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RESEARCH ARTICLE

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Antimicrobial resistance, biofilm formation, and virulence gene profiles of foodborne bacterial isolates from animal-based foods

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ABSTRACT. This study aimed to investigate the antibiotic resistance profiles, biofilm-forming capacities, and virulence characteristics of bacteria isolated from 86 animal-based food products, including 25 raw milk, 25 cheese, and 36 raw chicken samples. A total of 40 isolates were identified, comprising 14 *Macrococcus caseolyticus*, 10 *Staphylococcus aureus*, 9 *Hafnia alvei*, and 7 *Salmonella* spp. isolates. The results demonstrated that slime production was detected in all isolates, while moderate to strong biofilm formation was observed in 90% of the isolates. Among Gram-negative bacteria, resistance to ampicillin was detected in 85.7% of *Salmonella* spp. and 45.5% of *H. alvei* isolates. However, all Gram-negative isolates were susceptible to chloramphenicol, ciprofloxacin, and streptomycin (100%). Regarding Gram-positive bacteria, *S. aureus* isolates exhibited 20% resistance to tetracycline and erythromycin, whereas *M. caseolyticus* isolates showed notably higher erythromycin resistance (57.1%). In contrast, all Gram-positive isolates were susceptible to gentamicin and chloramphenicol (100%). Virulence gene analysis revealed that among the *S. aureus* isolates tested, 30% were positive for *icaA* and 70% carried *These posts must be removed.*. Furthermore, among the seven *Salmonella* spp. isolates analyzed, 85.7% were positive for the *mgtC* gene, while all isolates (100%) harbored the *sopB* gene. In conclusion, the detection of *S. aureus* and *Salmonella* spp. in animal-based food products, particularly isolates exhibiting strong slime production, biofilm-forming ability, and antibiotic resistance, represents a significant public health concern. These findings emphasize the critical importance of maintaining strict hygiene and sanitation practices throughout the production, processing, storage, and distribution stages under appropriate cold chain conditions.

Keywords: *S. aureus, Salmonella spp., antibiotic resistance, virulence, biofilm*

INTRODUCTION

Foodborne diseases constitute a major global public health concern, causing significant morbidity and mortality across all age groups and posing substantial challenges to socioeconomic development. According to the World Health Organization, approximately 600 million cases of foodborne illness occur annually due to contaminated food [1, 2]. These diseases generally arise from contamination during food production, processing, transportation, and storage by various pathogenic microorganisms, including bacteria, viruses, parasites, and fungi, as well as their associated mycotoxins, in addition to host-related factors. Among these agents, bacterial pathogens are recognized as the leading causes of foodborne illnesses worldwide. The most significant bacterial pathogens associated with foodborne outbreaks include *Salmonella* spp., *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Staphylococcus aureus*, and several other clinically important microorganisms [3, 4].

Salmonella is recognized as one of the most significant foodborne pathogens worldwide, causing an estimated 200 million to 1 billion infections annually, including approximately 93 million cases of gastroenteritis and 155,000 deaths, with nearly 85% of infections associated with foodborne transmission [5,6]. As one of the leading etiological agents of foodborne diseases, *Salmonella* is predominantly associated with foods of animal origin, particularly raw poultry, raw meat, eggs, and related products [7,8,9]. Furthermore, cross-contamination occurring during food production and processing facilitates the transfer of bacterial pathogens to these products [10]. Epidemiological data from these sources are essential for developing effective global monitoring and control strategies across the food chain [11,12]. The virulence of *Salmonella* species is largely associated with surface antigens, including the Vi antigen, as well as porins and siderophores. These

virulence determinants contribute to the severity of infection symptoms, such as diarrhea, fever, and abdominal cramps, and may lead to severe complications, including septicemia and death. Moreover, contamination occurring during the production, processing, storage, and distribution stages of animal-based food products, particularly under inadequate cold chain conditions, significantly increases the risk of *Salmonella* transmission and proliferation [13,14].

S. aureus is recognized worldwide as an important cause of food poisoning due to the consumption of raw, undercooked, or improperly processed foods. In the food industry, *S. aureus* may contaminate meat and dairy products during production, processing, or storage, leading to food intoxication by producing enzymes and toxins. [15]. Staphylococcal food poisoning is associated with the consumption of foods containing sufficient amounts of one or more preformed enterotoxins [16]. The high incidence of staphylococcal food poisoning is due to inadequate pasteurization/decontamination of the originally contaminated product source or contamination during preparation and processing by persons who are carriers of the organism [17,18]. Staphylococcal poisoning usually occurs in raw retail meat and meat products, poultry and egg products, milk and milk products, salads, bakery products, especially cream-filled pastries, cakes, and sandwich fillings [19,20].

Biofilms are structured microbial communities attached to surfaces and embedded in an extracellular polymeric matrix. By secreting extracellular polymeric substances, bacteria facilitate colonization and attachment to surfaces by binding water within the biofilm. This helps to protect bacteria against toxic compounds, phage contamination, osmotic stress, disinfectants, and antibiotics [21]. Biofilm is very important both medically and industrially as it causes contamination in medical instruments and implants, industrial water pipes, food production machines and packaging surfaces, and water systems. The types of microorganisms that form biofilms in food processing environments vary according to production area, usage patterns, and the materials from which equipment is made. Biofilm formations caused by *Staphylococcus* spp., *Enterobacter* spp., *Listeria* spp., *Lactobacillus* spp., *Streptococcus* spp. in the milk and dairy products industry, *L. monocytogenes*, *Salmonella* spp., *Vibrio parahaemolyticus, Campylobacter* spp., *E. coli* O157:H7 microorganisms in the meat and poultry products industry pose a great danger to human health. Among these biofilm-forming pathogenic bacteria, *S. aureus* and *Salmonella* spp. species, which are the subject of our study, interact with food surfaces, causing food spoilage, the emergence of animal-based foodborne diseases, shortening the shelf life of foods, and, as a result, economic losses [22].

Antibiotic resistance is defined as the inability of bacteria to be affected by one or more antibiotics or the transformation of an antibiotic-sensitive bacterial strain into a resistant one through one of several resistance mechanisms. Antibiotic resistance in bacteria is gradually increasing due to factors such as the frequent use of antibiotics in society and their use in the food industry [23]. In the development of antibiotic resistance, oxygen concentration in the biofilm, pH, and the ease of transfer of antibiotic resistance genes play important roles. In studies, the lower growth rate of biofilm-forming bacteria compared to planktonic bacteria is a reason for decreased antibiotic susceptibility [24].

Although *S. aureus* has many virulence factors, biofilm formation and hemolysin enzymes are very important. Although many bacterial and external factors affect adhesion and accumulation, the production of an extracellular polysaccharide adhesin known as intercellular polysaccharide adhesin (PIA) or polymeric N-acetyl-glucosamine (PNAG), synthesized by enzymes encoded by the *icaA* operon, is the best understood biofilm mechanism of *Staphylococcus* [25]. *S. aureus* produces many exoproteins that cause disease by colonization of the host cell. The main function of these exoproteins is to prepare host tissues for microbial growth. *S. aureus* produces hemolysins, which are divided into four groups (alpha, beta, delta, and gamma) and many cytotoxic molecules such as Panton-Valentin leukocidin (PV-leukocidin). Gamma hemolysin is encoded by *hlgA, hlgB,* and *hlgC* genes in the chromosomal *hlg* locus [26].

Adhesion of *Salmonella* spp. to the epithelium of the digestive canal is formed by flagella and fimbriae on the cell surface. When it adheres to the intestinal epithelium, it forms T3SS (Type 3 secretion system), a complex multiprotein structure that increases endothelial adhesion and invasion. T3SSs are needle-like protrusions on the bacterial cell wall that inject bacterial proteins into eukaryotic cells. The T3SS mechanism encodes virulence genes that mediate attachment, invasion, and toxicity in *Salmonella*. *sopB*, one of the effector proteins located in the T3SS, has an important role in causing inflammation by activation of the secretory pathway (attack of neutrophils to the infection site) and, as a result, it causes increased fluid flow to the digestive tract by changing the ion balance in the cells, resulting in diarrhea in humans and cattle [27].

This study aimed to determine the presence of pathogenic bacteria isolated from animal-based food products and to evaluate their antibiotic resistance, biofilm formation, slime production, and virulence characteristics. Although previous studies have investigated these properties separately, comprehensive data evaluating them simultaneously in isolates recovered from different animal-based foods remain limited. In addition to major foodborne pathogens such as *S. aureus* and *Salmonella* spp., this study also included *M. caseolyticus* and *H. alvei* to provide further insight into emerging food-associated bacteria and their potential public health risks.

MATERIALS AND METHODS

Between September 2018 and May 2019, a total of 86 animal-based food samples, including 25 raw milk, 25 cheese, and 36 raw chicken samples, were collected from various markets, street vendors, and bazaars in Ankara to evaluate the presence of foodborne pathogenic bacteria and their potential public health risks. The samples were transported to the laboratory under cold chain conditions in sterile containers and analyzed on the same day.

Isolation of *S. aureus* and *Salmonella* spp.

S. aureus isolation was performed by the method specified by the Turkish Standards Institute. 0.1 mL of the dilutions prepared from food samples using Buffered Peptone Water (BPW) was added to Egg-Yolk Tellurite-Baird-Parker Agar (BPA) (Oxoid, England) and incubated at 37°C for 24-48 hours. Phenotypic identification tests (Gram staining, oxidase, catalase, hemolysis, and coagulase) were performed on the black-colored, clear, and bright zone colonies formed on BPA after incubation on blood agar (Oxoid, England) at 37°C for 24-48 hours. Isolates identified as *S. aureus* were stored in Brain Heart Infusion Broth (Oxoid, England) with 30% glycerin (Merck, Germany) at -20°C. *S. aureus* ATCC 29213 was used as a reference strain in all experiments [28].

Salmonella spp. were isolated by the method specified by the Turkish Standards Institute. Dilutions of food samples in Buffered Peptone Water (BPW) for pre-enrichment were incubated at 37°C for 24 hours. 1 mL of BPW was transferred into Rapport Vassiliadis Broth (RVS). After incubation at 41.5°C for 24 hours, 0.1 mL of RVS was taken, and *Salmonella* suspected colonies formed after incubation on XLT4 Agar at 37°C for 24-48 hours were cultured on Nutrient Agar. Lactose, glucose, sucrose, gas, H2S, urea, indole, VP, lysine decarboxylase, and β-galactosidase tests were performed to identify the culture biochemically. *Salmonella* isolates were preserved in Brain Heart Infusion (BHI) Broth (Oxoid, England) with 30% glycerin (Merck, Germany) at -20 °C. *S. Typhimurium* ATCC 14028 was used as a reference strain in all experiments [29].

Species-level identification of suspected bacterial isolates was confirmed by MALDI-TOF mass spectrometry at the National Microbiology Laboratory of the Microbiology Reference Laboratory and the Biological Products Department of the Ministry of Health. As a result of our investigations, 14 *M. caseolyticus*, 10 *S. aureus*, 9 *H. alvei,* and 7 *Salmonella* spp. isolates were detected in animal-based food samples. Subsequently, antimicrobial resistance and biofilm-forming ability were evaluated in all identified Gram-positive and Gram-negative isolates.

Antibiotic Resistance of Bacterial Isolates

Antibiotic resistance of isolates was determined using the Kirby-Bauer disc diffusion method, as reported by CLSI (The Clinical and Laboratory Standards Institute, 2018). Isolates were incubated in Nutrient Broth, adjusted to a 0.5 McFarland ($1.5x10^8$ CFU/mL) standard, and inoculated onto Mueller–Hinton agar. Antibiotic discs were selected according to the bacterial group and incubated at 37 °C for 18–24 h. *S. aureus* and *M. caseolyticus* isolates were tested with ampicillin (10 μg), erythromycin (15 μg), gentamicin (10 μg), tetracycline (30 μg), vancomycin (10 μg), chloramphenicol (10 μg), penicillin (10 μg), and clindamycin (2 μg). *Salmonella* spp. and *H. alvei* isolates were tested with ampicillin (10 μg), nalidixic acid (30 μg), nitrofurantoin (300 μg), kanamycin (30 μg), tetracycline (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), and streptomycin (10 μg). The antibiotic inhibition zone diameters were evaluated by comparing them with those reported by the CLSI, 2018 [30].

Biofilm Production of Bacterial Isolates

Biofilm production of Gram-positive and Gram-negative bacterial isolates was studied using the microtiter plate method described by Christensen [31]. The 24-hour cultures of isolates in TSB (Tryptic Soy Broth) supplemented with 0.25% glucose (GTSB) (200 μl) were incubated in 96-well microtiter plates (Nunc, Wiesbaden, Germany) at 37°C for 24 hours. The liquid medium containing planktonic cells was washed twice with phosphate-buffered saline (PBS), the air-dried wells were stained with 0.1% crystal violet (200 µl) for 30 minutes, and the plates were washed with sterile PBS. After re-drying, 200 µl ethanol acetic acid (95:5) was added to the plates, the stain was dissolved, and optical densities were measured spectrophotometrically at 570 nm (BioTek Instruments, Inc., USA). Biofilm formation was evaluated according to the absorbance scales shown in Table 1 [32].

Table 1. Evaluation scale of biofilm production

OD value	Adhesion value	Biofilm degree
OD Control > OD MB	Nonadhere	0
OD Control < OD MB < 2 OD Control	Weak Adhere	I
2 OD Control < OD MB < 4 OD Control	Moderately Adhere	II
4 OD Control < OD MB	Strong Adhere	III

OD: Optical density, OD, MB: Optical density of microbial biofilm

Slime Formation of Bacterial Isolates

Slime production of Gram-positive and negative bacteria was determined on Congo red agar (sucrose 50 g/L, BHI broth 37 g/L, Congo red 0,8 g/L, agar 10 g/L). After incubation at 37 °C for 24 hours, black-coloured colonies were considered slime-positive, and pink-red colored colonies were considered slime-negative [33].

Genomic DNA Isolation

The manufacturer's genomic DNA isolation protocol for the High Pure PCR Template Preparation Kit (Roche, Germany) was followed. Stock cultures were inoculated into a 5 mL Nutrient Broth medium and incubated at 37°C for 18 hours. At the end of incubation, 1.5 mL of bacterial suspension was taken into 2 mL disposable tubes and centrifuged at 5000 x g for 10 minutes. After centrifugation, the supernatant was removed, and lysis buffer, proteinase K (Roche, Germany), washing buffer, and elution buffer were added to the pellet in the amounts and order specified in the protocol. DNA isolation was performed by incubation and centrifugation. The purity and quantification of the resulting bacterial DNA samples were determined by absorbance measurements using a micro-volume spectrophotometer (BioTek, Epoch, USA) [34].

Detection of IcaA, hlg, *MgtC*, and *SopB* Genes by Polymerase Chain Reaction (PCR)

Conventional PCR was used to detect the presence of *icaA* and *hlg* genes in *S. aureus* isolates and *MgtC* and *SopB* genes in *Salmonella* spp. isolates. For amplification, a PCR mixture was prepared using 5XApta Taq Fast PCR Master Mix. The appropriate annealing temperature for each gene was selected based on the primers' melting temperatures, and amplification was performed on a thermal cycler (Applied Biosystems). In the cycle, 94 °C 4 min for initial denaturation, 30 cycles of 94 °C 45 s denaturation, 52 °C 30 s binding, 72 °C 1 min elongation, and 72 °C 7 min for final elongation were used. A 1% agarose gel was used for visualization; a 1 kb DNA ladder was used as a marker. 1 μL Runsafe dye (Cleaver) and 5 μL amplicon were mixed and loaded into the wells, respectively. The agarose gel was run at 80 V for 45 minutes and visualized with Epi Blue in the imaging device (Azure Biosystems C200). The primer sequences used for PCR of the *IcaA*, *hlg*, *MgtC*, and *SopB* genes are given in Table 2.

Table 2. Primer sequences

Target genes	Primer sequence	Amplicon size
icaA	F 5'-CCTAACTAACGAAAGGTAG-3', R 5'-AAGATATAGCGATAAGTGC-3'	1315 bp [35]
hlg	F 5'-GCCAATCCGTTATTAGAAAATGC-3', R 3'-CCATAGACGTAGCAACGGAT-5'	937 bp [36]
mgtC	F 5'-TGACTATCAATGCTCCAGTGAAT-3', R 5'-ATTTACTGGCCGCTATGCTGTTG-3'	677 bp [37]
sopB	F 5'-CAACCGTTCTGGGTAAACAAGAC-3', R 5'-AGGATTGAGCTCCTCTGGCGA-3'	1348 bp [38]

RESULTS

A total of 86 animal-based food samples, including 25 raw milk, 25 cheese, and 36 raw chicken samples, were collected from various retail sources. As a result of our experiments, 14 *M. caseolyticus*, 10 *S. aureus*, 9 *H. alvei,* and 7 *Salmonella* spp. isolates were obtained (Table 3).

Table 3. Number and percentages of isolates obtained from animal-based food samples.

Animal-based food	Analyzed samples (n)	*S. aureus*		*M. caseolyticus*		*Salmonella* spp.		*H. alvei*	
		Isolates (n)	%	Isolates (n)	%	Isolates (n)	%	Isolates (n)	%
Raw milk	25	10	40	-	-	-	-	-	-
Cheese	25	-	-	14	56	-	-	-	-
Raw Chicken	36	-	-	-	-	7	19.44	9	25
TOTAL	86	10	40	14	56	7	19.44	9	25

Biofilm production and slime formation were determined in 14 *M. caseolyticus*, 10 *S. aureus*, 9 *H. alvei,* and 7 *Salmonella* spp. isolates from animal-based foods.

Table 4. Biofilm production, antibiotic resistance, and virulence gene profile of Gram-positive bacteria isolated from animal-based food samples

No	Isolate no	Biofilm production	Antibiotic resistance and virulence gene profile
1	S6 *S. aureus*	III (strong)	AM, E, K, *icaA, hlg*
2	S7 *S. aureus*	III (strong)	DA, P, *hlg*
3	S9 *S. aureus*	II (moderate)	DA, E, K, *hlg*
4	S10 *S. aureus*	III (strong)	DA, P, i*caA*
5	S11 *S. aureus*	III (strong)	DA, TE, K, *hlg*
6	S13 *S. aureus*	II (moderate)	TE, K
7	S15 *S. aureus*	II (moderate)	K, *hlg*
8	S16 *S. aureus*	III (strong)	DA, P, *icaA, hlg*
9	S17 *S. aureus*	III (strong)	DA, P, K
10	S26 *S. aureus*	III (strong)	DA, K, *hlg*
11	P27 *M. caseolyticus*	III (strong)	E, P, DA
12	P76 *M. caseolyticus*	0	TE,K
13	P79 *M. caseolyticus*	0	E, DA
14	P82 *M. caseolyticus*	0	
15	P83 *M. caseolyticus*	III (strong)	AM, P, K
16	P86 *M. caseolyticus*	III (strong)	AM, TE, DA
17	P91 *M. caseolyticus*	III (strong)	E, P, K
18	P92 *M. caseolyticus*	III (strong)	AM, TE, P
19	P93 *M. caseolyticus*	II (moderate)	E, DA, K
20	P97 *M. caseolyticus*	II (moderate)	E, P, K
21	P98 *M. caseolyticus*	0	TE
22	P99 *M. caseolyticus*	II (moderate)	E, DA, K
23	P100 *M. caseolyticus*	III (strong)	AM, E, P
24	P102 *M. caseolyticus*	II (moderate)	E, DA

AM: ampicillin, E: erythromycin, TE: tetracycline, P: penicillin, K: clindamycin, NA: Nalidixic Acid, F: nitrofurantoin

Table 5. Biofilm production, antibiotic resistance, and virulence gene profile of Gram-negative bacteria isolated from animal-based food samples

No	Isolate no	Biofilm production	Antibiotic resistance and virulence gene profile
1	Y37 *Salmonella* spp.	III (strong)	AM, NA, *mgtC, sopB*
2	Y44 *Salmonella* spp.	II (moderate)	AM, F, TE, *mgtC*
3	Y45 *Salmonella* spp.	II (moderate)	AM, NA, F, *mgtC*
4	Y47 *Salmonella* spp.	II (moderate)	AM, NA, F
5	Y48 *Salmonella* spp.	III (strong)	AM, NA, F, *mgtC*
6	T50 *Salmonella* spp.	III (strong)	NA, F, *mgtC*
7	T51 *Salmonella* spp.	II (moderate)	AM, F, *mgtC*
8	T61 *H. alvei*	III (strong)	AM
9	T65 *H. alvei*	II (moderate)	
10	T70 *H. alvei*	III (strong)	AM, F
11	T75 *H. alvei*	II (moderate)	
12	D1 *H. alvei*	III (strong)	AM
13	C2 *H. alvei*	II (moderate)	
14	C3 *H. alvei*	III (strong)	AM
15	C4 *H. alvei*	III (strong)	F
16	Y5 *H. alvei*	III (strong)	

AM: ampicillin, E: erythromycin, TE: tetracycline, P: penicillin, K: clindamycin, NA: Nalidixic Acid, F: nitrofurantoin

According to the results presented in Table 4, among the 24 Gram-positive bacterial isolates obtained from animal-based food samples, 13 isolates (54.1%) exhibited strong biofilm-forming ability, whereas 7 isolates (29.1%) were determined to possess moderate biofilm-forming capacity. Among the *S. aureus* isolates obtained from animal-based food samples, both strong and moderate biofilm formation capacities were detected. Specifically, seven isolates exhibited strong biofilm-forming ability, while three showed moderate biofilm-forming ability. In contrast, no biofilm-negative isolate was identified among the *Salmonella* spp. isolates, and both moderate and strong biofilm formation were observed (Table 5).

Similarly, *M. caseolyticus* isolates demonstrated variable biofilm-forming capacities, including strong, moderate, and negative biofilm profiles. Four *M. caseolyticus* isolates were determined to be biofilm-negative, whereas the remaining isolates exhibited moderate or strong biofilm production. Among the *H. alvei* isolates, all isolates showed either moderate or strong biofilm-forming ability, and no biofilm-negative isolate was detected.

Overall, Gram-negative bacterial isolates (*Salmonella* spp. and *H. alvei*) exhibited higher rates of moderate and strong biofilm formation compared to Gram-positive bacterial

isolates (*S. aureus* and *M. caseolyticus*). Furthermore, biofilm-negative isolates were detected only among Gram-positive bacteria, whereas all Gram-negative isolates demonstrated measurable biofilm-forming capacity.

In our study, eight antibiotics were tested for *S. aureus* isolates, and high resistance to penicillin (40%) and clindamycin (70%) was determined. Eight antibiotics were tested against *M. caseolyticus* isolates, and penicillin and clindamycin showed the same resistance rate (42.8%). The highest resistance was determined as erythromycin (57.1%). Eight antibiotics were tested for *Salmonella* spp. isolates and high resistance rates were determined for nalidixic acid (71.5%), nitrofurantoin (85.7%), and ampicillin (85.7%). Eight antibiotics were tested for *H. alvei* isolates, and resistance to ampicillin (45.5%) and nitrofurantoin (22.2%) was determined. Resistance profiles of all isolates are presented in Tables 4 and 5.

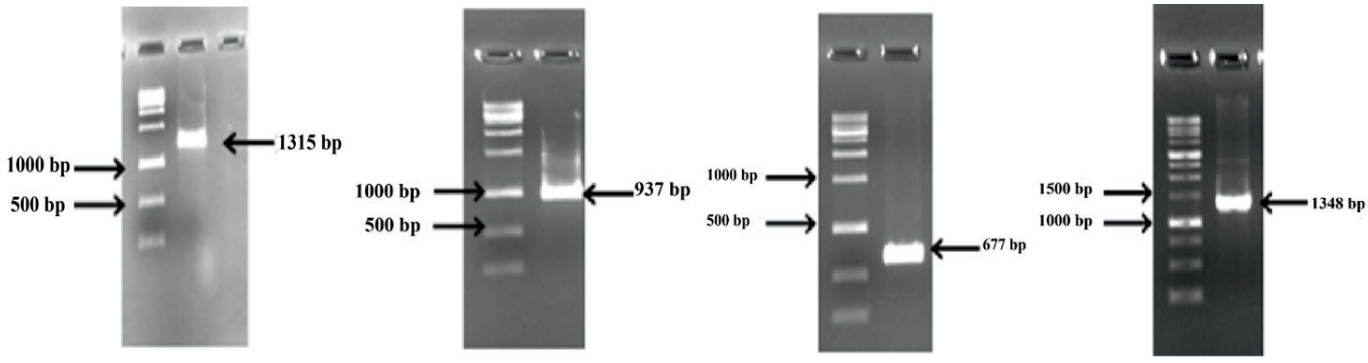

Fig. 1. Images of *icaA*, *hlg*, *mgtC*, *sopB* genes on agarose gel, respectively. (Photographs were taken by Meryem Burcu Külahcı and Serdar Coşkun).

After species identification by MALDI TOF mass spectrometry, *S. aureus* and *Salmonella* spp. isolates were included in the molecular study as animal-based food pathogens in our study. *M. caseolyticus* and *H. alvei* isolates included in antibiotic resistance, slime, and biofilm studies were not included in the molecular study. Among the *S. aureus* isolates tested for *icaA* and *hlg*, 3/10 (30%) were positive for *icaA* and 7/10 (70%) were positive for *hlg*. Among the *Salmonella* spp. isolates tested for *mgtC* and *sopB*, 6/7 (85.7%) were positive for *mgtC* and all isolates, 7/7 (100%), were positive for *sopB* (Figure 1).

DISCUSSION

S. aureus and *Salmonella* spp. are important food pathogens, and their presence in foods, besides causing food infections, raises concerns about the production of slime factor and biofilm, antibiotic resistance, and the spread of this resistance. In our study, the rate of *S. aureus* and *Salmonella* spp. isolated from 86 animal-based food samples was 11.6% and 8.1%, respectively.

In a study conducted by Normanno et al. in Italy in 2007, 168 (31%) of 437 raw milk samples tested positive for *S. aureus* [39]. In a study conducted on dairy products in Italy, *S. aureus* was detected in mozzarella-type (25%) and soft-type cheeses (18.9%) [40]. In a study conducted in Türkiye, *S. aureus* was isolated at 33.33% from raw milk and 27.9% from cheese collected from the Eskişehir and Kütahya regions [41]. Although the presence of *S. aureus* was determined in raw milk (40%) and cheese (56%) samples collected in our study (Table 3), the rates of *S. aureus* in the samples studied were similar to those in other studies. *Salmonella* spp. were isolated from 80 (21.1%) of 378 raw chicken meat, heart, gizzard, and liver samples collected in Debre Zeit and Addis Ababa, Ethiopia [42]. In our study, 7 (8.1%) *Salmonella* spp. were isolated from 86 animal-based food samples (Table 3). In a study by Aarestrup et al., *Salmonella enterica* serovar Schwarzengrund was isolated from 7 of 7 chicken meat samples collected in Denmark and 44 of 48 samples collected in Thailand [43]. In this study, *Salmonella* spp. were isolated at a much higher rate compared to our study. Çetinkaya et al. detected *Salmonella* spp. contamination in only 1 of 168 raw chicken samples, which was lower than the rate observed in our study [44].

In the food industry, bacterial biofilm formation in food processing environments and on processing materials poses a significant risk of contaminating ready-to-eat foods. Biofilms formed in these environments can cause problems such as food spoilage and reduced shelf life, the presence of infectious bacteria, reduced heat transfer, corrosion, and deterioration of metal surfaces. In addition, due to the biofilm's multi-layered structure, bacteria within it can develop antimicrobial resistance as oxygen levels decrease at the bottom, and the effectiveness of some antibiotics and disinfectants declines. For this reason, *Salmonella* and *S. aureus*, which are very important in the food industry, become resistant to disinfectants and antibiotics, and are protected from many external factors when they form biofilms [45].

In their 2012 study, Gündoğan and Ataol found 50% slime positivity in 6 *S. aureus* isolates from minced meat samples [46]. In the present study, slime production was detected at a

higher rate. While no slime production was observed among 22 *Salmonella* isolates from poultry and clinical samples in Tunisia in 2014 [47], slime positivity was observed in all 16 *Salmonella* spp. and *H. alvei* isolated in our study.

In a study of various ready-to-eat food samples in China, biofilm formation was detected in 33 (61.1%) of 54 *S. aureus* isolates from meat and dairy products. Biofilm formation was strong in 3.7%, moderate in 1.9%, and weak in 56.6% of the isolates [48]. In our study, strong and moderate biofilm production rates were higher (Table 4). In the same study, antibiotic resistance rates among *S. aureus* isolates were penicillin (90.7%), erythromycin (46.3%), clindamycin (40.7%), and tetracycline (22.2%), which are similar to those in our study (Table 4). In another study conducted by Xing et al. in China, among 87 ready-to-eat food products, 78.1% of erythromycin and 40.6% of tetracycline resistance were found in 128 *S. aureus* isolates [49].

In a study by Wang et al., 104 samples collected from various parts of a chicken slaughterhouse yielded 23 Salmonella isolates, of which 5 formed strong biofilms [50]. In Iran, 34.5% of 282 *Salmonella enterica* serotype *enteritidis* isolates from chicken meat, live chicken, and human feces were strong biofilm producers [51]. In our study, strong biofilm was observed in all *Salmonella* isolates.

Frequent use of β-lactam (penicillin), macrolide (erythromycin), and lincosamide (clindamycin) group antibiotics for the prophylaxis and treatment of bacterial infections in farm and poultry farming can lead to the development of antibiotic-resistant bacteria. It has been emphasized in many studies that the spread of antibiotic resistance through direct and indirect routes, as a result of raw and cooked foods cross-contaminated with antibiotic-resistant bacteria reaching consumers, may pose a serious public health risk [52, 53].

In a study by Ağay and Kimiran, resistance to nitrofurantoin and tetracycline was found in 66.7% of Salmonella isolates from different sources. Compared with our results, the nitrofurantoin resistance rate was similar, whereas the tetracycline resistance rate was lower [54]. Gargano et al. found 33.3% resistance to tetracycline and 21.6% resistance to ampicillin in 16 (26.6%) *Salmonella* isolates obtained from a 2021 study of 60 animal-based foods [55], which supports the results of our study (Table 4).

In a study conducted with 104 chicken carcass samples, Wang et al. isolated *Salmonella* spp. from 23 samples. They found 78% resistance to ampicillin, 70% to tetracycline, 61% to chloramphenicol, and 13% to ciprofloxacin in *Salmonella* spp. isolates [50]. These results were consistent with the ampicillin and tetracycline resistance rates in our study, but it was remarkable that no resistance was observed to ciprofloxacin or chloramphenicol. In the study conducted by Reza Khaltabadi Farahani et al. in 2018 with 282 chicken meat, feces, and egg samples, the 78.6% nalidixic acid resistance they found in the *Salmonella enterica* serotype *enteritidis* species they isolated is highly similar to the nalidixic acid resistance (75%) determined in our study [51]. When the results of our study shown in Table 4 are compared with the results of Babacan and Karadeniz, ampicillin (57.15%) and nitrofurantoin (82.85%) resistance rates were similar, tetracycline resistance (82.85%) was found to be higher in our study, chloramphenicol resistance was not found in our study, whereas resistance was found at a rate of 57.14% in Babacan and Karadeniz's study [56].

Unlike Staphylococci, Macrococci do not cause human or animal diseases and are typically isolated from animal skin, milk, and meat products. However, the physiological properties of this microorganism are largely unknown, and few studies have been conducted on Macrococci [57]. MacFadyen et al. (2016) detected the presence of 33 methicillin-resistant *M. caseolyticus* in bulk tank milk samples from dairy farms in England and Wales. *Macrococci* are closely related as veterinary pathogens but are increasingly recognized as potential determinants of methicillin resistance that can be passed on to pathogenic Staphylococci [58]. Blaiotta et al. (2004) detected Coagulase-negative staphylococci (CNS) and *M. caseolyticus* in two types of traditionally fermented sausages (salsiccia and soppressata lucana) produced in Basilicata, Southern Italy [59].

208 Gram-positive bacterial isolates were obtained from 35 local Minas cheese samples from 7 different brands. Of these, 140 were *Enterococcus*, 30 were *Staphylococcus,* and 38 were *M. caseolyticus* [60]. In a 2016 study by Dugat et al., H. alvei was isolated from diet soft French cheeses with reduced salt content. They determined that reducing the salt percentage in the diet of soft French cheeses will reduce the growth of some pathogenic bacteria [61]. In Poland in 2012, Szweda et al. phenotypically and genotypically studied the biofilm-forming ability of 132 *S. aureus* isolates from bovine mastitis. They found that most of the isolates, 76 out of 132 *S. aureus,* formed biofilms. Both *icaA* and *icaD* genes were detected in 100% of the tested isolates [62].

In Brazil in 2014, Castelani et al. investigated biofilm formation and virulence genes of *S. aureus* in cows with mastitis. In this context, they investigated biofilm formation on Congo red agar, the presence of *icaA* and *icaD* genes by polymerase chain reaction (PCR), and antimicrobial resistance of *S. aureus* isolates. In a PCR study of mastitic cows and their secretions, the *icaA* and *icaD* genes were detected in 98% and 100% of the isolates, respectively. However, only 55.5% of all isolates were biofilm-positive on Congo red agar. They found that cows may carry antibiotic-resistant isolates capable of forming biofilms, posing a threat to public and animal health [63]. Literature indicates that bacterial agents account for one-third of foodborne infections. *Salmonella* spp. ranks first among these agents, and *S. aureus* ranks second [64]. Taken together with previous reports, our findings suggest that *H. alvei* should be considered an emerging food-associated microorganism [65].

As a result of our study, the isolation of *S. aureus* and *Salmonella* spp. as important food pathogens, the high rates of slime factor and biofilm production by these bacteria, and the high rates of resistance to various antibiotics tested are of serious importance for food hygiene and public health. Furthermore, these findings underscore the significance of *Macrococcus* and *Hafnia* regarding the spread of antibiotic resistance in foods. Therefore, strict hygiene and sanitation measures should be maintained throughout the cold chain, from production to consumption, for animal-based food products.

CONCLUSION

In conclusion, this study demonstrated the presence of foodborne *S. aureus* and *Salmonella* spp. in animal-based food products and revealed that these isolates exhibited notable antibiotic resistance, biofilm-forming ability, slime production, and virulence-associated genes. The high prevalence of moderate to strong biofilm formation among both Gram-positive and Gram-negative isolates highlights the potential of these bacteria to persist in food processing environments and contribute to the spread of antimicrobial resistance. Furthermore, the detection of virulence genes such as *icaA*, *hlg*, *mgtC*, and *sopB* emphasizes the potential public health risks associated with contaminated animal-based foods.

The original contribution of this study lies in the integrated evaluation of antimicrobial resistance, slime production, biofilm formation, and virulence-associated genes in bacterial isolates recovered from different animal-based foods, including emerging food-associated bacteria such as *M. caseolyticus* and *H. alvei*. In addition, the combined use of conventional phenotypic methods, MALDI-TOF mass spectrometry, and molecular analyses strengthened the reliability and comprehensive characterization of the bacterial isolates. These findings highlight the importance of maintaining strict hygiene and sanitation practices throughout the food production chain, promoting rational antibiotic use in animal husbandry, and ensuring continuous surveillance of foodborne pathogens.

This study has several limitations. The relatively small sample size and the restriction of sampling to a specific geographical region may limit the generalizability of the findings. In addition, the limited number of bacterial isolates reduced the comprehensiveness of species-specific evaluations. Molecular analyses were restricted to selected virulence genes, while the absence of serotyping, advanced molecular typing methods, and minimum inhibitory concentration (MIC) testing limited the detailed characterization of the isolates and their antimicrobial resistance profiles.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

AUTHORSHIP CONTRIBUTIONS

Concept: S.C., M.B.K., N.G., Design: S.C., M.B.K., Data Collection or Processing: S.C., M.B.K., Analysis or Interpretation: S.C., M.B.K., Literature Search: S.C., N.G., Writing: S.C., M.B.K.

FINANCIAL DISCLOSURE

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