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Prevalence of *Staphylococcus aureus* in Traditional Ice Cream in Iran: A Systematic Review and Meta-analysis

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KEYWORDS

Staphylococcus aureus;
Traditional ice cream;
Prevalence;
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ABSTRACT: *Staphylococcus aureus* is known to be the third leading cause of food poisoning in the world. Traditional ice cream is one of the dairy products in Iran and some countries in the world that should be controlled for *Staphylococcus aureus* contamination. The purpose of this review and meta-analysis is to determine the contamination of traditional ice cream with *Staphylococcus aureus* in different regions of Iran. Search terms "*Staphylococcus aureus*", "traditional ice Cream", and "Iran" were used in Science Direct, Pubmed, Scopus, Google Scholar, and SID, Magiran Magazine until 2018 published without time limitation selected. To review the original articles and remove duplicate articles and review titles and abstracts, articles that have the required qualities for this research were studied in this study. Data were analyzed by the random effects model in STATA (version 11) and MedCalc (version 13) software. Publication bias and sensitivity analysis were assessed by funnel and influence plots. According to a sample size of 3811 from 35 articles, the prevalence of *Staphylococcus aureus is* estimated 32% in Iran (95% CI: 25-38%, $p<0.001$). The heterogeneity in the estimation of the pooled prevalence among the studies demonstrated; Cochran Q test: 1577.39, $p< 0.001$, I^2 = 97.84%. Based on Begg's adjusted rank correlation test, publication bias was statistically significant ($p <0.05$). Epidemiological data is useful to determine the distribution of *Staphylococcus aureus* contamination and provide a broad picture of the prevalence of traditional ice cream Iran and can be used as an important indicator in the model of risk assessment of microbiologic.

INTRODUCTION

Food safety is one of the main goals of public health in the world. Regarding the improvement of food safety, the prevalence and incidence of food poisoning caused by microbial, chemical, and toxin contamination in food are still common in many countries of the world [1-6]. Biological agents are one of the most important sources of contamination so about 70% of infectious diseases spread through unsafe food to humans and have imposed a heavy economic and health burden on human life. There are now more than 250 cases of diseases due to

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food being known and bacteria are the most important and most common cause of these diseases [7]. Based on the World Health Organization report, there is about 1.5 billion diarrhea in children under age 5, of which about 5 million people die [8]. In Iran, there are also about 70,000 children under age 5 annually due to diarrhea is often associated with foodborne pathogens them lose their lives [9]. During the last decade, the prevalence of food-borne diseases in developing countries with poor health and in developed countries has been increasing with a high standard of health, while infections and food poisoning are mainly not reported. For this reason, it is not possible to determine the exact number of infections, especially in developing countries. Food pathogens are a serious threat to public health in countries that can be prevented from taking into account the principles of health in various food processes [10]. Milk and dairy products are among the main groups in the food pyramid because of their high nutritional value and the size of the consumer's age group and the supply of many human nutrients, especially calcium and vitamin B2 [11-12]. Per capita, dairy consumption in Iran is 15-30 kg per year [13] and ice cream is one of the most important dairy products and is one of the milk substitutes in the diet, especially in children and adolescents, which is largely due to sweets and variation, flavor, and coolness, especially in warm months of the year, are in many people. According to the US per capita of ice cream consumption, it was 7.5 kilograms in 2000. Although there are no accurate statistics available in Iran, traditional ice cream has a high consumption [14]. Ice cream not only eliminates part of the nutritional needs but also neutralizes free radicals and prevents cancer and increases various microorganisms [15]. Considering the amount and general acceptance of ice cream, microbial safety is very important because microbial contamination of ice cream can lead to food poisoning, especially in children and other vulnerable people. Raw milk and dairy products are one of the most important ways of transmitting food poisoning [14].

Traditional foods have been introduced around the world. These food have special characteristics, which are different from other similar products of the same type due to the use of traditional materials (raw materials of the original products), the traditional composition, or the traditional type of production or process method [16]. There is a great concern about the microbial contamination of traditional food. Traditional ice cream is one of the most popular foods produced in hot seasons in Iran, and its production process differs from industrial ice cream [17]. Ice cream is a frozen dairy food composed of milk or cream, sugar, flavoring, and color agent. In Iran two different ways to make ice cream: the traditional method and industrial production. The traditional method points out the manufacturing of open ice cream and this method is generally manufactured in small-scale production units which don't follow completely a standard producer for the production of ice cream. Usually, pasteurization is not done due to a lack of facilities in the production of traditional ice cream [18-19]. In Iran materials such as raw milk, sugar, rose water, orchis, saffron, colorants, and flavors are used for the production of traditional ice cream. To produce this product, first, heat the milk slightly and then add it to the sugar and orchis mixture and then add the rose water, saffron, coloring matter, and flavoring and pour the mixture into the ice machine[20].

Ice cream is a good environment for the growth of microbial because of its high nutritional value, optimum pH, and long shelf life [9]. All ingredients in ice cream can contribute to the ingestion of microbes into the foodstuff and increase its microbial load [21] contamination of devices, equipment, and containers, the health status of production and sale, personal hygiene can all cause an increase in the microbial burden of ice cream [14, 17, 22]. Microbial contamination can occur in each stage of its production and maintenance and it can lead to poisoning [17]. One of the main contamination factors of traditional ice cream in Iran is the use of unpasteurized milk and its lack of adequate heating and lack of sanitary supervision in production, which is often done manually and is at high risk of contamination, which causes food poisoning due to present the microorganisms or toxins they produce [12, 23-24]. According to studies conducted in Iran, the most important bacteria that cause food poisoning are *E. coli*, *Staphylococcus aureus* (*S. aureus*), Salmonella, Listeria monocytogenes, and Campylobacter, which can be transmitted to humans through the milk and milk products and lead to zoonosis (diseases or infections

transmitted between the livestock and human) with a high death rate [25]. Food poisoning with *S. aureus* is a common cause of foodborne disease in the world [26]. Common contamination of dairy products contaminated milk from staphylococcal mastitis (Mastitis) and food they are produced manually and, in the case of non-compliance with the health principles, there is a potential for transmission of *S. aureus* bacteria and contamination of the final product [27-28].

S. aureus is a common and important pathogen associated with serious community and hospital diseases such as acute gastroenteritis, skin infections, infectious shock, septicemia, and pneumonia, and has long been considered a major public health problem [29-30]. This bacterium is scattered in nature, its primary source in the skin and mucous membranes of mammals and birds [31], and its place of residence is more in the skin, nose, and upper respiratory tract of humans and animals, and in water, dust, milk, food, sewage is also found and is one of the main sources of human food contamination [12, 32]. The presence of this bacterium in food and the consumption of its toxin in food causes symptoms of Staphylococcal food poisoning (such as nausea, abdominal cramps, diarrhea, and vomiting) for 1 to 6 hours after consuming contaminated food [27, 33-35]. *S. aureus* bacteria disappear at the pasteurized temperature, but the presence of heat-resistant enterotoxin in the foodstuff even at very low levels (20 ng to 1 µg) can cause signs of food poisoning, depending on the type of enterotoxin. If there are 105 bacteria per gram of nutrition, the bacteria will have the opportunity to grow and produce an enterotoxin, and even if the bacteria is lost in the heat, due to the resistance of enterotoxin to the heat, the active toxin remains and leads to The occurrence of food poisoning [26]. The existence of this bacterium in the foodstuff usually indicates contamination by bacteria in the skin, hands, mouth, and nose of people who deal with food or as a result of the entry of respiratory droplets through sneezing and coughing [36]. The bacterium is present in 20 to 30% of the human population is permanent and stable and 60% of people are alternate. Therefore, people who are involved in food processing and distribution centers can transfer bacteria to food in case of non-compliance with health issues [26]. It should also be noted that *S. aureus* is unable to compete with natural flora in raw foods, and contamination of food with this bacteria is related to inappropriate methods for the production of cooked and processed foods, and then maintenance under conditions that allow bacteria to grow and produce enterotoxin [27]. Outbreaks of *S. aureus* enterotoxin it is always a serious threat to dairy products, and frequent reports of contamination of these products with this bacterium clearly show the importance of this pathogen [37]. Among various foods, traditional ice creams are due to the high percentage of milk falsification and because of their production processes in places and shops of the city, mainly in manual and machine are at high risk of infection with this bacterium [38].

MATERIALS AND METHODS

Study Selection

This research is a systematic review of the prevalence of *S. aureus* in traditional ice cream in Iran, which is carried out through a review of articles and a meta-analysis of available resources. Review documents are limited to internet searches that have been in foreign and internal journals in the Scopus, Pubmed, Google Scholar, Science Direct, SID, Iran medex, Irandoc, Magiran databases, regardless of the time limit until the end of 2019. Articles were searched using the systematic method and valid keywords in both Persian and Latin languages**.** Because some internal databases, to search operators(OR, AND, NOT) sensitivity did not show, only by keyword Search Inside "*Staphylococcus aureus*" was to be found with high sensitivity. But to search the international sites, the keywords "*Staphylococcus aureus*" and the equivalent of "*S.aureus*", "Traditional ice cream" and "Prevalence" were used. The keywords in Mesh are standardized and eventually searched. In addition, the list of selected references, to find the relevant studies was screened.

Selection criteria and quality assessment

First of all, articles related to the topic are reviewed by two researchers individually in the relevant database with related keywords and check out reviewed titles, abstracts, and full texts for defining articles that adhere to entry criteria. After completing the search, a list of abstract articles was prepared by the researchers. First, the titles

and abstracts of all the articles searched in the databases are selected based on their full relevance to the subject matter. Subsequently, the abstracts of the articles were reviewed for appropriate studies. If the two scholars did not agree on the selection of the article, the judgment would be vested in the third person. Articles reported in English or Persian language were considered with English abstracts were indexed in the relevant databases and the criteria for entering the phrase were from 1) Use of standard methods culture methods, antibody-based results (ELISA), and molecular techniques) to detect *S. aureus*. 2) Present existing data on the prevalence of *S. aureus*. 3) Food Samples considered. The criteria for identifying Iranian writers were either the author or the place of work, as well as the authors' affiliation. In addition, a study conducted by non-Iranian writers on populations or Iranian samples also entered the study. Also, studies that did not use standard methods were excluded, repeated reports, and articles whose samples were taken from environmental sources or sample sources were not known, articles written in Farsi with Persian abbreviation and did not identify *S. aureus* bacteria. Qualified studies qualifications were independently judged by two authors according to the Joanna Briggs Institute or STROBE. Finally, studies with quality of over 60% were included in this study.

Data extraction

Subsequently, the full text of the articles was examined. In the next step, all articles were evaluated using the STROBE checklist. By reviewing the text of the remaining articles, the articles that were completely related to the purpose of this study were selected and then a checklist of the information necessary for the research (authors' names, area of the study, the year of the study, the experimental method used, the sample size, the number of the positive samples infected and the prevalence of *S. aureus* bacteria for all studies that were the primary evaluation)**.** The final assessment was prepared. The final checklist was reviewed by the researchers and finally the articles related to the title of the research were entered into the STATA software and the meta-analysis process. In the first stage of the search, 91 articles were found, based on entry and exit criteria, finally, 35 articles entered the final stage.

Statistical analysis

Considering that the main index of study in the current study was prevalence, its variance was calculated by binomial distribution and 95% confidence interval for the ratio was calculated. The weighted average was used to combine the prevalence of different studies. The study was weighted proportional to the inverse variance. The homogeneity of the groups was estimated using Cochran's Chi-square test. Funnel Chart, Begg Rank Correlation Test, and Egger Weighted regression test was used to estimate the bias distribution ($p < 0.05$ represents the bias distribution). Heterogeneous potential sources were evaluated using sensitivity analysis, regression, and subgroup analysis based on the location of the study and diagnostic methods. Sensitivity analysis was conducted to determine whether the removal of any study had a significant effect on the overall prevalence rate. Due to the high difference in the prevalence rates in different studies (heterogeneity of studies) and the significance of the I^2 heterogeneity index, the random effects method was used in the meta-analysis. To estimate the overall meta-analysis prevalence, STATA 11.0 (STATA Corp, College Station, TX) software was used and P-value less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Figure 1 demonstrates the study selection process of the literature search**.** In a primary screening process, 91 studies were identified. At last, 35 cross-sectional studies were included in the final meta-analysis based on the exclusion/inclusion criteria, among which 23 had reported in the Persian language.

All of the included studies had published between 1970 and 2019. The total sample size is 3811 subjects. *Jazayreri et al*. (2000) reported the highest prevalence of *S. aureus* in Tehran province with 85% and *Hazhir et al.* (2005) reported the lowest prevalence in Kurdistan province with 1.7%. Characteristics of included studies in the systematic review and meta-analysis are presented in Table 1.

Based on the random effect results, the pooled estimation of the prevalence of *S. aureus* bacteria in Iranian traditional cheese was 0.32 (95% C.I: 0.25- 0.38). The heterogeneity in estimating the pooled prevalence among

the studies was shown; Cochran Q test: 1577.39, p-value< 0.001, I^2 = 97.84% (Figure 2 & Table 2). Based on Egger's regression and Begg's adjusted rank correlation tests, publication bias was statistically significant (*p-value<0.001*, *p-value=0.010*, respectively) (Figure 3).

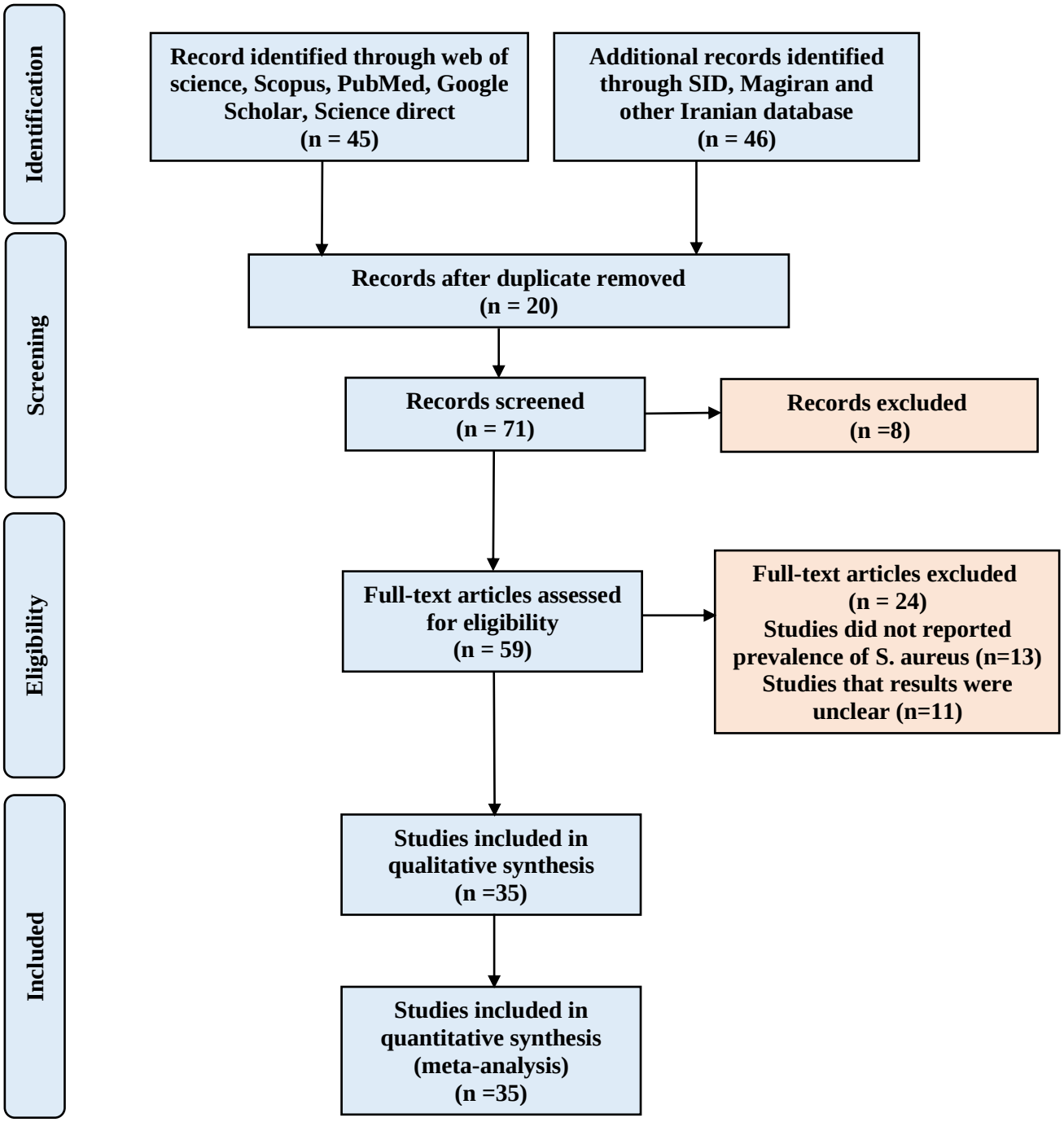

Figure 1. Flowchart for literature search and study selection

Table 1. Characteristics of included studies in the meta-analysis

ID study	First author	Publication year	Location	Sample size	Positive *S. aureus*	References
1	Malaki.M	1970	Tehran	297	30	[39]
2	Abolghasem Jazayeri	2000	Tehran	70	60	[40]
3	Mahmoud Jamshidian	2001	Ahvaz	100	61	[41]
4	Aziz allah Poormahmoodi	2002	Yasuj	70	20	[42]
5	Mohammad Reza Shadan	2003	Zahedan	250	16	[22]
6	Hossein Mokhtarian	2004	Mashhad	100	67	[43]
7	Laleh Hoveyda	2004	Tehran	100	74	[44]
8	Amir Shakerian	2005	Shahr-e-Kord	200	114	[20]
9	Mohammad Saleh Hazhir	2006	Kurdistan	170	3	[12]
10	Seyed Shahram Shekarforoush	2006	Shiraz	70	26	[45]
11	Mohammad Mahdi Soltan Dallal	2008	Tehran	241	41	[46]
12	Alisha Akya	2008	kermanshah	80	23	[36]
13	Ali Jazayery Moghadas	2008	Semnan	136	53	[47]
14	Abolfazl Naeimabadi	2008	Bojnourd	21	17	[48]
15	Hossein Mokhtarian	2009	Gonabad	100	4	[49]
16	Mahdi Sadeghi	2009	Gorgan	25	14	[50]
17	Maryam Ekhtelat	2009	Ahvaz	120	38	[51]
18	Mohammad Rezaei	2009	Arak	70	35	[24]
19	Arasb Dabbagh Moghaddam	2010	Rasht	120	7	[52]
20	Ebrahim Rahimi	2010	Isfahan,Chaharmahal va Bakhtyari,and Khuzestan provinces	55	5	[30]
21	Leili Mohammadi	2010-2011	Zahedan	188	82	[19]
22	P Azadnia	2011	Khormoj	50	10	[18]
23	Behnaz Haeri Behbahani	2011	Tehran	125	5	[14]
24	Hasan Hasanzadazar	2011	Urmia	144	4	[53]
25	Hamid Mirzaei	2012	Maragheh	99	41	[54]
26	Maryam Salehian	2013	Sari	50	14	[55]
27	Mahvash Anvarinejad	2013	Maragheh	99	41	[٥٤]
28	Nasser Panahi	2014	Urmia	100	16	[56]
29	Sima Karimzadeh Sadegh	2015	Urmia	40	18	[57]
30	Seyed Sajjad Khoramrooz	2015	Yasuj	30	2	[9]
31	Mohadeseh Abolhasannezhad	2015	Birjand	96	27	[58]
32	Ashraf Kariminik	2015	Rafsanjan	40	2	[59]
33	Yasser Shahbazi	2015	Kermanshah	120	27	[٦٠]
34	Ali Heshmati	2016	Hamedan	114	57	[61]
35	Ramin Abri	2017	East azerbaijan	122	26	[62]

Table 2. Status of *S. aureus* prevalence among included studies in Iran

Status	No. of studies	Pooled Prevalence	95% CI			Test for heterogeneity	
			Lower	Upper	P-value	I^2	P-value
Random	35	0.276	0.191	0.369	<0.001	97.84	<0.001

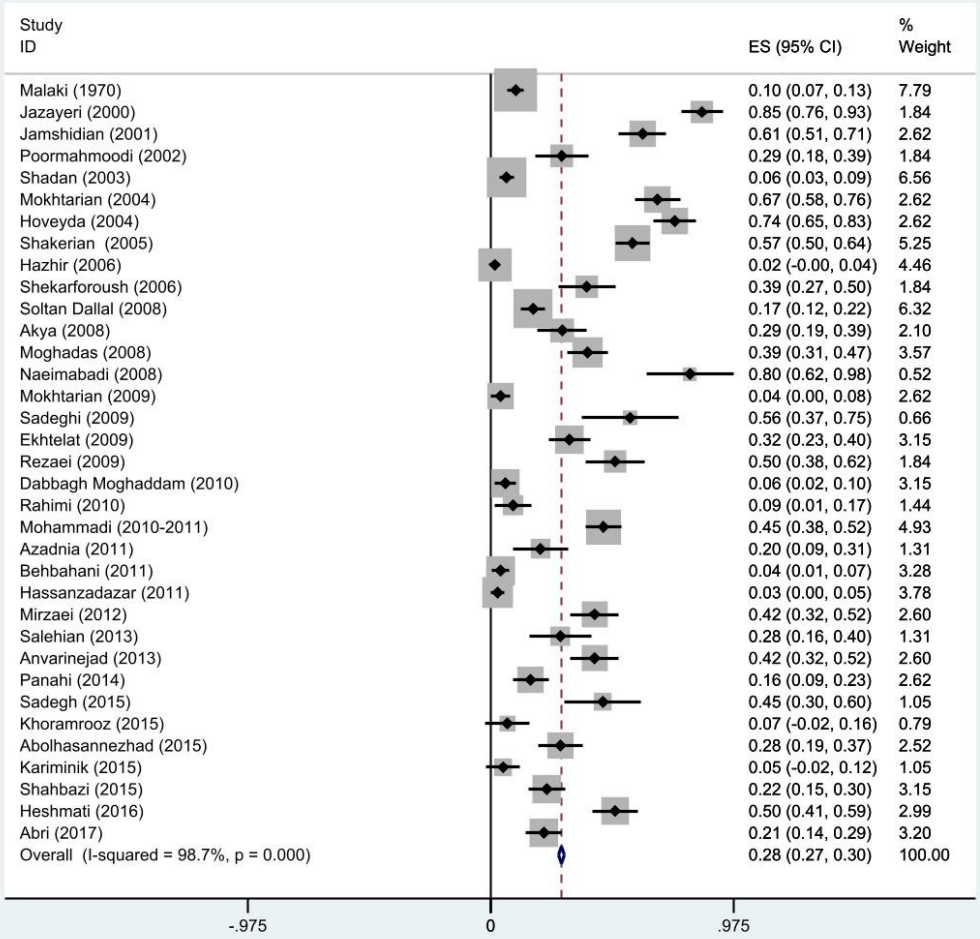

Figure 2. Forest plot of meta-analysis for pooled prevalence with 95% C.I.

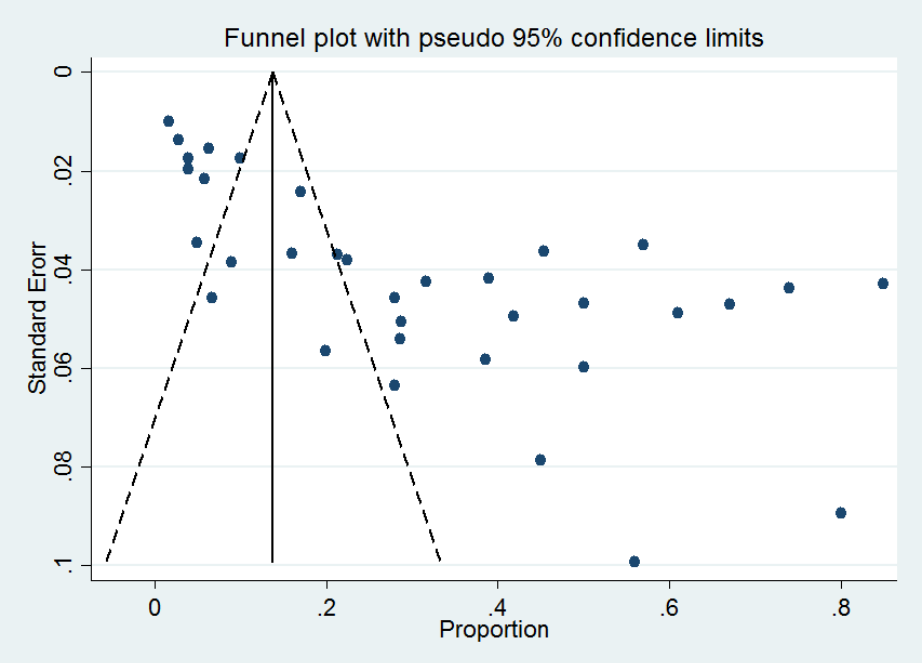

Figure 3. Assessing publication bias by Begg's funnel plot with pseudo 95% C.I.

The high occurrence of *S.aureus* in traditional dairy products could be due to a contaminated environment with infected animal wastes or the methods of producing and storing unsanitary materials [30]. Traditional ice cream is not applied due to the high percentage of corruption of milk due to the lack of pasteurization and the suitability of milk heating during the production of this product to maintain its desired taste [12, 63]. Also, the presence of *S. aureus* in ice cream can be caused by poor hygienic measures workers [61]. This microorganism is naturally found on the hands, nasal cavity, and human skin [61]. Since many ice creams are often consumed by children and are also popular among adults and make food poisoning, the study of the microbial status of this product is necessary and very useful [12].

In the present study, from 35 collected articles without a time limit up to 2019, the prevalence of *S. aureus* in Iranian traditional ice cream has been reported in 32% of Iran. Ice cream is one of the most important causes of food poisoning, especially in children, and one of the most important sanitary indicators is contamination with *S. aureus* and *Escherichia coli* [47]. In a study in Tehran by Jazayeri and colleagues in 2000 highest prevalence, 85% reported that the reason may be a lack of sanitation services, physical space, and food warehouse [40]. In another study by Hazhir et al., in Kurdistan, the lowest prevalence of *S.aureus* in traditional ice cream was reported at 1.7 % in 2005, probably due to observance of hygiene standards during production and distribution [12]. Microbial quality and antibiotic resistance of *S. aureus* and *Escherichia coli* were isolated from traditional ice cream in Hamedan in 2016 and reported the prevalence of *S. aureus* in traditional ice cream at 50%, it may be due to poor hygiene practices of handlers [61]. So ice cream production processes in places and shops in the city for manual and machines depending on the production environment, personal health workers, equipment, and transportation are at high risk of contamination [58, 53]. The possible source of s*taphylococcus* in the dairy product may be contaminated equipment or water, non-sanitary storage conditions, infectious discharges, and nasal discharge of people involved with production [59]. On the other hand, the use of unpasteurized milk for traditional ice cream production is one of the main microbial contaminants. Also suitable thermal processing was not applied during the manufacturing of these products to maintain their desirable taste [61]. The primary components used in the preparation of ice cream, including sugar, orchis, and vanilla are effective in microbial contamination entry [55]. Various studies have been conducted on the prevalence of *S. aureus* worldwide. In a 2004 study in Turkey, 15% of samples of *Staphylococcus spp*. were not conforming to the TFC standard. The presence of *S. aureus* may have resulted from insufficient pasteurization of milk, or human exposure [21]. In another study in Egypt in 2009, the prevalence of 50% have been reported [64] and another study in Turkey found that the primary mixture of ice cream had the lowest amount of microbial contamination and the highest amount of sold ice cream microbial contamination to all microbial groups, and prevalence rate of *S.aureus* 55% and both indicating secondary contamination and poor health of workers [17]. In another study in Egypt on packaged ice cream and streets without packaging in 2016, the prevalence of this bacterium is 28% in non-packaged ice cream and probably due to its presence in the open air and dust [65]. In another study in Egypt on traditional ice cream with different flavors carried out in 2018, the incidence of *S. aureus* has been reported to be 22% may be S.aureus present on the skin, eyes, throat, and intestinal tract. From these sources, droplets containing microbe might be produced during coughing, talking, and sneezing, the organism finds its way into air and dust, onto clothing, and in other places from which it may settle on ice cream [61, 63]. Despite the presence of reports of *S. aureus* of 22% in samples taken in Kathmandu, 38% in Libya and 12.2% in Vietnam, and 36% in Pakistan, some studies have shown that not separating this bacterium from samples of traditional ice cream probably due to hygiene during production [24]. Although the results of some of Iran's internal studies are consistent together and with those of other studies in other countries but the difference in the prevalence of *S.aureus* in traditional ice cream appears to be due to the observance rate of hygiene standards during production and distribution. Also, this bacterium is not a good competitor for other bacteria and is not able to grow well when other bacteria

are present. Therefore, the reasons mentioned may justify the inconsistency of the results obtained in different studies in Iran's internal and other countries [42]. By surveying the results obtained and the results of similar studies in Iran and elsewhere in the world, regardless of the different amounts of contaminated reports, it can be seen that the non-hygiene of traditional ice creams and the non-conformity of their microbial properties with existing standards [51]. Since this bacteria is primarily contaminated by contaminated milk and by secondary contact with hands and oral secretions [20]. Also, by confirming the increased likelihood of various microbial contamination in ice cream and transferring to consumers, more attention is paid to the quality of hygiene that depends on the pasteurization or the application of adequate heat to the initial mixture of ice cream and the observance of health standards during the production stages [51]. Among the critical points in the process of production of traditional ice cream are the receive, storage, transportation of raw materials, especially milk, people in contact with the product, as well as temperature and production time is of particular importance. The control of these critical points (CCP) requires compliance with the principles (GMP) based on critical control (pasteurization temperature, production time, and product pH) and compliance with milking hygiene, transportation, and personal hygiene of the personnel.The fulfillment of the above-mentioned requirements is based on the knowledge and training of milk producers and distributors, especially food safety staff, regarding the identification of pollution points, and the application of appropriate and hygienic technology related to the proper production and maintenance of this product [24]. In this study, we have tried all the papers related to the prevalence of *S. aureus* in traditional ice cream that will be included in Iran. In this regard, the results of the study were summarized using meta-analysis. According to the research, no systematic review and meta-analysis have been done on this subject. And as a restriction for the current research, gathering information from all parts of the country was not available.

CONCLUSIONS

The results of this study show that the prevalence of *Staphylococcus aureus* is 33% in Iranian traditional ice cream according to studies up to 2018. The prevalence of *S. aureus* in Iran may be comparable with similar countries in the world. In addition, according to the traditional ice cream contamination by *S. aureus* in Iran, sanitary conditions have during the process and the use of pasteurized milk in the ice cream production process to be monitored**.** The presence of this bacterium in traditional ice cream may pose a potential risk, such as illness in the consumer, especially in vulnerable groups such as children, so effective information on the risk of using traditional ice cream is imperative. Good hygiene practices should improve the hygienic quality of ice cream, especially in all steps, after pasteurization, and at the retail level. An increased level of staff training and knowledge transfer, especially concerning food safety, handling, maintenance, and machine cleaning and equipment may improve the situation. Further improvements need to be researched in personnel hygiene and the general hygiene conditions of premises. According to the Hazard Analysis Critical Control Point (HACCP), the mandatory adoption of a food safety management system is needed to improve the quality of traditional ice cream . The quality of raw materials must be prioritized before processing, producing, and storing products in appropriate conditions, and also, to prevent infections and toxins caused by pathogenic microorganisms, ice-cream workers and sellers should be checked regularly to maintain good health. The results of the current study provide broad epidemiological data about pollution, the distribution overview of the prevalence *S. aureus* among Iran's traditional ice cream**,** and can be used as an important input in the modeling of microbiological risk assessment**.** The control of bacteria in food by the Ministry of Health or other relevant organizations seems necessary.

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Conflicts of interest

The authors declare no conflict of interest.

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