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High Occurrence and Antimicrobial Resistance of *Staphylococcus aureus* Isolates from Unpacked Ice Creams

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ABSTRACT

Aim: The objective of this study was to determine the occurrence and antimicrobial resistance pattern of *Staphylococcus aureus* strains, as one of the important foodborne pathogens, isolated from unpacked ice creams.

Materials & Methods: A total of 122 unpacked ice cream samples were randomly collected from different localities in East Azerbaijan province and transferred to the laboratory using a cool box and screened for the presence of *S. aureus* strains. Also, the isolates resistance to antibiotics was determined by disk diffusion method.

Findings: In total, 21.3% of the ice creams samples were contaminated with *S. aureus* strains. Furthermore, antibiotic susceptibility testing revealed that the highest resistance was against penicillin and erythromycin, whereas the highest susceptibility was observed against gentamicin and rifampin. A warning issue was the significant resistance to vancomycin.

Conclusions: The relative high isolation and antimicrobial resistance rates detected in *S. aureus* strains isolated from unpacked ice creams underline the necessity for applying strict standards at all processing steps by food control agencies and emphasize the need for educational efforts for those personnel involved in products preparation procedures in order to promote food hygiene. It is worth noting that the emergence of resistance to vancomycin, as the last line of treatment for staphylococcal infections, is a worrying global health concern. Moreover, this study highlighted that poor adherence to personal hygiene and health principles during the food products preparation and/or storage could be a potential factor in the spread of pathogenic bacteria and resistance genes in the community.

Keywords: *Staphylococcus aureus*, Drug resistance, Ice cream, Foodborne diseases, Iran

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[1] Occurrence of Bacillus cereus and *Staphylococcus aureus* organisms... [2] Determination of microbiological contamination sources ... [3] Slime production, DNase activity and antibiotic resistance of... [4] *Staphylococcus aureus* food-poisoning outbreak... [5] Microbial contamination of traditional ... [6] Food animals and antimicrobials: Impacts on human health. Clin Microbiol Rev. [7] Clinical and Laboratory Standard Institute (CLSI)... [8] Food born disease control: A transnational challenge. Emerging Infect Dis. [9] Microbial quality of ice cream sold openly by retail outlets in Turkey. [10] Determination of enterotoxigenic and... [11] Isolation of Staphylococcus aureus... [12] Bacteriological and molecular studies on toxigenic Staphylococcus... [13] Microbial quality and chemical composition of... [14] Microbial quality and antimicrobial resistance of Staphylococcus aureus and Escherichia... [15] Microbiological quality of ice cream ... [16] Enterotoxigenic *Staphylococci* and their toxins in restaurant foods. Trends Food Sci Tech. [17] Prevalence of antibiotic resistant bacteria in milk sold in Accra. [18] Bacteriological quality analysis of ice cream produced by the small factories of Dhaka city. [19] Prevalence of enteric bacteria ...[20] Assessment of prevalence and antibiotic resistance of *Staphylococcus aureus* in raw ... [21] Evaluation of antimicrobial resistance ... [22] Prevalence and pattern of antibiotic sensitivity of methicillin-sensitive and methicillin-resistant... [23] Genetic characterization of methicillin resistant and sensitive, vancomycin intermediate *Staphylococcus aureus* strains isolated from different Iranian hospitals. [24] Vancomycin-resistant Staphylococcus aureus in the United States, 2002–2006. Clin Infect Dis. [25] Epidemiological survey of the first case of vancomycin-resistant *Staphylococcus aureus* infection in Europe. [26] Epidemiology of vancomycin and oxacillin resistant *Staphylococcus aureus* clinical isolates in Urmia. [27] Antimicrobial resistance of *Staphylococcus aureus* isolated from bovine, sheep, and goat raw milk. [28] Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethiopia. Ann Clin Microbiol Antimicrob.

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Introduction

Ice cream is a nutritious dairy product consumed by different age groups, particularly children, during summer time [1]. This frozen dairy product is a good medium for microbial growth, which can transfer infections and threat humans' health due to its nutrient content, neutral pH (6-7), and long-time storage [2]. *Staphylococcus aureus* is among the most common foodborne pathogenic bacteria due to its capability of producing heat-stable enterotoxins. *S. aureus* is considered as the commensal of skin and mucous membranes of animals and humans. Generally, about 20-30% of healthy people in the community carry *S. aureus* in their nasopharynx, which can cause infection if the opportunity is provided. Food poisoning caused by *S. aureus* is characterized with sudden onset of nausea, vomiting, abdominal cramps, diarrhea, and even hospitalization in immunocompromised individuals such as children, elderly, pregnant women, patients with cancer, and etc [3-4]. Ice cream may be contaminated with *S. aureus* through different ways; the risk of such contaminations is rather high in traditional ice creams due to the use of unpasteurized milk, flavors, and contaminated additives. On the other hand, since such products are distributed manually in scoops and sold in open containers with no hygienic packaging, the consequent probability of these foods contamination by pathogenic and spoilage bacteria increases [5]. In addition, increased bacterial resistance to different antibiotics is today a worrying global concern in health sectors and a great threat for public health. In addition to discriminative use of antibiotics for therapeutic purposes, non-therapeutic applications of antibiotics as an additive to the poultry and livestock food, not only indirectly transfer antibiotics to humans through the food chain but also are considered as another reason for the emergence of antibiotic-resistant bacteria [6]. Hence, paying attention to the food health promotion is of great importance in order to reduce the contamination and to inhibit the increasing trend of antibiotic-resistance in microorganisms.

Objectives: The current study aimed to evaluate the occurrence and antibiotic-resistance pattern of *S. aureus* strains isolated from Iranian traditional ice cream samples in East Azerbaijan province, Iran.

Materials and Methods

Sample collection: The current descriptive cross sectional study was conducted during spring and summer in 2017 in East Azerbaijan province, Iran. A total of 122 traditional ice cream samples were collected from different regions of the province and transferred to the microbiology laboratory of local food and drug organization and evaluated based on the following instructions compiled in this filed by Iranian National Standards Organization.

Microbiological analyses: A total of 10 g ice cream was diluted with 90 mL of Ringer serum; then a 10-mL of 1:10 dilution was inoculated into the selective enrichment Giolitti Cantoni broth (Merck, Germany) and incubated at 37°C for 24-48 hrs. Then a loopful of culture was streaked on a Baird Parker agar plate (Merck; Germany) and incubated at 37°C. The suspicious colonies appeared round, black, and glossy with a tiny clear halo were Gram stained. The results of biochemical tests such as positive catalase, positive coagulase, and the presence of yellow colonies with yellow zones on mannitol salt agar were considered to differentiate *S. aureus* strains from other *Staphylococci* species.

Antibiotic susceptibility testing: The antibiotic susceptibility testing was performed against gentamicin, erythromycin, penicillin, rifampin,

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tetracycline, and vancomycin for all the bacterial isolates using the standard disc diffusion method according to the Clinical & Laboratory Standards Institute (CLSI) guidelines [7]. Briefly, a standard inoculum of each isolate adjusted to 0.5 McFarland was swabbed on Muller-Hinton agar (Merck; Germany), then antibiotic discs were dispensed after drying the plate and incubated at 37°C for 24 hrs.

Reference strain: The reference strain of *S. aureus* ATCC 25923 was used for quality control.

Findings

In the current study, a total of 122 traditional ice cream samples were collected, of which 26 (21.3%) samples were identified to be contaminated with *S. aureus* strains. The isolates antimicrobial susceptibility testing results showed the highest resistance to penicillin (100%), followed by erythromycin (87.5%), and tetracycline (75%). Surprisingly, 12.5% of the *S. aureus* strains isolated in the current study were resistant to vancomycin. Furthermore, 100% of the isolates were fully susceptible to gentamicin and rifampin.

Discussion

Foodborne infectious diseases have been recently raised as a public health concern and as a significant cause of mortality due to the globalization of political economy in the late 20th century and the increased migrations and travels among different communities [8]. *S. aureus* is one of the most common causes of foodborne illnesses.

In addition, antibiotic resistance is one the most critical problems of World Health Organization (WHO) and considered as the main challenge of the current century, which severely threatens humans' lives. Discriminative use of antibiotics for therapeutic purposes along with non-therapeutic applications of them as growth factor and stimulus in livestock and poultry industries are considered as one of the main causes of increased antibiotic resistance and their transmission to humans through food chain[6].

The aforementioned issues highlighted the importance of evaluating microbial contamination of food products and the antibiotic resistance patterns of the isolated bacteria. The present study aimed to evaluate the occurrence of *S. aureus* strains, as the main cause of food poisoning, in traditional ice cream samples and to identify its antibiotic resistance pattern. The importance of the current study is doubled due to the high consumption of ice cream among children as the high-risk group, and due to the fact that such products are usually sold unpackaged at retail outlets with high bare-hand contact, increasing the risk of contamination and transferring antibiotic resistant bacteria through these products to humans [9].

The present study results revealed the high staphylococcal contamination levels of traditional ice creams in the area under study; however, limited studies have been conducted on bulk ice cream in some parts of the world. In a similar study carried out by Gujukoglu et al. (2013), the prevalence of *S. aureus* strains was reported 23% in traditional ice creams [10]. Gundogan et al. (2006), isolated *S. aureus* strains from 26.6% of traditional ice cream samples [3]. In a study by Samir et al. (2018) in Egypt, *S. aureus* strains were isolated from 22% of ice cream samples through conventional phenotypic methods and from 15% of samples according to the PCR technique [11]. Their result was in agreement with another study result in the same country [12]. In another study conducted by Shahbazi et al. [13], 50 traditional ice cream samples were tested, of which 22.5% were contaminated with *S. aureus* strains. The

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aforementioned studies results were in line with those of the current study. In the other studies by Ghadimi et al. (2017) [14], Ahmed et al. (2009) [15], and Anvarinejad et al. (2013) [5], the prevalence of *S. aureus* strains in traditional ice creams was reported as 50, 45, and 41.8%, respectively, indicating that contamination rates reported in these studies were higher than those reported in the current study. Generally, different studies results indicated the high prevalence of *S. aureus* strains in traditional ice creams. Since these bacteria are a part of normal flora in nasopharynx and nasal cavity of many people, they can be directly transferred to other people through contaminated skin injuries as well as coughing and sneezing [16]. Hence, poor adherence to personal hygiene principles by workers and lack of using gloves and masks during the production and selling procedures result in the transmission of these pathogens to humans. In addition, *S. aureus* is capable of transmitting through contaminated water, soil, air, and dusts [17]. On the other hand, milk and dairy products such as ice cream are nutritionally rich and ideal environments for the growth and proliferation of pathogenic bacteria, which may even cause spoilage in these products; therefore, the risks of contamination of dairy products provided from the milk of cows with mastitis by *S. aureus* strains and transferring the pathogens to humans are very high [18-19]. These results underline the necessity for applying strict standards at all processing steps by food control agencies and emphasize the need for educational efforts for those personnel involved in products preparation procedures in order to promote food hygiene.

All the bacteria isolated in the current study were resistant to penicillin; in addition, 87.5, 75, and 12.5% of the isolated strains were resistant to erythromycin, tetracycline, and vancomycin, respectively. Although the prevalence of antibiotics resistance evaluated in the current study was significantly problematic, the emergence of *Staphylococci* with moderate or complete resistance to vancomycin is an alarming and worrisome problem. Since vancomycin is among the few last-line antibiotics administered to treat Gram-positive infections, transmission of resistance genes from such resistant species to clinical ones may cause devastating health consequences. In addition, all the isolated *S. aureus* strains in the current study were sensitive to gentamicin and rifampin. In a study by Dehghani et al. (2016) on the prevalence and antibiotic-resistance pattern of *S. aureus* strains isolated from raw and pasteurized milk samples in Sari, Iran, all the isolated *S. aureus* strains were sensitive to vancomycin, gentamicin, and cotrimoxazole [20]. Also, another study by Gundogan et al. (2006), showed that all the isolated *S. aureus* strains were sensitive to vancomycin, while there was high resistance to penicillin and methicillin [3]. In a study by Ghadimi et al. (2017) on traditional ice cream samples, 82.5 and 45.6% of the isolated *S. aureus* strains were resistant to penicillin and erythromycin, respectively, while 79, 100, and 100% were sensitive to rifampin, gentamicin, and ciprofloxacin, respectively [14]. The aforementioned studies results were consistent with those of the current study; similar studies on clinical samples also reported similar results. A study by Ragbelti et al. (2016), indicated high sensitivity to gentamicin and rifampin, while resistant to penicillin was reported in 100% of the clinically isolated *S. aureus* strains [21]. In a study by Alborzi et al. (2000), all of the isolated strains were sensitive to vancomycin and rifampin [22]. In another study by Havaei et al. (2012), reduced sensitivity to vancomycin was reported in 97% of *S. aureus* clinical strains isolated from different patients [23]. Although

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the evaluations and comparisons showed a great consistency between the current study and previous studies results, but unlike the previous studies, the current research indicated the emergence of resistance to vancomycin in 12.5% of the isolated *S. aureus* strains. Vancomycin-resistant *S. aureus* was first reported in 2002 in the USA [24], followed by different studies from all around the world, including Iran, reporting the increase in the number of *S. aureus* strains or other Gram-positive bacteria with moderate to complete resistance to vancomycin [25, 26]. Therefore, since vancomycin is the last-line antibiotic administered for methicillin-resistant *S. aureus* associated infections, the isolation of *Staphylococci* with complete or even moderate resistance to this antibiotic is a serious alarm for probable failure in the treatment of *S. aureus* infections because in the case of outbreaks, this fact may be a great threat for global health. Another critical point in the current study was *S. aureus* strains high sensitivity to gentamicin and rifampin, which are among those antibiotics not commonly used or less used in veterinary for therapeutic and non-therapeutic purposes [27-28]; whereas a high resistance was observed against antibiotics such as penicillin, erythromycin, and tetracycline, which have high applications for therapeutic and non-therapeutic purposes in veterinary.

Conclusion

Evaluation of the current study and other similar studies findings suggest that the food chain is probability the main route of transferring such bacteria from the environment and animals to humans' gastrointestinal system. Unfortunately, despite the high consumption of antibiotics in veterinary, lack of adequate monitoring on the proper administration of such medicines in this sector as well as non-therapeutic use of them as growth stimulus resulted in the increased antibiotic resistance as a global dilemma. To overcome this dilemma, the cooperation of veterinary and health sectors seems necessary; in addition, ignoring the significant role of Iran Veterinary Organization and other organizations active in the food health have undesirable and irreversible outcomes on microbial resistance and finally on the public health. Hence, in order to promote public health, the contributing organizations should cooperate.

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