

## Pathogenic potential of *Staphylococcus aureus* strains isolated from unpackaged ice cream samples sold in Istanbul

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### ABSTRACT

Ice cream is a widely consumed refreshing food, especially in the summer months, and it creates a suitable environment for microbial growth due to its high nutritional value, appropriate pH, and long storage time. This study aimed to evaluate the incidence of enterotoxigenic and methicillin-resistant *Staphylococcus aureus* in unpackaged ice cream samples sold in Istanbul, Turkey.

For that purpose, 101 unpackaged ice cream samples were collected and evaluated for coagulase activity, DNase activity, and methicillin resistance. *Staphylococcus aureus* is determined in 66 (65.3%) samples with a mean load of  $3.2 \pm 1.0$  log cfu/mL. 34.6% of samples exceeded the Turkish Food Codex (2009) limit, which ranged between  $10^2 - 10^3$  cfu/g. It was found that 61 (64.8%) of the *S. aureus* strains were coagulase positive, 21 (22.3%) were DNase positive, and 18 (19.1%) had methicillin resistance. The present study's findings revealed that unpackaged ice cream samples in Istanbul have high enterotoxin-producing potential with a high *S. aureus* load. The presence of Methicillin-resistant strains may represent a potential hazard to public health.

**Keywords:** Ice cream, *Staphylococcus aureus*, Coagulase, DNase, Methicillin resistance

## Introduction

Ice cream is a dairy product obtained by freezing pasteurized milk, cream, skimmed milk solids, sugar, emulsifier, stabilizer, colour, and flavouring mixtures (Fetsch et al., 2014). High nutritional value, nearly neutral pH, and extended storage conditions are convenient factors for microbial growth in ice cream (Gücükoğlu et al., 2013). The increased consumption of ice cream in summer can create a suitable environment for producing pathogens and mediate the transmission of pathogenic microorganisms in forming food-borne diseases (Zhang et al., 2022). Microbial contamination may occur due to insufficient pasteurization during the ice cream production process, the use of contaminated raw materials and additives, tools and equipment, and the lack of hygienic conditions during distribution (Salehian et al., 2013). Several studies pointed out that *Staphylococcus aureus* is among the most common microorganisms in ice cream (Zhang et al., 2022).

*Staphylococcus aureus* is a Gram-positive, coagulase-positive, facultative anaerobic bacterium and can tolerate 0-20% salt concentration at a temperature of 6-48 °C, pH 4-10 (Cretenet et al., 2011). Although it is a pathogenic microorganism, it is naturally found in humans' skin flora and nasal mucosa, the main factors in contamination are humans and milk obtained from human animals with mastitis (Al-Bahry et al., 2014). *Staphylococcus aureus* is considered the most common cause of food poisoning worldwide due to the potential of enterotoxigenic strains to produce staphylococcal enterotoxins in food. Enterotoxin-producing *S. aureus* strains also have lecithinase, coagulase, thermonuclease and DNase enzyme activities (Sergelidis & Angelidis, 2017). Food poisoning symptoms begin 2-5 hours after ingestion of the toxin, and the main symptoms are nausea, vomiting, abdominal pain and fatigue (Fetsch et al., 2014). The occurrence of *S. aureus* in foods above 10<sup>5</sup> cfu/g can present a potential risk to public health due to enterotoxin production that can cause food poisoning (Martin et al., 2016).

The type of strain, food composition, temperature, physical and chemical parameters and the presence of inhibitors play a role in the formation of toxins (İrfan & İşeri, 2004). Dairy products, including cheese, milk, and ice cream, are often contaminated by antibiotic-resistant and enterotoxigenic *S. aureus*. Zhang et al. (2022) found the rate of *Staphylococcus* enterotoxins in dairy products as 39.31% in a meta-analysis study. Besides, the pathogenicity of *S. aureus* strains could be related to the secretion of extracellular toxins and enzymes such as DNase and coagulase (Zhang et al., 2022). Gündoğan et al. found DNase activity in 94.5% of *S. aureus* in ice cream samples (Gündoğan et al., 2006). Hunt et

al. reported 49 of 63 coagulase-positive *S. aureus* in raw milk (Hunt et al., 2012).

Antibiotic resistance is a worldwide health problem (Lee et al., 2018). The development of antibiotic resistance in pathogenic microorganisms has been associated with the therapeutic overuse of antibiotics and their use as growth promoters in animal production (Samir et al., 2018). Methicillin-resistant *S. aureus* (MRSA) is an essential cause of hospital-acquired infections, which can be transmitted through the consumption and processing of unpasteurized dairy products; identifying MRSA strains in contaminated food is of great importance in public health. (Algammal et al., 2020). Several studies indicated the presence of Methicillin-resistant *S. aureus* strains in dairy products (Al-Ashmawy et al., 2016; Gücükoğlu et al., 2013; Güçlü et al., 2022; Perry et al., 2004; Zhang et al., 2022).

This study aimed to investigate the enterotoxigenic and methicillin-resistant potential of *Staphylococcus aureus* strains isolated from unpackaged ice cream samples sold in the Istanbul markets.

## Materials and Methods

In the study, 101 unpackaged ice cream samples, including milk (n=25), chocolate (n=23), lemon (n=18), caramel (n=17), strawberry (n=15), banana (n=2) and cherry (n=1) were obtained from different manufacturers in district of Istanbul where are Beykoz, Umraniye, Uskudar, Sariyer and Besiktas, between August-September 2015 with random sampling method. All samples were preserved in ice packs (4- 8 °C) and transported to the laboratory. The microbiological analyses began immediately upon arrival under aseptic conditions. Microbiological analyses were performed in the Istanbul Medipol University Food Microbiology Laboratory.

### Isolation and identification of *Staphylococcus aureus* strains

The isolation and analyses of *S. aureus* were performed by the methodology referred to by Normanno et al. (2005) with minor modifications. Ten grams of each ice cream sample were diluted with 90 mL physiological saline solution and homogenized with a stomacher (Interscience-Bag Mixer® 400) for 60 seconds. Further dilutions were also obtained from the homogenate. 0.1 mL of dilutions were inoculated onto sterile Baird Parker Agar (BPA, Fluka) medium containing egg-yolk tellurite (Merck) and incubated at 37 °C for 24-

48 hours under aerobic conditions. Black, shiny, convex colonies with clear and dull halos were considered typical of *S. aureus*, whereas colonies with black colour without zones were atypical of *S. aureus*. Typical and atypical colonies were selected and purified in Tryptic Soy Broth medium and stored at -20°C in media containing 30% glycerol. Gram stain, catalase, and mannitol fermentation tests were performed to identify the isolates (Normanno et al., 2005).

### Coagulase Test

The coagulase test was performed on typical and atypical *S. aureus* isolates. 0.5 mL of rabbit blood plasma with EDTA (diluted 1:5) and 0.1 mL of isolates placed into the sterile tube and incubated at 37 °C. The tubes were observed for 6 hours for clot formation. Isolates where clotting did not occur were incubated for an additional 24 hours. Clotted samples were evaluated as coagulase-positive *S. aureus* (Sperber & Tatini, 1975). *Staphylococcus aureus* ATCC 25923 strain was used as a positive control.

### DNase Test

A deoxyribonuclease (DNase) test was performed to confirm the isolates' pathogenicity potential. 24-hour active cultures were inoculated into DNase Test Agar (Merck) medium and incubated for 24-48 hours at 37°C under aerobic conditions. After incubation, 1 mL of 30% HCl solution was transferred onto the colonies. Clear zones around the bacterial colonies indicated DNase-positive colonies (Winn et al., 2006).

### Meticillin Resistance Test

In order to control the methicillin resistance properties of typical and atypical *S. aureus* isolates, 25-hour active cultures inoculated into Orsab (Oxoid) Agar medium and incubated at 37°C for 24-48 hours. Following incubation, the isolates that produced blue colonies were evaluated as methicillin-resistant (Perry et al., 2004).

## Results and Discussion

In the present study, out of 101 ice cream samples, the incidence rate of *S. aureus* was 65.3% (n=66). and the load were ranged between <2 and 6.02 log cfu/g with a mean 3.2±1.0 log cfu/g. The prevalence of *S. aureus* strains in the examined ice cream samples, including milk, chocolate, lemon, caramel, strawberry, banana and cherry species, were represented in Table 1. According to the Turkish Food Codex Communiqué on Microbiological Criteria (28157), the maximum number of *S. aureus* in ice creams should not exceed 10<sup>2</sup> – 10<sup>3</sup> cfu/g. The latest communiqué on microbiological criteria for ice cream was published in 2011. According to the 2011 criteria, *Enterobacteriaceae*, *Salmonella* and *L. monocytogenes* bacteria species are expected to be detected in ice creams. However, there is no declared data for *S. aureus*; therefore, the data were obtained from 2009 criteria (Turkish Food Codex, 2011; Anonymous, 2009). As seen in Table 1, 65.3% of samples contain *S. aureus* strains, whereas 34.6% of samples (n=35) exceed the Turkish Food Codex limit.

**Table 1.** Prevalence of *S. aureus* in the ice cream samples.

Examined samples	Number of samples	Positive samples		Log cfu/g			Samples above Turkish Food Codex				
		No.	Proportion	Min	Max	Mean ± SD	no	%	Coagulase (%)	DNase (%)	Methicillin Resistance (%)
Ice cream, milk	25	19	0.76	<2	5.4	3.3±0.97	9	36.0	64.0	12.0	20.0
Ice cream, chocolate	23	19	0.82	<2	6.02	3.49±1.17	13	56.5	53.8	38.4	23.0
Ice cream, Lemon	18	8	0.44	<2	3.7	2.65±0.67	2	11.1	63.6	18.1	18.1
Ice cream Caramel	17	14	0.82	<2	5.5	3.25±1.19	8	47.0	68.4	21.0	5.2
Ice cream Strawberry	15	4	0.26	<2	4.6	3.4±0.85	2	13.3	71.4	14.2	42.8
Ice cream, banana	2	1	0.50	<2	3.51	2.75±1.06	1	50.0	100	0	0
Ice cream, cherry	1	1	1	2.3	2.3	2.3	0	0	100	0	0
Total	101	66	0.65	<2	6.02	3.2±1.0	35	34.6	64.8	22.3	19.1

The high occurrence of *S. aureus* strains in unpackaged ice cream samples may be related to inadequate pasteurization of milk, use of contaminated raw materials, additives, tools and equipment, ambient air, working personnel, packaging materials, and failure to provide appropriate conditions during distribution (Shaheen et al., 2018). The presence of *S. aureus* in ice cream is an essential issue for public health, and researchers worldwide from different countries have focused on this field. Gucukoglu et al. (2013) reported that 23% of ice creams contained *S. aureus* between  $2.0 \times 10^2$  and  $4.0 \times 10^3$  in Samsun province, Turkey (Gücüköğlü et al., 2013). Güçlü et al. investigated the microbiological quality of ice creams in Turkey during the COVID-19 pandemic. They found that the *S. aureus* load ranged between 0 -  $3.2 \times 10^3$ , and 5.3% of ice cream samples exceeded the upper limit according to the Turkish Food Codex limit. The results also concluded that despite COVID-19 hygiene rules, high contamination was observed in ice cream samples (Güçlü et al., 2022).

Similarly, Samir et al. (2018) determined that 22% of ice cream samples were positive for *S. aureus* in Qena City, Egypt (Samir et al., 2018). Rahimi et al. detected no strains of *S. aureus* in commercial ice cream, whereas 9.1% of traditional ice cream samples contained *S. aureus* in Iran (Rahimi, 2013). Consistent with our results, Ahmed et al. found a prevalence of *S. aureus* in ice cream, 74%, with a mean load of  $3.74 \pm 1.03 \log \text{ cfu/g}$  (Ahmed et al., 2019). The researchers of these studies indicated that the *S. aureus* loads of the ice creams may attributed to contamination of milk during milking, environmental contamination with animal wastes, unsanitary production and storage conditions, unpasteurized milk from the infected mammary glands of dairy animals (Ahmed et al., 2019; Gücüköğlü et al., 2013; Rahimi, 2013; Samir et al., 2018). Zhang et al. examined 240 ice cream samples in Shaanxi, China, and only 4.2% contained *S. aureus*; however, no sample exceeded the permissible limits of Chinese Standards. Zhang et al. also noted that, despite the low detecting rate of *S. aureus* (0.8%), its spreading potential in the food chain should not be ignored, especially regarding MRSA infection (Zhang et al., 2022). In addition to all these results, evaluating the *S. aureus* presence and load in terms of ice cream species and location is essential. Gucukoglu et al. indicated chocolate species had the lowest *S. aureus* load, whereas Güçlü et al. reported no *S. aureus* in fruit species. Like Güçlü et al., ice cream samples with lemon and cherry had the lowest *S. aureus* load among all samples, which can be attributed to the acidity of fruits. Also, no *S. aureus* was reported in commercial ice creams, whereas *S. aureus* presence was reported by Ahmed et al. (74%) in Egypt, Gucukoglu et al. (23%) in Turkey, Samir et al. (22%) in Egypt, Rahimi in Egypt (9.1%) and Zhang et al. in China (4.2%).

The high prevalence rate reported by Ahmed et al. in Egypt may attributed to handling operations and improper storage conditions. Although Zhang et al. stated low prevalence in China, MRSA strains may represent a health hazard due to pathogenicity potential (Ahmed et al., 2019; Gücüköğlü et al., 2013; Rahimi, 2013; Samir et al., 2018; Zhang et al., 2022).

This study isolated 36 typical and 58 atypical *S. aureus* strains from 66 (65.3%) ice cream samples. It was found that all (n=94) *S. aureus* isolates obtained from ice cream samples were Gram-positive, had typical staphylococcal (grape cluster) appearance in microscopic examination and also catalase positive.

In addition to these results, to evaluate the pathogenicity potential of *S. aureus* strains is important. DNase, coagulase and Methicillin resistance test results were also given in Table 1. The positive reactions for DNase and methicillin resistance tests were also given in Figure 1 and Figure 2, respectively.

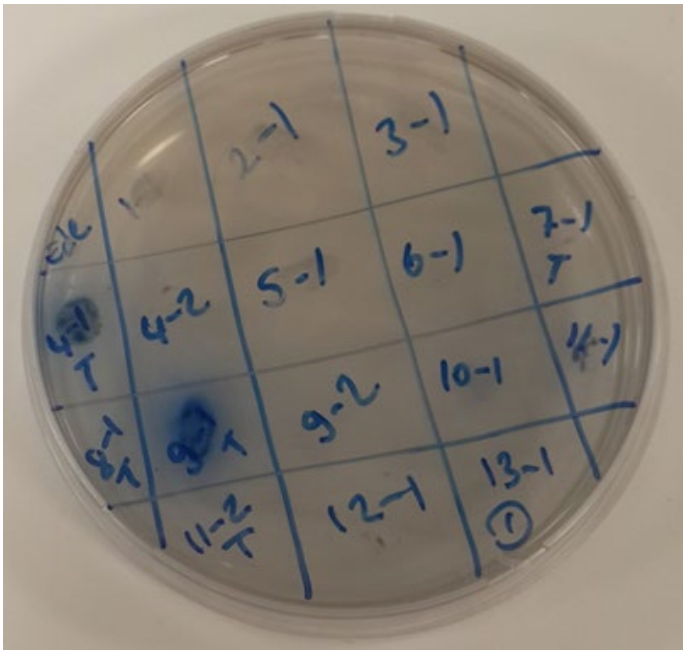
DNase measures the ability of microorganisms to synthesize the heat-stable DNase enzyme. The enzyme decomposes deoxyribonucleic acid (DNA) in the cell nucleus by depolymerizing it. DNase activity distinguishes pathogenic staphylococci from nonpathogenic resident flora members (Kateete et al., 2010). DNase activity was tested in isolated strains in the present study (Table 1) for that purpose.

DNase activity was shown in 22.3% (n=21) of *S. aureus* isolates. DNase-positive isolates may pose a threat to public health in terms of enterotoxin production potential. Meyrand et al. found an excellent correlation between DNase activity and enterotoxin production potential and suggested the test to confirm enterotoxin results (Meyrand et al., 1999). Bartolomeoli et al. also reported DNase activity in all *S. aureus* strains isolated from raw milk (Bartolomeoli et al., 2009). Besides DNase activity, coagulase-positive staphylococci strains also potentially produce enterotoxins (Bingöl & Toğay, 2017). It was seen that 64.8% of *S. aureus* isolates were coagulase-positive. Staphylococcal intoxication occurs after enterotoxigenic staphylococci reach at least  $10^5 \text{ cfu/g}$  (Veras et al., 2008). In this study, *S. aureus* growth over  $10^5 \text{ cfu/g}$  was observed in 5 samples, and these strains were coagulase-positive. Enterotoxins production risk of *S. aureus* is higher in these five samples than in all.

As presented in Table 1, the present study detected MRSA in 19.1% of *S. aureus* isolates in ice cream samples. Additionally, 4.25% of isolates were Methicillin-resistant, coagulase-positive and DNase-positive, which indicates the pathogenicity potential.



**Figure 1.** Figure 1. DNase test results in *S. aureus* isolates.



**Figure 2.** Methicillin resistance test results in *S. aureus* isolates.

Similar to our results, Ahmed et al. (Ahmed et al. 2019) also found MRSA in 15.4% of dairy products, whereas Al-Ashmawy et al. found 53%, which was relatively high (Al-Ashmawy et al., 2016). On the contrary, Zhang et al. detected

only 2 MRSA isolates from 240 ice cream samples. However, Zhang et al. indicated that even though the detection rate was low (%0.8), it can not be ignored because of its spread in the food chain (Zhang et al., 2022). Masihinejad et al. reported a significant correlation between the number of enterotoxin genes and antibiotic resistance in *S. aureus* isolates in creamy pastries. (Masihinejad et al., 2023). Since ice cream is not heated before consumption, MRSA can enter the digestive tract by ingesting contaminated foods and may colonize the digestive tract (Lee, 2003). MRSA also can complicate the treatment of *S. aureus* strains in foods and increase the risk of infection. Therefore, it is essential to apply food safety measures, hygiene practices, and appropriate food processing methods. Controlling the use of antibiotics can limit the occurrence of methicillin resistance (Lee et al., 2018). Antibiotic use regulation is essential in controlling pathogens given the human-animal-food environment. Suggested solutions to reduce the demand for antibiotics may include Antimicrobial Management Programs, determining the dose and status of inappropriate antibiotic use, reducing unnecessary antibiotic use, prescription, and developing new antibiotics (Findik, 2022). Food safety problems should be solved given the 'One Health' approach, which involves cooperation and collaboration among the many professional disciplines and organizations with the intersection of human, animal, and environmental health (Qian et al., 2023).

## Conclusion

In conclusion, the present study revealed a high presence of *S. aureus* strains isolated from different ice cream samples in Istanbul, Turkey. The presence of *S. aureus* in ice creams may attributed to insufficient pasteurization, contaminated equipment, poor personnel hygiene, or inappropriate storage conditions. Also, it was determined that some of the *S. aureus* isolates were coagulase positive and DNase positive, indicating the potential to produce enterotoxin and was Methicillin-resistant. MRSA-containing ice creams increase the risk of food poisoning and MRSA infections and may pose risks to the community. Regulating the use of antibiotics in a human-animal-food-environment context and establishing solutions based on the One Health perspective is required. These results represent health hazards for consumers and indicate the need for improved hygiene standards in the production and distribution process. It was concluded that the application of the Hazard Analysis and Critical Control Points (HACCP) system is essential in the ice cream production process.

### Compliance with Ethical Standards

**Conflict of interests:** The author(s) declares that for this article, they have no actual, potential, or perceived conflict of interest.

**Ethics committee approval:** Authors declare that this study includes no experiments with human or animal subjects. Ethics committee approval is not required for this study.

**Data availability:** Data will be made available on request.

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