistance in *S. aureus* isolates isolated from meat samples has been reported in other studies conducted in Turkey as well as in studies conducted in countries around

the world such as Greece, Nigeria, Iran, China, and Italy [15, 25, 36-38, 44, 45]. A high rate of resistance has recently been detected in *S. aureus* isolates against macrolide group antibiotics [46]. In this study, erythromycin, a macrolide group antibiotic, was used and 24 of the isolates (41.37%) were found to be resistant. Hasanpour et al. determined that *S. aureus* isolates obtained from beef in Iran were 37.50% resistant to erythromycin.

Additionally, in the same study, it was reported that the isolates were resistant to 12 different antibiotics (ampicillin, ceftriaxone, amoxicillin–clavulanic acid, tetracycline, azithromycin, oxacillin, and penicillin) [47]. Another study conducted in Nigeria reported that *S. aureus* isolates had a 15.70% resistance rate to erythromycin [48]. According to CLSI criteria, cefoxitin antibiotic is preferred over oxacillin antibiotic in determining methicillin resistance due to more reproducible results. In our study, MRSA resistance of *S. aureus* isolates was not detected. However, the resistance profile of *S. aureus* isolates against the antibiotic methicillin was determined [49]. In this study, *S. aureus* isolates were found to be highly resistant to 25 (43.10% resistant or moderately resistant) methicillin antibiotics. The main reason for the difference observed between studies in the literature may be the difference in antibiotic groups used in the treatment of infections caused by *S. aureus*, especially in food-producing animals, in different geographical regions.

The ability of *S. aureus* to cause infection is increased by the ability of the bacterium to form biofilm, which is the extracellular matrix layer, an important virulence factor [50]. In the food industry, data on biofilm production of *S. aureus* isolates are limited. It is known that *S. aureus* isolates can form biofilms by spreading on foods of animal origin and equipment in the food slaughtering environment, such as slaughterhouses. It is known that after biofilm production, its resistance to various disinfectants and antibiotics increases and creates a basis for cross contamination [51].

Various phenotypic and genotypic methods are used to detect biofilm production, such as standard tube, Congo Red Agar, microtiter plate methods and molecular validations [52, 53]. In this study, biofilm production was detected by the microtiter plate method, which is a phenotypic test, and the genotypic method. According to the OD values obtained according to the microtiter method, we found that 34 of 58 *S. aureus* isolates (58.62%) formed biofilms. In studies conducted in the literature, it was determined that biofilm production according to the microtiter method was 50%, 60% and 95.5% in *S. aureus* isolates originating from milk [54], chicken meat [55] and ground beef [37], respectively. Poly-N acetyl β16 glucosamine (PNAG) surface polysaccharide, which is involved in intercellular adhesion of the biofilm layer and synthesized by proteins encoded by the intercellular adhesion operon (*icaADBC* genes), is an important structure. In this study, biofilm production of *S. aureus* isolates was analyzed by PCR targeting *icaA* and *icaD*. In PCR analysis, the presence of the *icaA* gene was detected in only 2 isolates and the *icaD* gene was detected in 3 isolates.

Various studies have stated that biofilm production is associated with the presence of *icaA* and *icaD* genes, especially for *S. aureus* isolates responsible for hospital-acquired infections or for *S. aureus* isolates isolated from daily milk and dairy products. There are very few studies on whether *S. aureus* isolates of meat origin genotypically produce biofilm. Therefore, the relationship between biofilm production and *ica* genes examined in the study can be evaluated in the light of data obtained from isolates other than ground beef and meatballs [30, 53, 56]. This is necessary because there is little data on these parameters for isolates originating from ground meat and meatballs. The results in this study were in contrast to Prenafeta et al. who reported that the *icaA* and *icaD* genes were positive in all isolates [54].

Vasudevan et al isolated 35 *S. aureus* from bovine mastitis. They reported that although a total of 24 of these isolates produced slime phenotypically, all of them had the ica locus [30]. Abbasi et al. determined that *S. aureus* isolates contained 65.38%, 57.69%, 50% and 42.30% of *icaD, icaA, icaB* and *icaC* genes, respectively [26]. Similar to our study, Avila-Novoa et al. reported that while biofilm production was

detected at a rate of 90.4% according to the microtiter plate method, the rate of isolates with *icaA* and *icaD* genes was much lower (4, 5.1%) [57]. These results show that *S. aureus* strains may have biofilm-forming capacities at different rates depending on the source from which they are isolated (human or food). In addition, other gene regions (*icaB* and *icaC*) need to be analyzed in order to obtain a clear result regarding genotypic biofilm production.

5. Conclusion

According to the findings of the current study, ground beef and meatball samples consumed in Amasya province of Turkey were found to be highly contaminated with *S. aureus*. As a result of the disc diffusion test, it was seen that some isolates were resistant to antibiotics. The possibility of other bacteria becoming resistant through mobile genetic elements as a result of widespread horizontal gene transfers between bacteria may be a concern for health and food safety.

The fact that more *S. aureus* isolates were obtained in meatball samples than in ground meat samples in the study reveals that hygiene rules are not taken into consideration, especially during the processing of food, and the possibility of contamination by carrier food handlers. Contamination caused by *S. aureus* can be reduced, especially by improving hygiene conditions. In addition, biofilm production of *S. aureus* isolates is a source of risk for the food industry. Therefore, in terms of public health, animal food samples should be examined at regular intervals for antibiotic resistance, and it is important to take the necessary sanitation and hygienic measures.

Article Information Form

Funding
The author (s) has no received any financial support for the research, authorship or publication of this study.

Authors' Contribution
The authors contributed equally to the study.

The Declaration of Conflict of Interest/ Common Interest
No conflict of interest or common interest has been declared by the authors.

The Declaration of Ethics Committee Approval
This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics
The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

References

[1] S. Parvin, Y. Ali, S. Talukder, A. Nahar, E. H. Chowdhury, T. Rahman, T. Islam, "Prevalence and multidrug resistance pattern of methicillin resistant *S. aureus* isolated from frozen chicken meat in Bangladesh," Microorganisms, vol. 9, no. 3, pp. 636, 2021.

[2] S. Karimi, H. Momtaz, "Molecular typing, phenotypic and genotypic assessment of antibiotic resistance and virulence factors amongst the *Staphylococcus aureus* bacteria isolated from raw chicken meat," Molecular Genetics, Microbiology and Virology, vol. 37, no. 4, pp. 226-241, 2022.

[3] D. Bae, D. M. Macoy, W. Ahmad, S. Peseth, B. Kim, J. W. Chon, G. R. Ryu, G. H. Ban, S. A. Kim, H. J. Kang, J. S. Moon, M. G. Kim, "Distribution and characterization of antimicrobial resistant pathogens in a pig farm, slaughterhouse,

meat processing plant, and in retail stores," Microorganisms, vol. 10, no. 11, pp. 2252, 2022.

[4] World Health Organization (2021), "Global Antimicrobial Resistance and Use Surveillance System (GLASS)", Available at: https://www.who.int/publications/i/item/9789240027336.

[5] Q. Tao, Q. Wu, Z. Zhang, J. Liu, C. Tian, Z. Huang, P. K. Malakar, Y. Pan, Y. Zhao, "Meta-analysis for the global prevalence of foodborne pathogens exhibiting antibiotic resistance and biofilm formation," Frontiers in Microbiology, vol. 13, pp. 906490, 2022.

[6] J. Kim, J. Ahn, "Emergence and spread of antibiotic-resistant foodborne pathogens from farm to table," Food Science and Biotechnology, vol. 31, no. 12, pp.1481-1499, 2022.

[7] V. K. Ahmed, I. M. Helal, M. M. Zaky, M. Abdulla, "Antimicrobial activity of *Actinomycetes* extracts against multidrug-resistant *Staphylococcus aureus* and *Salmonella* spp. isolated from meat," Alfarama Journal of Basic & Applied Sciences, vol. 3, no. 2, pp. 283-299, 2022.

[8] T. Asfaw, D. Genetu, D. Shenkute, T. Shenkutie, Y. E. Amare, B. Yitayew, "High levels of multidrug-resistant and beta-lactamase-producing bacteria in meat and meat contact surfaces, Debre Berhan Town, Ethiopia," Infection and Drug Resistance, vol. 16, no. 1, pp. 1965-1977, 2023.

[9] M. G. Basanisi, G. Nobili, G. La Bellaa, R. Russo, G, Spano, G. Normanno, G. La Salandraa, "Molecular characterization of *Staphylococcus aureus* isolated from sheep and goat cheeses in southern Italy," Small Ruminant Research, vol. 135, pp. 17-19, 2016.

[10] A. Fetsch, S. Joler, "*Staphylococcus aureus* as a foodborne pathogen," Current Clinical Microbiology Reports, vol. 5, pp. 88–96, 2018.

[11] M. Yousefi, M. Farshidi, M. A. Sani, L. Payahoo, "Investigation of the microbial quality and the occurrence of Shiga-like-toxin-producing *Escherichia coli* and methicillin resistant *Staphylococcus aureus* in traditional cheeses produced in northwest of Iran," Nutrition & Food Science, vol. 3, pp. 407-417, 2018.

[12] S. Gürbüz, Ç. Güngör, "Determination of some foodborne pathogens and residual nitrate and nitrite in traditional fermented sausages in Turkey," International Food Research Journal, vol. 27, no. 1, pp. 39-46. 2020.

[13] P. Visciano, F. Pomilio, R. Tofalo, L. Sacchini M. A Saletti, E. Tieri, M. Schirone, G. Suzzi, "Detection of methicillin resistant *Staphylococcus aureus* in dairy cow farms," Food Control, vol. 46, pp. 532-538, 2014.

[14] J. M. Kwiecinski, A. R. Horswill, "*Staphylococcus aureus* bloodstream infections: pathogenesis and regulatory mechanisms," Current Opinion in Microbiology, vol. 53, pp. 51-60, 2020.

[15] P. Şanlıbaba, "Prevalence, antibiotic resistance, and enterotoxin production of *Staphylococcus aureus* isolated from retail raw beef, sheep, and lamb meat in Turkey," Internatol Journal Food Microbiology, vol. 361, pp. 109461, 2022.

[16] W. Sankomkai, W. Boonyanugomol, K. Kraisriwattana, J. Nutchanon, K. Boonsam, S. Kaewbutra, W. Wongboot, "Characterisation of classical enterotoxins, virulence activity, and antibiotic susceptibility of isolated from Thai fermented pork sausages, clinical samples, and healthy carriers in northeastern Thailand," Journal of Veterinary Research, vol. 64, no. 2, pp. 289-297, 2020.

[17] G. K. Sivaraman, S. S. Grupta, S. Visnuvinayagam, T. Muthulakshmi, R.

Elangovan, V. Perumal, G. Balasubramanium, T. Lodha, A. Yadav, "Prevalence of *S. aureus* and/or MRSA from seafood products from Indian seafood products," BMC Microbiology, vol. 22, no. 1, pp. 233, 2022.

[18] N. Guo, B. Xue, Y. Shen, T. Zhang, "Target-based screening for natural products against *Staphylococcus aureus* biofilms," Critical reviews in Food Science and Nutrition, vol. 63, no. 14, pp. 2216-2230,2023.

[19] M. T. Kashef, N. M. Saleh, N. H. Assar, M. A. Ramadan, "The antimicrobial activity of ciprofloxacin-loaded niosomes against ciprofloxacin-resistant and biofilm-forming *Staphylococcus aureus*," Infection and Drug Resistance, vol. 13, pp. 1619-1629, 2020.

[20] S. Derakhshan, M. Navidinia, F. Haghi, "Antibiotic susceptibility of human associated *Staphylococcus aureus* and its relation to agr typing, virulence genes, and biofilm formation," BMC Infectious Diseases, vol. 21, pp. 627, 2021.

[21] C. Wang, Y. Xiong, C. Bao, Y. Wei, Z. Wen, X. Cao, Z. Yu, X. Deng, G. Li, Q. Deng, "Antibacterial and anti-biofilm activity of radezolid against *Staphylococcus aureus* clinical isolates from China," Frontiers in Microbiology, vol. 14, pp. 1131178, 2023.

[22] J. Gajewska, W. Chajecka-Wierzchowska, "Biofilm formation ability and presence of adhesion genes among coagulase-negative and coagulase-positive *Staphylococci* isolates from raw cow's milk," Pathogens, vol. 9, no. 8, pp. 654, 2020.

[23] J. Gajewska, W. C. Wierzchowska, A. Zadernowska, "Occurrence and characteristics of *Staphylococcus aureus* strains along the production chain of raw milk cheeses in Poland," Molecules, vol. 27, no. 19, pp. 6569, 2022.

[24] D. Ashraf, R. A. Ombarak, A. Samir, A. B. Abdel-Salam, "Characterization of multidrug-resistant potential pathogens isolated from milk and some dairy products in Egypt," Journal of Advanced Veterınary and Animal Research, vol. 10, no. 2, pp. 275-283, 2023.

[25] T. Cebeci, N. Gündoğan, "Enterotoxin production and antibiotic resistance profile of *Staphylococcus aureus* isolated from meat samples," Hitit Medical Journal, vol. 3, no. 2, 13-19, 2021.

[26] K. Abbasi, E. Tajbakhsh, H. Momtaz, "Antimicrobial resistance, virulence genes, and biofilm formation in *Staphylococcus aureus* strains isolated from meat and meat products," Journal of food safety, vol. 41, no. 2, pp. 1-10, 2021.

[27] S. Yoon, Y. K. Park, T. S. Jung, S. B. Park, "Molecular typing, antibiotic resistance and enterotoxin gene profiles of *Staphylococcus aureus* isolated from humans in South Korea," Microorganisms, vol. 10, no. 3, pp. 642, 2022.

[28] K. Zhang, J. Sarling, B. L. Chow, S. Elsayed, Z. Hussain, D. L. Church, D. B. Gregson, T. Louie, J. M. Conly, "New quadriplex PCR assay for detection of methicillin resistance and simultaneous discrimination of *S. aureus* from coagulase-negative *Staphylococci*," Journal Clinical Microbiology, vol. 42, no. 11, pp. 4947-4955, 2004.

[29] J. A. McClure, J. M. Conly, V. Lau, S. Elsayed, T. Louie, W. Hutchins, K. Zhang, "Novel multiplex PCR assay for detection of the *Staphylococcal* virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from resistant *Staphylococci*," Journal Clinical Microbiology, vol. 44, no. 3, pp. 1141-1144, 2006.

[30] P. Vasudevan, M. K. M. Nair, T. Annamalai, K. S. Venkitanarayanan, "Phenotypic and genotypic characterization of bovine mastitis isolates

of *Staphylococcus aureus* for biofilm formation," Veterinary Microbiology, vol. 92, no. 1-2, pp.179-185, 2003.

[31] Clinical laboratory standart institute, (CLSI), "Performance Standards for antimicrobial susceptibility testing. Twentieth edition," Informational Supplement M100-S22, Wayne, PA, 2012.

[32] T. Saba, M. Sajid, A. A. Khan, R. Zahra, "Role of intracellular adhesion *icaAD* and *agr* genes in biofilm formation in clinical *S. aureus* isolates and assessment of two phenotypic methods," Pakistan Journal of Medical Science, vol. 34, no. 3, pp. 633-637, 2018.

[33] K. Kolodziejczak, A. Onopiuk, A. Szpicer, A. Poltorak, "Meat analogues in the perspective of recent scientific research: a review," Foods, vol. 11, no. 1, pp. 105, 2022.

[34] L. Grispoldi, P. A. Popescu, M. Karama, V. Gullo, G. Poerio, E. Borgogni, P. Torlai, G. Chianese, A. G. Fermani, P. Sechi, B. Cenci-Goga, "Study on the growth and enterotoxin production by *Staphylococcus aureus* in canned meat before retorting," Toxins, vol. 11, no. 5, pp. 291, 2019.

[35] T. Thwala, E. Madoroba, A. Basson, P. Butaye, "Prevalence and characteristics of *Staphylococcus aureus* associated with meat and meat products in African countries: a review," Antibiotics, vol. 10, no. 9, pp. 1108, 2021.

[36] E. O. Igbinosa, A. Beshiru, I. H. Igbinosa, A. G. Ogofure, T. C. Ekundayo, A. I. Okoh, "Prevalence, multiple antibiotic resistance and virulence profile of methicillin resistant *Staphylococcus aureus* (MRSA) in retail poultry meat from Edo, Nigeria," Frontiers in Cellular and Infection Microbiology, vol. 13, pp. 1122059, 2023.

[37] D. Komodromos, C. Kotzamanidis, V. Giantzi, S. Pappa, A. Papa, A. Zdragas, A. Angelidis, D. Sergelidis, "Prevalence, infectious characteristics and genetic diversity of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in two raw-meat processing establishments in Northern Greece," Pathogens, vol. 11, no. 11, pp. 1370, 2022.

[38] M. Rajaei, M. H. Moosavy, S. N. Gharajalar, S. A. Khatibi, "Antibiotic resistance in the pathogenic foodborne bacteria isolated from raw kebab and hamburger: phenotypic and genotypic study," BMC Microbiology, vol. 21, pp. 272, 2021.

[39] E. Keyvan, H. Özdemir, "Sığır karkaslarında *Staphylococcus aureus*'un varlığı, enterotoksijenik özellikleri ve antimikrobiyal dirençliliği," Ankara Üniversitesi Veteriner Fakültesi Dergisi, vol. 63, pp. 17-23, 2016.

[40] N. Gündoğdu, A. Devren, "Protease and lipase activity of *Staphylococcus aureus* obtained from meat, chicken and meatball samples," Gazi University Journal of Science, vol. 23, no. 4, pp. 381-384, 2010.

[41] M. H. Chaia, T. A. M. Faiqa, S. M. Z. Ariffinb, Z. Suhailia, M. Z. Sukimana, M. F. Ghazali, "Prevalence of methicillin resistant *Staphylococcus aureus* in raw goat milks from selected farms in Terengganu, Malaysia," Tropical Animal Science Journal, vol. 43, no. 1, pp. 64-69, 2020.

[42] A. M. Edris, A. A. A. Maarouf, R. A. E. Amin, E. A. I. Bahbah, "Prevalence of *Staphylococci* in meat products with special reference to methicillin-resistant *Staphylococcus aureus* (MRSA) at Kaliobia Governorate," Benha Veterinary Medical Journal, vol. 35, no. 2, pp. 364-374, 2018.

[43] G. A. Ballash, A. L. Albers, D. F. Mollenkopf, E. Sechrist, R. J. Adams, T. E. Wittum, "Antimicrobial resistant bacteria recovered from retail ground meat products in the US include a *Raoultella*

ornithinolytica co-harboring *bla*$_{KPC-2}$ and *bla*$_{NDM-5}$," Scientific Reports, vol. 11, pp. 14041, 2021.

[44] S. Wu, J. Huang, Q. Wu, J. Zhang, F. Zhang, X. Yang, H. Wu, H. Zeng, M. Chen, Y. Ding, J. Wang T. Lei, S. Zhang, L. Xue, "*Staphylococcus aureus* isolated from retail meat and meat products in China: incidence, antibiotic resistance and genetic diversity," Frontiers in Microbiology, vol. 9, pp. 2767, 2018.

[45] M. G. Basanisi, G. La Bella, G. Nobili, S. Tola, M. A. Cafiero, G. La Salandra, "Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from retail meat in south Italy," Italian Journal of Food Science, vol. 32, no. 2, pp. 410-419, 2020.

[46] Z. T. Baghbaderani, A. Shakerian, E. Rahimi, "Phenotypic and genotypic assessment of antibiotic resistance of *Staphylococcus aureus* bacteria isolated from retail meat," Infection and Drug Resistance, vol. 13, pp. 1339-1349, 2020.

[47] D. A. Hasanpour, L. Khaji, S. M. Sakhaei, Z. Mashak, D. F. Safarpoor, Y. Safaee, A. Hosseinzadeh, I. Alavi, E. Ghasem, F. M. Rabiei, "One-year prevalence of antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* recovered from raw meat," Tropical Biomedicine, vol. 34, no. 2, pp. 396-404, 2017.

[48] P. Fowoyo, S. Ogunbanwo, "Antimicrobial resistance in coagulase-negative *Staphylococci* from Nigerian traditional fermented foods," Annals of Clinical Microbiology and Antimicrobial, vol. 16, no. 4, pp. 1-7, 2017.

[49] A. Kılıç, E. Doğan, S. Kaya, M. Baysallar, "Yedi yıllık sürede klinik örneklerden izole edilen *Staphylococcus aureus* suşlarında *mecC* ve Panton-Valantine lökosidin gen varlığının araştırılması," Mikrobiyoloji Bülteni, vol. 49, no. 4, pp. 594-599, 2015.

[50] R. Parastan, M. Kargara, K. Solhjooc, F. Kafilzadeh, "*Staphylococcus aureus* biofilms: structures, antibiotic resistance, inhibition, and vaccines," Gene Reports, vol. 20, pp. 100739, 2020.

[51] A. C. Silva-de-Jesus, R. G. Ferrari, P. Panzenhagen, C. A. ConteJunior, "*Staphylococcus aureus* biofilm: the role in disseminating antimicrobial resistance over the meat chain," Microbiology, vol. 168, pp. 001245, 2022.

[52] L. E. Alcaraz, S. E. Satorres, R. M. Lucero, O. N. P. Centorbi, "Species identification, slime production and oxacillin susceptibility in coagulase-negative staphylococci isolated from nosocomial specimens," Brazilian Journal of Microbiology, vol. 34, no. 1, pp. 45-51, 2003.

[53] A. Ciftci, A. Findik, E. E. Onuk, S. Savasan, "Detection of methicillin resistance and slime factor production of *Staphylococcus* aureus in bovine mastitis," Brazilian Journal of Microbiology, vol. 40, no. 2, pp. 254-261, 2009.

[54] A. Prenafeta, M. Sitjà, M. A. Holmes, G. K. Paterson, "Communication: Biofilm production characterization of *mecA* and *mecC* methicillin-resistant *Staphylococcus aureus* isolated from bovine milk in Great Britain," Journal of Dairy Science, vol. 97, pp. 4838–4841, 2014.

[55] A. E. M. A. Morshdy, A. E. Tharwat, A. M. A. Merwad, N. A. M. Abdallah, T. Saber, "Prevalence, phenotypic-genotypic resistance and biofilm formation of *Staphylococcus aureus* in chicken meat with reference to its public health hazard," Veterinary Medicine and The One Health Concept, vol. 60, pp. 413–424, 2023.

[56] M. E. A. Bissong, C. N. Ateba, "Genotypic and phenotypic evaluation of biofilm production and antimicrobial resistance in *Staphylococcus aureus* isolated from milk, NorthWest province, South Africa," Antibiotics, vol. 9, pp. 156, 2020.

[57] M. G. Avila-Novoa, M. Iñıguez-Moreno, O. A. Solıs-Velazquez, J. P. Gonzalez-Gomez, P. J. Guerrero-Medina, M. Gutierrez-Lomel, “Biofilm Formation by *Staphylococcus aureus* isolated from Food Contact Surfaces in the Dairy Industry of Jalisco, Mexico,” Journal of Food Quality, vol. 2018, pp. 1746139, 2018.