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Prevalence, antibiotic resistance, and biofilm formation of coagulase-positive staphylococci in Izmir Tulum Cheese

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Abstract

Coagulase-positive staphylococci (CPS) are the main causative bacterial agents of staphylococcal food intoxication, posing a significant public health risk and causing infections in humans and animals. In this study, a hundred Izmir Tulum Cheese samples were collected from various retail outlets in the Izmir province. CPS isolates from cheese samples were identified using standard cultural methods. The phenotypic antibiotic resistance of CPS isolates was determined using the agar disk diffusion test method, while their biofilm formation capacity was assessed using the colorimetric method. In the study, CPS was isolated from 30 out of 100 analyzed Izmir Tulum Cheese samples (30%), and it was determined that 27 of these samples (27%) had CPS levels exceeding the maximum acceptable limit of 10^3 CFU/g set by the Turkish Food Codex Microbiological Criteria Regulation. Antimicrobial resistance analysis revealed that among the 30 CPS isolates, 90% were resistant to penicillin, while resistance rates to other commonly used antibiotics were 83.3% for clindamycin, 56.7% for ciprofloxacin, and 53.3% for tetracycline. Additionally, 76.7% of the isolates were multidrug-resistant, meaning they were not easily killed by different antibiotics, which limits treatment options. Furthermore, 83.3% of the CPS isolates had the capacity for biofilm formation, highlighting its impact on food safety. These findings emphasize the need for stricter hygiene protocols, controlled antibiotic use, and innovative strategies to combat biofilms in dairy production.

Keywords: Coagulase-positive Staphylococci, Antimicrobial Resistance, Biofilm Formation, Izmir Tulum Cheese, Food Safety

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INTRODUCTION

Cheeses are dairy products that are produced and consumed on a global scale (Tilocca et al., 2020). In Türkiye, tulum cheese is among the most widely consumed varieties. Its name is derived from the Turkish word 'tulum,' meaning 'goat or sheep skin,' which refers to the material traditionally used for packaging and maturation (Hayaloglu et al., 2007). Izmir Tulum Cheese, a geographically indicated product, is among Turkey's leading traditional cheese varieties. The cheese is produced in the Aegean region using milk derived from sheep, goats, and cows. In the traditional production process, starter cultures are not used, and the cheese is aged in brine. (Yerlikaya and Akbulut, 2019). Since there are no starter cultures in the cheese, it can age with the help of the natural milk microbiota. The quality of cheese depends on the composition of its microbiota (Coelho et al., 2022). Although the production process is similar to that of Erzincan Tulum Cheese, the ripening of Izmir Tulum Cheese in brine, filled tins differentiates it from other tulum cheese varieties. In producing Izmir Tulum Cheese, raw milk is either used directly or pasteurized at 60–68°C for 30 minutes before being cooled to the fermentation temperature

(27–37°C) and allowed to coagulate for 45–60 minutes. After curd cutting and whey drainage, the curd is shaped and salted. The cheese is then ripened in tins filled with 12–14% brine at 4–6°C for 90–180 days, with periodic turning, until it is ready for consumption (Koca, 1996; Yerlikaya and Akbulut, 2019).

Raw milk is the primary source of contamination in cheese production (André et al., 2008). Pasteurization eliminates harmful and spoilage bacteria in raw milk, but heat-resistant toxins made by some microorganisms can still change the microbiological quality (Penna et al., 2021). Mainly, staphylococcal enterotoxins produced by CPS, such as *Staphylococcus aureus* (*S. aureus*), are generally heat-resistant and can remain in food even after thermal processes like pasteurization (Balaban and Rasooly, 2000; Angulo et al., 2009; Larkin et al., 2009). These heat-resistant enterotoxins pose a significant risk of food poisoning for consumers (Ferreira et al., 2016; Calahorrano-Moreno et al., 2022).

Bacteria belonging to the *Staphylococcus* genus are Gram-positive, non-motile, and non-spore, forming facultative anaerobic cocci that typically form grape-like clusters (Götz et al., 2006). At least nine *Staphylococcus* species, including *S. aureus*, *S. intermedius*, *S. pseudointermedius*, *S. delphini*, *S. lutrae S. schleiferi* subsp. *coagulans*, *S. hyicus*, *S. argenteus*, and *S. schweitzeri*, have been identified. Among these CPS *S. aureus* is the most common cause of foodborne illnesses (Esemu et al., 2023). In addition to foodborne illnesses, *S. aureus*, a member of the CPS group, can cause purulent skin and soft tissue infections by leading to wound infections, pneumonia, meningitis, and bacteremia (Pereira et al., 2022; Ryan and Ray, 2004).

According to the Turkish Food Codex Microbiological Criteria Regulation, the maximum acceptable limit for CPS in cheese is 10^3 CFU/g (Anonymous, 2025). However, studies conducted in Türkiye indicate that CPS contamination in cheese often exceeds this threshold. Unal Turhan, (2019) detected CPS in all 20 traditional cheese samples analyzed, with 45% exceeding the regulatory limits. Similarly, Gökmen et al. (2017) found CPS in 10% of 100 cheese samples they examined. The presence of *S. aureus* in various cheese samples has been reported at different rates: 48% (Gundogan and Avci, 2014), 12.5% (Can et al., 2017), 22% (Kayili and Sanlibaba, 2020), 37.5% (Güngören et al., 2022) and a study conducted in Aydın province reported that 18 out of 100 tulum and white cheese samples (18%) contained *S. aureus* (Taşcıoğlu, 2022).

Staphylococcal food poisoning caused by cheese is a significant global issue. Several factors contribute to this problem, including the pathogen's high salt tolerance (capable of growing in salt concentrations of 10% and even 20%), the use of raw milk contaminated with *S. aureus* without pasteurization, insufficient activity of starter cultures (Yıldırım et al., 2016), improper cooling and storage conditions, and contamination of processed foods by food handlers who are infected or natural carriers of *S. aureus*, either through direct hand contact or respiratory secretions.(Fernandes et al., 2022). Figure 1. shows the primary contamination sources of CPS in dairy processing.

Besides foodborne microbial diseases, another significant concern in food safety is the development of antimicrobial resistance resulting from the misuse and abuse of antimicrobial agents in humans and animals (de Souza Paiva et al., 2021). The unregulated access to veterinary antibiotics used for therapeutic and prophylactic purposes in animal husbandry, insufficient knowledge about antibiotic dosage and resistance development, excessive use of antibiotics, and the addition of antibiotics to animal feed play a key role in the development of antibiotic resistance (Bacanlı and Başaran, 2019; Tiseo et al., 2020).

CPS is commonly detected in milk samples and is associated with mastitis, a widespread disease in dairy cattle. Excessive use of intramammary antibiotics in mastitis treatment has led to the emergence of antibiotic-resistant bacteria and the horizontal transfer of resistance genes to other bacteria (Capurro et al., 2010; Vanderhaeghen et al., 2010).

The use of antimicrobials in animal husbandry significantly contributes to the development of antibiotic resistance in both human and animal pathogens, posing a considerable threat to public health, especially regarding infections caused by multidrug-resistant bacteria (Kupczyński et al., 2024). Antibiotic-resistant bacteria, including methicillin-resistant *S. aureus* (MRSA), are widespread in both communities and healthcare settings and have developed resistance to various antibiotics like tetracycline, tobramycin, and gentamicin (Silva et al., 2014). Increased antibiotic-resistant infections remain a global issue, resulting in treatment failures, higher morbidity and mortality rates, and increased healthcare costs (Spellberg et al., 2008; Arefi et al., 2014; Uddin et al., 2021). Furthermore, resistance genes that can spread through food via environmental factors directly risk human health (Endres et al., 2023). Morar et al. (2021) recommend establishing an integrated surveillance system throughout the food chain to address this.

Biofilms can persist despite cleaning and disinfection efforts, making their removal even more difficult (Yang et al., 2012). Moreover, the biofilm matrix enhances bacterial antibiotic resistance, increasing food contamination risk (Kroning et al., 2016). Bacterial biofilms that form on food processing surfaces and equipment can spread to other areas or directly contaminate food, acting as a persistent source of contamination (Kasnowski et al., 2010). Given these risks, effective biofilm prevention and control strategies are essential to ensuring food safety in processing environments.

In conclusion, the presence of CPS in dairy products is significant due to its potential to produce enterotoxins, which poses a major concern for product and consumer safety. Literature on the microbiological quality parameters of Izmir Tulum Cheese is limited (Büyükyörük and Soyutemiz, 2010; Şen et al., 2023). This study aims to examine

the prevalence, antimicrobial resistance profiles, and biofilm formation capacities of CPS isolated from Izmir Tulum Cheese in the city center and surrounding districts and assess their impact on food safety. Furthermore, the hypothesis of a statistically significant correlation between antimicrobial resistance and biofilm production ability will be tested.

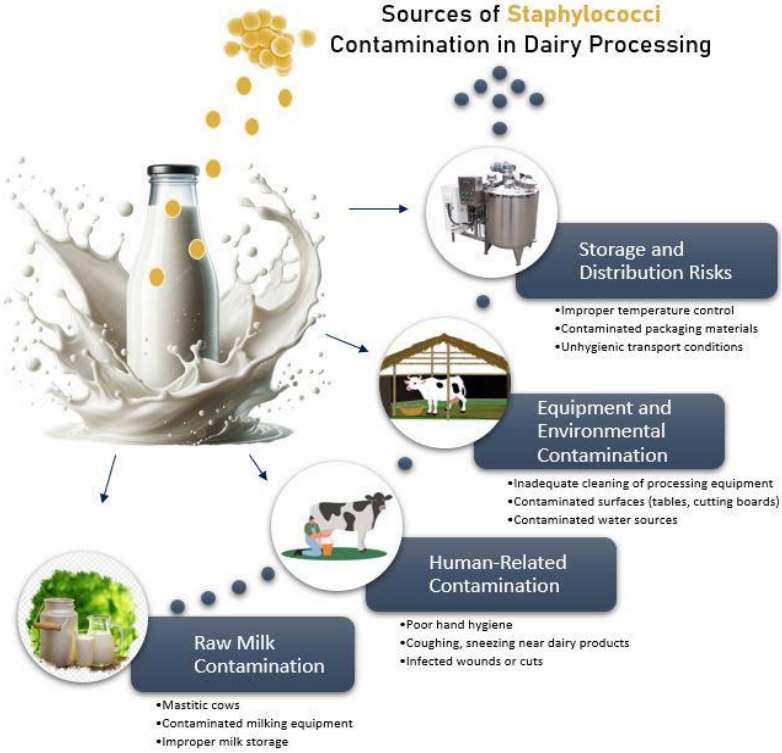

Figure 1. Primary contamination sources of CPS in dairy processing

MATERIALS AND METHODS

Material

A total of 100 Izmir Tulum Cheese samples were obtained from several local markets, commercial establishments, and retail outlets in the Izmir province center and surrounding districts (Dikili, Bergama, Aliağa) between August and September 2024. The sample size was determined based on representativeness, considering Izmir's significance in cheese production and consumption, feasibility due to resource and time constraints, and alignment with previous studies. However, some limitations exist, including the restricted geographical scope, as samples were collected only from specific districts, the non, random sampling method, which may affect generalizability, and the sample size, which, while sufficient for analysis, could be expanded for broader insights. Cheese samples made from a mixture of goat, cow, and sheep milk and matured for at least 90 days were transported to the laboratory under cold chain conditions (2–8 ºC). To minimize potential food safety and contamination risks, sterile sample collection containers and monitoring forms were used during sampling. The work plan is given as a graphical abstract in Figure 2.

Coagulase-positive Staphylococci Analysis

Under aseptic procedures, 25 grams of cheese samples were placed in a sterile stomacher bag, to which 225 milliliters of Maximum Recovery Diluent (MRD, Merck, 112535) were added and homogenized for two minutes. Appropriate dilutions of 1/10, 1/100, and 1/1000 from the homogenized sample were prepared by mixing one milliliter of the sample with nine milliliters of sterile dilution fluid. 0.3,0.3,0.3,0.4 mL of each dilution (1 mL in total) was transferred to three separate Petri dishes containing Baird Parker Agar (BPA, Merck, 105406) supplemented with 5% Egg Yolk Tellurite Emulsion (Merck, 103785) and spread quickly with a sterile Drigalski spatula without touching the sides of the petri dish. The Petri dishes were maintained at laboratory temperature for 15 minutes to permit the inoculum to be absorbed by the medium. Subsequently, the BPA medium was inverted and incubated at 35,37°C for 48±2 hours. Following the incubation period, typical and suspicious colonies with a diameter of 2,3 mm, black in color, shiny, and forming a transparent zone around them were evaluated for a coagulase test using a commercial latex test kit (OXOID Staphylase Test Kit, DR0595). All samples were studied at a biosafety level of 2 cabinets. Coagulase-positive colonies were counted, and results were calculated in CFU/g (ISO 6888,1:2003).

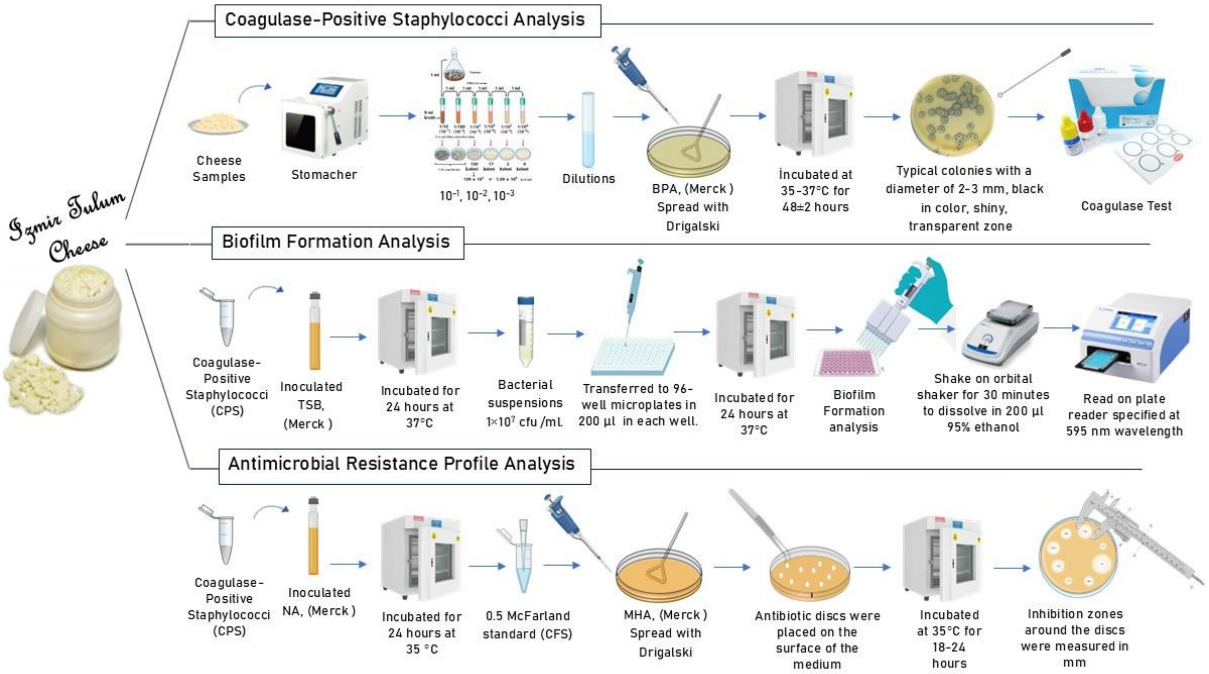

Figure 2. Graphical abstract

Antimicrobial Resistance Profile Analysis

Antibiotic selections were made by choosing one representative antibiotic from each group used to treat staphylococcal diseases in dairy animals, with phenotypic zone diameters available as specified by the Clinical and Laboratory Standards Institute (CLSI, 2020) documents. The antimicrobial susceptibility of CPS isolates was determined using the Kirby- Bauer disc diffusion method (Yao et al., 2021). In the tests, discs selected from seven different antibiotic groups were used: cefixime (5 µg), sulfamethoxazole/trimethoprim (25 µg), tetracycline (30 µg), penicillin G (10 µg), gentamicin (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg) and clindamycin (2 µg) (Bioanalyse). For antimicrobial susceptibility testing, bacterial suspensions were prepared from fresh and pure cultures grown on nutrient agar (NA, Merck, 105450) under aerobic conditions at 35 °C by 0.5 McFarland standard (Biomerieux, 70900). 0.1 mL of these suspensions were taken and spread inoculated on Mueller, Hinton agar (MHA, Merck, 103872) plates. Subsequently, antibiotic discs were placed on the surface of the medium. The plates were incubated at 35°C for 18,24 hours, after which the inhibition zones around the discs were measured in millimeters. The *S. aureus* ATCC 29213 strain was employed as the positive control. The results were classified according to the CLSI (2020) criteria as susceptible (S), intermediate susceptible (I), or resistant (R).

Biofilm Formation Analysis

In this experiment, CPS bacteria obtained from cheese samples were incubated with 5 mL Tryptic Soy Broth (TSB, Merck, 105459) containing 1% glucose for 24 hours at 37°C to obtain fresh cultures. Then, bacterial suspensions at approximately 1×10^7 CFU/mL density were prepared and transferred to 96 well microplates in 3 replicates with 200 µL in each well. The microplates were incubated at 37°C for 24 hours. A TSB solution containing 1% glucose was the negative control, while the positive control was the *S. aureus* ATCC 29213 strain. After the incubation period, the wells were washed three times with 250 µL of physiological saline to remove any unbound bacteria, then dried and proceeded to the next step. To facilitate fixation, 200 µL of 99% methanol was added to each well and allowed to stand for 15 minutes. Then, the methanol was aspirated, the microplates were dried, and the staining stage was started. The dried wells were stained with 200 µL of a 0.1% crystal violet solution for five minutes. The excess stain was removed by rinsing with tap water, and the microplates were then allowed to air dry. The stained biofilms were shaken on an orbital shaker for 30 min to dissolve in 200 µL of 95% ethanol and transferred to the measurement stage. The optical density of the resulting solution was determined at a wavelength of 595 nm (OD595) (Li and Tang, 2004). According to the measurement results, biofilm formation ability was evaluated as weak, medium, and strong. ODc = Mean OD of negative control + (3X SD value of the standard deviation of negative control); OD: Optical Density; ODc: Optical Density cut, off value; OD MB: Microorganism Biofilm Optical Density; SD: Standard Deviation. As a result of the measurements, biofilm formations are scored as 0, +, ++, +++. Measurement result; OD MB ≤ ODc: Nonbiofilm formation and score value 0, ODc < OD MB ≤ 2 X ODc: Weak biofilm, score value +1, 2 X ODc < OD MB ≤ 4 X ODc: Moderate biofilm, score value +2, 4 X ODc < OD MB: Strong biofilm, score value +3 (Kim et al., 2019; Yılmaz, 2020).

Statistical Analysis

The distributions between CPS levels, antimicrobial resistance rates, and biofilm formation abilities were presented with frequency and percentage analyses. Frequency and 95% Confidence Interval (CI) were calculated for categorical variables. The relationship between antibiotics and biofilm formation capacity was evaluated by Fisher's exact test. All analyses were performed SPSS Statistics v29.0.1 package program (SPSS Inc., Chicago, Ill., USA).

RESULTS AND DISCUSSION

Coagulase- positive Staphylococci Analysis Results

In this study, CPS was isolated from 30 out of 100 analyzed Izmir Tulum Cheese samples (30%). It was determined that 27 of these samples (27%) had CPS levels above the maximum CPS limit (10^3 CFU/g) accepted in cheese according to the Turkish Food Codex Microbiological Criteria Regulation (Anonymous, 2025). The CPS results (ranging from 2.0×10^1 to 6×10^5 CFU) are presented in Table 1, while the contamination levels and distribution of these 30 CPS-containing İzmir Tulum Cheese samples are shown in Table 2.

Table 1. CPS results in Izmir Tulum Cheese

Sample no	Coagulase-positive staphylococci (CFU/g)	Sample no	Coagulase-positive staphylococci (CFU/g)	Sample no	Coagulase-positive staphylococci (CFU/g)
1	2.0×10^1	**11**	$7{,}0\times10^3$	**21**	3.6×10^4
2	1.8×10^2	**12**	1.4×10^4	**22**	3.7×10^4
3	7.1×10^2	**13**	1.5×10^4	**23**	4.9×10^4
4	4.0×10^3	**14**	1.6×10^4	**24**	5.1×10^4
5	4.5×10^3	**15**	1.6×10^4	**25**	6.4×10^4
6	4.6×10^3	**16**	2.0×10^4	**26**	6.6×10^4
7	5.0×10^3	**17**	2.1×10^4	**27**	6.8×10^4
8	$5{,}0\times10^3$	**18**	$2{,}6\times10^4$	**28**	1.0×10^5
9	$5{,}0\times10^3$	**19**	$2{,}8\times10^4$	**29**	4.0×10^5
10	$6{,}0\times10^3$	**20**	2.9×10^4	**30**	6.0×10^5

Table 2. Prevalence of CPS in Izmir Tulum Cheese

Total samples	Number with CPS (%)	Number of samples with CPS (%) at different levels	
		$<10^3$ CFU/g	$\geq10^3$ CFU/g
100	30 (30)	3(3)	27 (27)

Gökmen et al. (2017), in a study on milk and dairy products, reported that they detected CPS in 10% of 100 cheese samples examined. Similarly, Sylejmani et al. (2015) detected 22% CPS in artisan cheese samples and reported that these values ranged between $2.0x10^2$ and $6.5x10^5$ CFU/g. Normanno et al. (2005) detected 23.7% CPS in cheeses produced from heat-treated milk sold in markets in Italy, and this rate was determined as 24.2% in ricotta cheese. Morar et al. (2013) found CPS in 34.8% of 51 cheese samples produced from fresh and matured raw milk, and more than 50% of these cheeses had CPS levels above 10^5 CFU/g. In a study conducted in Egypt, CPS was detected in 76% of 50 cheese samples (Endres et al., 2023). The current study revealed that 27% of Izmir Tulum Cheese exceeded the maximum level (10^3 CFU/g) specified in the Microbiological Criteria Regulation terms of CPS. These include contaminated raw milk, recontamination after pasteurization, poor storage conditions, and staff not following proper hygiene practices. Inadequate personnel adherence to hygiene protocols can markedly contribute to contamination in food production (Kousta et al., 2010; Sospedra et al., 2012). Moreover, while standard pasteurization can inactivate CPS, it cannot eliminate pre-formed enterotoxins. These toxins are recognized as the causative agents of foodborne illnesses, even at exceedingly low levels (Medeiros et al., 2019). Unal Turhan (2019) detected CPS in all 20 traditional cheese samples analyzed and reported that 45% exceeded the Turkish Food Codex limits. In a study conducted in Romania, CPS was found in 35.5% of traditional cheeses obtained from raw milk, and 68.1% of these samples had CPS levels above 10^5 CFU/g (Rosengren et al., 2010). Radoslava et al. (2010) detected CPS in 20.48% of 415 cheese samples collected from local markets, and Normanno et al. (2005) reported that 20.7% of 3,097 milk and milk product samples were contaminated with CPS. In a study conducted in Brazil, CPS was detected in 43% of samples taken from raw milk, curd, matured cheese, and colonial cheeses (Grecelle et al., 2020). The prevalence of CPS can vary across regions and studies, with factors such as hygiene conditions, contamination levels, and the procedures applied during food processing influencing this variability.

Antimicrobial Resistance Profile Analysis Results

Antibiotics used for treatment or protection in animals promote the development of antimicrobial resistance in pathogens and normal flora bacteria. Bacteria carrying resistance genes can pass into the human flora through food and cause transmission of this problem to humans (Barton, 2000). This situation represents a substantial risk to

both food safety and public health. Our findings are consistent with those observed in other studies (Ferreira et al., 2016; Kürekçi, 2016; Da Silva, 2021; Kizanlik and Goksoy, 2024), with penicillin exhibiting the most significant level of resistance (90%). In addition, phenotypic resistance to antibiotics from three or more different groups was observed in 23 (76.7%) of the thirty CPS isolates for which antibiogram analyses were performed. Similarly, Endres et al. (2023) observed that all *S. aureus* strains obtained from cheese samples exhibited multidrug resistance. In this study, antibiotic resistance rates were found to be high for clindamycin (83.3%), ciprofloxacin (56.7%), and tetracycline (53.3%) antibiotics, respectively. Table 3 presents the antibiotic susceptibility and multidrug resistance profiles of the 30 CPS isolates, while Table 4 shows the phenotypic susceptibility percentages of the isolates to antibiotics.

Table 3. Antibiotic susceptibility and multidrug resistance profiles of CPS isolates

Sample No.	Penicillins (P)	Sulfonamides (SXT)	Tetracyclines (TE)	Aminoglycosides (CN)	Quinolones (CIP)	Lincosamides (DA)	Macrolides (E)	M D R
1	R	S	R	S	R	R	I	MDR
2	R	S	R	S	S	R	S	MDR
3	R	S	R	S	R	R	I	MDR
4	R	I	R	R	R	R	R	MDR
5	R	R	I	S	I	R	R	MDR
6	R	R	R	R	R	R	R	MDR
7	R	S	R	R	I	R	I	MDR
8	R	R	R	S	R	R	R	MDR
9	R	R	R	S	R	R	I	MDR
10	R	S	R	R	R	R	R	MRD
11	R	S	S	S	I	S	S	
12	R	R	I	I	R	R	I	MDR
13	R	R	I	R	I	R	I	MDR
14	R	R	R	R	R	R	R	MDR
15	R	S	I	I	I	R	I	
16	S	S	S	S	S	S	S	
17	R	I	I	I	R	R	I	MDR
18	R	I	R	I	I	I	I	
19	R	S	I	S	I	R	I	
20	R	R	I	R	R	R	I	MDR
21	R	R	R	I	R	R	I	MDR
22	R	R	I	S	R	R	I	MDR
23	R	I	R	S	R	I	I	MDR
24	S	S	S	S	S	I	S	
25	S	R	S	R	S	R	I	MDR
26	R	S	R	S	I	R	I	MDR
27	R	I	I	I	I	R	I	
28	R	R	R	I	R	R	R	MDR
29	R	R	R	R	R	R	I	MDR
30	R	I	I	I	R	R	R	MDR
Total								**(23/30)**
R	27	13	16	9	17	25	8	
I	-	6	10	8	9	3	18	
S	3	11	4	13	4	2	4	

*Resistant (**R**; Red); Intermediate Susceptible (**I**; Orange); Susceptible (**S**; Green), Multidrug resistance (**MDR**; Blue), MDR Negative (**Grey**), penicillin G **(P)**; trimethoprim/sulfamethoxazole (**SXT);** tetracycline (**TE**); gentamicin (**CN);** ciprofloxacin (**CIP**); clindamycin (**DA);** erythromycin (**E**)

Table 4. Phenotypic susceptibility percentages of CPS isolates to antibiotics

Antibiotic Class	Antibiotic Name	R [%]	R 95% CI	I [%]	I 95% CI	S [%]	S 95% CI
Penicillins	P	90	[79.2- 100]	0	[0- 0]	10	[0- 20.8]
Sulfonamides	SXT	43.3	[25.7- 60.9]	20	[5.9- 34.1]	36.7	[19.6- 53.8]
Tetracyclines	TE	53.3	[35.5- 71.1]	33.3	[16.5- 50.1]	13.3	[1.2- 25.4]
Aminoglycosides	CN	30	[13.6- 46.4]	26.7	[10.9- 42.5]	43.3	[25.7- 60.9]
Quinolones	CIP	56.7	[39.0- 74.4]	30	[13.6- 46.4]	13.3	[1.2- 25.4]
Lincosamides	DA	83.3	[70.0- 96.6]	10	[0- 20.8]	6.7	[0- 15.8]
Macrolides	E	26.7	[10.9- 42.5]	60	[42.6- 77.4]	13.3	[1.2- 25.4]

*penicillin G **(P)**; trimethoprim/sulfamethoxazole (**SXT);** tetracycline (**TE**); gentamicin (**CN);** ciprofloxacin (**CIP**); clindamycin (**DA);** erythromycin (**E**); **R**: Resistant; **I**: Intermediate Susceptible**; S**: Susceptible; 95% Confidence Interval: **95% CI**

In parallel with our findings, Endres et al. (2023) reported 50% resistance to tetracycline group antibiotics. Tetracycline, widely used in human and animal health due to its broad, spectrum effect, causes an increase in resistance rates due to its use as a growth factor (Aydin et al., 2011). The tetracycline resistance rate obtained was higher than the previously reported resistance rates between 6.9% and 30% in animal foods in Turkiye (Yücel and Anıl, 2011; Can et al., 2017; Hızlısoy et al., 2018). Antibiotic-resistant foodborne isolates pose serious health

threats to consumers, indicating inadequacies in hygiene practices. Uncontrolled and widespread use of antimicrobials, especially in developing countries, is one of the leading causes of this problem (Aslim, 2008). The extensive use of penicillin, particularly for animal treatment and prevention, plays a significant role in developing resistance. In the early years of penicillin treatment, all staphylococcal strains were susceptible to this antibiotic. However, in time, due to the production of β- lactamase, these bacteria acquired resistance to penicillin to a significant extent (Barton, 2000; Enright, 2003). The presence of residues of penicillins, which are included in the β, lactam antibiotic group, in dairy products can lead to serious health issues such as allergic reactions and anaphylactic shock (da Silva Abreu, 2021). Yücel and Anıl (2011) reported penicillin (13.9%), methicillin (11.9%), gentamicin (5%), and erythromycin (3.7%) resistance rates in cheese isolates. Morar et al. (2021) determined resistance rates of 53.1% for penicillin, 30.6% for clindamycin, and 22.4% for erythromycin. Samaržija et al. (2007) found that 54% of *S. aureus* isolates in cheese samples were resistant to penicillin. William and Withers (2010) reported 33% penicillin resistance, while Rola et al. (2016) found a resistance rate of 69.2%. Borelli et al. (2006) detected penicillin resistance in 70% of cheese isolates.

Mahdavi and Isazadeh (2019) reported that 21 *S. aureus* isolates from 100 cheese samples showed resistance to several antibiotics, with the highest resistance to penicillin. Similarly, Demirsıkan and Tuncer (2021) detected penicillin resistance in 60% of Tulum cheeses sold in Isparta province. To conclude, the antibiotic resistance profiles of *S. aureus* isolates from foods of animal origin, such as cheese, are critical for food safety. Differences in resistance profiles are influenced by factors such as changes in hygiene levels, production technologies, and sensitivity of detection methods used (Papadopoulos et al., 2019). Increasing molecular characterization studies is important regarding the similarity of virulence properties of strains isolated from foods with clinical strains (Kürekçi, 2016; Rodrigues et al., 2017). One of the most urgent issues of contemporary concern is antimicrobial resistance (AMR), which poses a significant threat to the well, being of humans and livestock. Various factors are believed to contribute to the emergence of antibiotic, resistant bacteria, including the inappropriate use of pharmaceuticals in the food industry, particularly within the dairy sector, and insufficient biosecurity protocols on farms. Consequently, to ensure the sustainability of dairy farming in the future and safeguard consumer health, it is imperative to implement strategies that effectively prevent the proliferation of AMR on agricultural premises. These strategies should emphasize reducing antimicrobial usage while upholding animal welfare and productivity standards (Neculai-Valeanu et al., 2024).

Biofilm Formation Results

The ability of *Staphylococcus* species to form biofilms provides resistance to antimicrobial and disinfectant agents and adverse environmental conditions such as desiccation, UV radiation, pH changes, osmotic shocks, and thermal stress. This characteristic can contribute to food contamination within industrial facilities, causing economic losses and facilitating the transmission of foodborne diseases (Chavant et al., 2007; Cha et al., 2013). Biofilms that adhere to surfaces and cannot be cleaned increase the risk of cross, contamination by acting as a reservoir for pathogenic microorganisms. This adversely affects the microbial quality of foods, shortens shelf life and can cause serious diseases when consumed (Flemming and Wingender, 2010). Extracellular DNA, polysaccharide components, teichoic acid, protein adhesives, minerals, and vitamins make up staphylococcal biofilms (Heilmann, 2011). Although biofilm formation has been widely studied in clinical conditions, especially on implants and medical materials, studies on biofilm formation in foods have been limited (dos Santos et al., 2014; Di Ciccio et al., 2015; Cruzado-Bravo et al., 2019). Figure 3 shows the biofilm-forming ability of CPS isolates obtained from cheese samples of the present study.

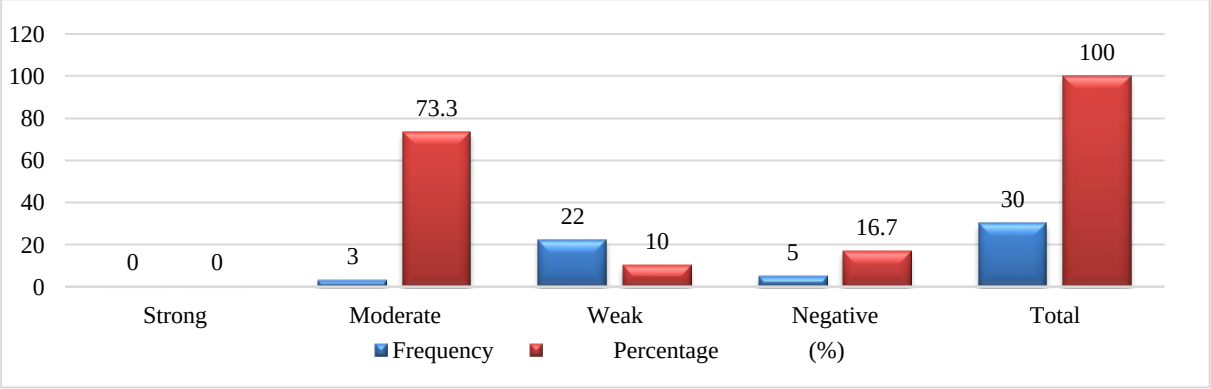

Figure 3. Frequency and percentage distribution of biofilm-forming capacity of CPS isolates

In the study, 25 out of 30 CPS had biofilm-producing capacity (83.3%) in total, which was negative (16.7%), moderate (10%), and weak (73.3%). In a study conducted in Turkey, 83.3% of 30 *Staphylococcus* isolates were capable of biofilm formation which resulted aligning the current study and the resistance rates of biofilm-forming isolates to various antibiotics were; penicillin (P) 73.3%, trimethoprim/sulfamethoxazole (SXT) 40%, tetracycline

(TE) 40%, gentamicin (CN) 26.6%, ciprofloxacin (CIP) 46.6%, clindamycin (DA) 66.6% and erythromycin (E) 20% (Gundoğan and Ataol, 2013). In another study conducted in Erzincan Tulum Cheeses, 37 (60.65%) of 61 *S. aureus* positive isolates were found to be capable of biofilm formation at a decent rate than the current study. Also, in the study, penicillin (P) exhibited the highest antibiotic resistance rate among biofilm-forming isolates, recorded at 45.90%. In addition, the highest rate of penicillin resistance in biofilm-forming isolates was found to be 25% (Özpınar and Gümüşsoy, 2013). In a study by Castro et al. (2020), 69.73% of *Staphylococcus* species isolated from raw milk, Minas artisanal cheese, and food workers' hands were found to form biofilms; while no strong biofilm-forming isolates were found in the study, the distribution according to biofilm capacity was reported as negative (18.42%), moderate (7.90%) and weak (73.68%) aligning the current study results. Unlike the present study, in which no isolates with strong biofilm-forming capacity were found, Gajewska et al. (2020) reported that 22.2% of 54 staphylococcal bacteria isolated from 30 milk samples were CPS, and all isolates exhibited biofilm-forming capacity, with 79.6% demonstrating strong biofilm formation. Another study in the Kayseri province found that 26% of *S. aureus* strains from 23 tulum cheese samples could form biofilms. Of these isolates, 2 had strong, 2 had moderate, and 2 had weak biofilm-forming ability (Akyol et al., 2023), which is lower rates than the current study. Table 5 presents the antibiotic resistance profiles of CPS isolates based on their biofilm production capacity, and the correlation between antibiotic resistance and biofilm formation was analyzed using Fisher's exact test.

Table 5. Antibiotic resistance profiles based on biofilm production capacity in CPS isolates.

	Moderate Biofilm Producer (n=3)			**Weak Biofilm Producer (n=22)**			**Negative (n=5)**		
Antibiotic	**R % [95% CI]**	**I % [95% CI]**	**S % [95% CI]**	**R % [95% CI]**	**I % [95% CI]**	**S % [95% CI]**	**R % [95% CI]**	**I % [95% CI]**	**S % [95% CI]**
P	66.7 [0.0-100.0]	0.0 [0.0-0.0]	33.3 [0.0-100.0]	90.9 [70.8-98.9]	0.0 [0.0-15.4]	9.1 [1.1-29.2]	100.0 [56.6-100.0]	0.0 [0.0-43.4]	0.0 [0.0-43.4]
SXT	33.3 [0.0-100.0]	0.0 [0.0-0.0]	66.7 [0.0-100.0]	50.0 [29.9-70.1]	36.4 [18.0-57.5]	13.6 [3.9-31.7]	20.0 [1.1-70.1]	20.0 [1.1-70.1]	60.0 [14.7-94.7]
TE	33.3 [0.0-100.0]	33.3 [0.0-100.0]	33.3 [0.0-100.0]	50.0 [29.9-70.1]	36.4 [18.0-57.5]	13.6 [3.9-31.7]	80.0 [29.9-98.9]	20.0 [1.1-70.1]	0.0 [0.0 - 43.4]
CN	33.3 [0.0-100.0]	0.0 [0.0-0.0]	66.7 [0.0-100.0]	31.8 [14.7-53.0]	31.8 [14.7-53.0]	36.4 [18.0-57.5]	20.0 [1.1-70.1]	20.0 [1.1-70.1]	60.0 [14.7-94.7]
CIP	66.7 [0.0-100.0]	0.0 [0.0-0.0]	33.3 [0.0-100.0]	54.5 [33.7-74.2]	36.4 [18.0-57.5]	9.1 [1.1-29.2]	60.0 [14.7-94.7]	20.0 [1.1-70.1]	20.0 [1.1-70.1]
DA	66.7 [0.0-100.0]	0.0 [0.0-0.0]	33.3 [0.0-100.0]	81.8 [59.7-94.8]	13.6 [3.9-31.7]	4.5 [0.1-22.8]	100.0 [56.6-100.0]	0.0 [0.0-43.4]	0.0 [0.0-43.4]
E	33.3 [0.0-100.0]	33.3 [0.0-100.0]	33.3 [0.0-100.0]	22.7 [8.6-42.8]	68.2 [45.1-86.1]	9.1 [1.1-29.2]	40.0 [5.3-85.3]	40.0 [5.3-85.3]	20.0 [1.1-70.1]

***R**: Resistant; **I**: Intermediate Susceptible**; S**: Susceptible; 95% Confidence Interval: **95% CI**; penicillin G **(P)**; trimethoprim/sulfamethoxazole (**SXT);** tetracycline (**TE)**; gentamicin (**CN);** ciprofloxacin (**CIP**); clindamycin (**DA);** erythromycin (**E**)

Based on Fisher's Exact test, significant correlations were found between biofilm formation and resistance to penicillin ($p = 2.82 \times 10^{-10}$), gentamicin ($p = 0.004$), clindamycin ($p = 3.56 \times 10^{-7}$), and erythromycin ($p = 0.00066$). However, no statistically significant differences were observed for trimethoprim/sulfamethoxazole, tetracycline, and ciprofloxacin ($p > 0.05$). Compared to the study by Pajohesh et al. (2022), which found significant correlations between strong biofilm formation and resistance to penicillin G, ampicillin, oxacillin, and gentamicin, the current study similarly identified significant associations with penicillin and gentamicin. However, unlike their findings, significant correlations with clindamycin and erythromycin were not observed. Despite these differences, both studies found no statistically significant relationship between biofilm formation and certain antibiotics ($p > 0.05$).

The results show that CPS species are often found in milk and dairy products. Their ability to form biofilms makes them more resistant to various conditions, increasing the likelihood of cross-contamination and foodborne illnesses. Additionally, the study demonstrates a correlation between biofilm formation and resistance to multiple antibiotics, further highlighting the challenges of controlling these bacteria in food environments.

CONCLUSION

This study's results indicate that CPS contamination was present in 30% of the samples, with 27% exceeding the microbiological limits set by Turkish food regulations. High levels of MDR (80%) and biofilm formation potential (73.3%) were identified, highlighting significant food safety risks. The development of antibiotic resistance and biofilm-forming capacity in coagulase-positive staphylococci isolated from cheeses was phenotypically examined. However, the genetic resistance profile was not examined, which is the study's limitations. To reveal the spread of antimicrobial resistance among bacteria via genetic means in food sources, further studies should be conducted, including molecular analyses such as *mecA* and *ica* gene detection, to better understand resistance and biofilm formation mechanisms. It is thought that increasing such studies is important in emphasizing the importance of animal-based food-borne infections resistant to antimicrobial treatment in terms of human health and increasing awareness and precautions in ensuring food safety from farm to table. The presence of CPS, such as *S. aureus*, in the human microbiota suggests that food handlers play a crucial role in contamination

during cheese production. The high antibiotic resistance observed, particularly against penicillin, clindamycin, ciprofloxacin, and tetracycline, indicates the potential misuse of antibiotics in dairy farming. Furthermore, the high percentage of CPS's biofilm-formation ability increases its resistance to cleaning agents, posing a risk for cross-contamination throughout the dairy production chain. To reduce these risks, stricter hygiene controls should be enforced in dairy processing environments, emphasizing the need for improved sanitation protocols targeting biofilms. Using new cleaning technologies that focus on breaking down biofilms and studying the genetic factors that help biofilms form is crucial for creating better antimicrobial treatments and managing these harmful bacteria. Also, the findings support the necessity for antibiotic stewardship programs in dairy farms, which align with Türkiye's Antimicrobial Resistance Action Plan. By addressing these challenges through evidence-based interventions, regulatory adjustments, and consumer education, food safety can be significantly enhanced, reducing the public health risks associated with CPS contamination in dairy products.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declared no actual, potential, or perceived conflict of interest in this research article.

Author contribution

The authors contribute equally to the present study. All the authors verify that the text, figures, and tables are original. The authors read and approved the final manuscript.

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