

Prevalence and Antibiotic resistance pattern of *Staphylococcus aureus* strains isolated from traditional cheese and pasteurized cheese at Maragheh city of Iran

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Abstract

Introduction: Milk and dairy products are a good food environment for bacteria to grow and can quickly become infected with a variety of bacteria and cause disease after being transmitted to humans. *Staphylococcus aureus* is one of the microbial agents in milk and dairy products that causes disease in humans. Due to the fact that traditional (unpasteurized) cheese is produced in Iran under unsanitary conditions, the aim of this study was to investigate the contamination of traditional cheeses and pasteurized cheeses against *staphylococcus aureus* and determining their antibiotic resistance pattern in Maragheh city.

Methods: In this cross-sectional study, which was performed from 80 samples (40 samples of traditional cheese and 40 samples of pasteurized cheese) from different centres selling dairy products in Maragheh city.

Results: The results are as follows: out of 40 samples of traditional cheese, 24 samples (62%), showed infection with *Staphylococcus aureus*, and out of 40 samples of pasteurized cheese, no sample showed infection with *Staphylococcus aureus*. Also, in the study of antibiogram results, methicillin resistance was reported in 4 samples, (16.64%) of tetracycline, Oxacillin and Co-trimoxazole in one sample (4.16%). Also, all isolated strains of *Staphylococcus aureus* were completely sensitive to the antibiotics Rifampin, clindamycin, Vancomycin, Tobramycin and Gentamicin.

Conclusion: Due to the high level of contamination of traditional cheeses consumed in this region with *Staphylococcus aureus* bacteria, their production and distribution should be under the control of relevant experts.

Keywords: *Staphylococcus aureus*, traditional Cheese, Antibiotic resistance, Antibiogram

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Introduction

Every year, hundreds of thousands of people suffer from food poisoning. One of the most important causes of food poisoning is *Staphylococcus aureus* ¹. Food poisoning occurs as a result of eating food containing heat-resistant enterotoxins, which are usually produced by *Staphylococcus aureus*. Enterotoxins exist in all kinds of food such as raw meat, vegetables, dairy products, etc., and the amount of bacteria required to cause food poisoning caused by enterotoxins is reported to be 10⁵-10⁸ bacteria. Although *Staphylococcus aureus* can be destroyed by pasteurization, the enterotoxins of this bacterium are resistant to pasteurization temperatures. On

the other hand, the thermal resistance of these toxins inside food is more than in laboratory culture environments. Therefore, after the heat stages of food preparation, they still maintain their biological activity and cause food poisoning ²⁻³. Despite the popularity of traditional cheeses to industrial cheeses, the important issue in the production of this dairy product is the use of raw milk, and due to contamination of milk during the production stages and after milking, there is a possibility of contamination of cheeses produced from this milk with various pathogenic microbes ⁴. *Staphylococcus aureus* is considered as the second or sometimes the third most

important cause of foodborne diseases ⁵. The genus *Staphylococcus* belongs to the Micrococcaceae family. Members of this genus are gram-positive, non-motile, spore-free, aerobic and facultative anaerobic ⁶. Due to the ease of growth, this bacterium can be isolated from various foods, including milk and dairy products and foods that require long manipulations ⁷. The survival of this microorganism in different types of cheese and the poisoning caused by their consumption have been well documented. People who are active in preparing food will transfer bacteria to food if they do not comply with health issues, and if there are 10^5 bacteria in each gram of food, the bacteria will have the opportunity to grow and produce enterotoxin ⁸. Due to the emergence of antibiotic-resistant strains in *Staphylococcus aureus* bacteria, the number of antibiotics available to treat these infections is decreasing every day. So that some strains have even shown resistance to a large number of antimicrobial compounds, including antibiotics and disinfectants ⁹. Considering the important role of dairy products in the food cycle of families and the use of traditional dairy products by villagers and their reluctance to use pasteurized dairy products, this research aims to investigate the level of contamination of traditional cheeses and pasteurized cheeses with *Staphylococcus aureus* and also to determine the pattern of sensitivity and antibiotic resistance was done.

Materials and Methods

The current research was cross-sectional, during which 80 samples (40 samples of traditional cheese and 40 samples of pasteurized cheese) were randomly collected from the distribution centers of traditional and pasteurized dairy products in Maragheh city and were examined for the presence of *Staphylococcus aureus*. In this way, 50g of each sample was taken and poured into a sterile container with a screw cap, and then the characteristics of the sample, such as the place of production, the date of sampling, were recorded on each of the containers, and it was transferred to the microbiology laboratory in a minimum time while maintaining the cold chain. First, to prepare the initial suspension of cheese, 5g of each sample was immersed in 25ml of sterile Ringer's serum and placed in a fixed place for 15 min. Then the sterile plastic containers containing the samples were placed on the Rotamix machine so that the samples form a complete suspension with Ringer's serum within 5 min at a speed of 200 rpm. Next to the flame, a portion of the solution was taken from each sample with a sterile ring and cultured inside the nutrient agar plates. After examining the colonies in nutrient agar plates with a sterile loop next to the flame, a separate colony was taken from each colony

and cultured in Baird-Parker agar, then the plates were kept in an incubator at 37°C for 24 h. For identification, gram staining, catalase test, coagulase test and mannitol fermentation in mannitol salt agar medium were used according to the National Standard of Iran (ISIRI) No. 1194.

After identifying the isolates, the pattern of antibiotic sensitivity and resistance to 10 antibiotics include Rifampin (5 mg), Clindamycin (2 mg), Vancomycin (30 mg), Oxacillin (1 mg), Tobramycin (10 mg), Ciprofloxacin (5 mg), Gentamicin (10 mg), Tetracycline (30 mg), Co-trimoxazole (25 mg) and Methicillin (5 mg) were determined by disc diffusion method on Mueller-Hinton agar culture medium (Table 1).

Table 1. Antibiotics that was used as disc diffusion.

Antibiotics	Abbreviation	Concentration(mg)
Rifampin	RA	5
Clindamycin	CC	2
Vancomycin	V	30
Oxacillin	OXA	1
Tobramycin	TOB	10
Ciprofloxacin	CP	5
Gentamicin	GM	10
Tetracycline	TE	30
Co-trimoxazole	STX	25
Methicillin	ME	5

Results

The results showed that after the end of incubation, out of 40 samples of pasteurized cheese, no type of colony grew in the culture medium. It was concluded that there was no case of *Staphylococcus aureus* bacteria in the sample of pasteurized cheeses studied and the contamination level of this cheese was reported as zero percent in this research. Out of 40 samples of traditional cheese, 9 samples showed no growth and 31 samples showed growth in Brad Parker Agar culture medium. After performing biochemical and confirmatory tests, 24 samples were found to be infected with *Staphylococcus aureus*. The results showed that 24 samples out of 40 traditional cheese samples were *Staphylococcus aureus* and the amount of these cheeses was reported as 62%.

To check and determine the antibiotic sensitivity of the studied strains, it was done according to the instructions

of the Clinical and Laboratory Standards Institute (CLSI). The results of the antibiogram test in this study showed that the isolated strains of *Staphylococcus aureus* in this study were completely sensitive to the antibiotics Rifampin, Clindamycin, Vancomycin, Tobramycin and Gentamicin. Only one of the isolated strains of

Staphylococcus aureus showed resistance to Tetracycline and Oxacillin antibiotics, (4.16%). The highest level of resistance reported in this study was related to the antibiotic Methicillin with four strains. Interstitial resistance were related to two antibiotics include Ciprofloxacin and Tetracycline ([Table 2](#)).

Table 2. Antibiogram test on *Staphylococcus aureus* strains isolated from traditional cheese.

Antibiotics	Sensitive		resistance		Interstitial resistance	
	number of samples	Percent	number of samples	Percent	number of samples	Percent
Rifampin	24	100				
Clindamycin	24	100				
Vancomycin	24	100				
Oxacillin	23	95.84	1	4/16		
Tobramycin	24	100				
Ciprofloxacin	23	95.84			1	4/16
Gentamicin	24	100				
Tetracycline	22	91.68	1	4/16	1	4/16
Co-trimoxazole	23	95.84	1	4/16		
Methicillin	20	83.36	4	16.64		

Discussion

The dairy industry has a major contribution to added value and employment in the agricultural sector. After meat and fruits, milk and its products rank third with 11.8% in terms of share of Iranian expenses ¹⁰. Despite the advances made in medical science and food technology in recent years, foodborne diseases are still considered as a major problem for human health. The increase in diseases and poisoning caused by food, along with the resulting economic and social problems has led to the expansion of various studies in the field of healthy food production. According to the United States Center for Disease Control (CDC), foodborne diseases caused by *Staphylococcus aureus* affect about 80 million people in this country every year, of which 325,000 people are hospitalized and 5,000 people die. According to our study of 40 samples of traditional cheese prepared from the distribution centers of traditional and pasteurized dairy products in Maragheh city of Iran, 62% contamination with *Staphylococcus aureus* was observed, which is consistent with high contamination. In a study conducted in Brazil by André et

al. in 2007, the results obtained from the samples of traditional cheeses collected showed 66.7% contamination with *Staphylococcus aureus* bacteria ¹¹. In a study conducted by Mollah Abbaszadeh et al. in Khoi city in 2011, the contamination level of *Staphylococcus aureus* bacteria in traditional cheeses of Khoi city of Iran was reported as 53.75% ¹² which was consistent with the result of our study. In another study conducted by Shokrian et al, regarding the contamination of traditional sheep cheeses with *Staphylococcus aureus* bacteria, they reported the contamination rate to be 57% in Shahrekord city ¹³. In a study conducted by Shekarforosh et al, they showed the contamination rate to be 36.6% ¹⁴. Due to the small number of samples, it seems that the level of contamination has also decreased. In a research conducted by Nikniaz and his colleagues in 2005 entitled the frequency of *Staphylococcus aureus* contamination of local cheeses in Tabriz city, they reported 68% contamination rate of Tabriz local cheeses with *Staphylococcus aureus* bacteria ¹⁵ which is consistent with

the results obtained from our study. In a study conducted by Giammanco et al in 2011, entitled the contamination of traditional Primosalo cheese with *Staphylococcus aureus* bacteria, the contamination rate of this cheese was reported as 4% ¹⁶. Considering the resistance of this bacteria and high salt concentrations, this low level of contamination compared to the present study can be due to the difference in health conditions in the production and supply stages of this type of cheese. In a study conducted by Arab Amiri et al, in Shahrood city in 2016 on dairy samples, they reported 48% contamination of traditional cheeses with *Staphylococcus aureus* bacteria ¹⁷. Aragon-ALego et al, in 2007 while analyzing 170 food samples including milk, cheese, ice cream, yogurt in the city of Rotucit, Brazil, reported that the contamination rate of the tested foods with *Staphylococcus aureus* bacteria was 15.1% ¹⁸. In our study, this rate was reported as 62%, which is acceptable considering the variety of samples in the above research. In a study conducted by Jorgensen et al, in Norway in 2005, the contamination rate of *Staphylococcus aureus* in cow's milk was 75%, goat's milk was 92.2%, and other raw dairy products were 37.8% ¹⁹ which was somewhat similar to the result of our study. In the study of Zakary et al, regarding the contamination of cheese, ice cream and raw milk with *Staphylococcus aureus* bacteria, they reported the contamination rate to be 40% ²⁰. The result of this study was the average contamination of different samples and the result of our study was specific to cheese. In a study conducted by Sadeghi Fard et al, in Ilam city in 2014 entitled of contamination of cheese with *Staphylococcus aureus* bacteria, they observed that 48.4% of the samples were contaminated ²¹. The result of this study is almost the same as the result of the present study. Since *Staphylococcus aureus* bacteria enter cheese due to non-observance of hygiene principles, it seems that one of the main reasons for contamination of traditional cheese with *Staphylococcus aureus* is the contamination of raw milk used in different stages of cheese making or contamination due to animals infected with *staphylococcus*. Also, of the 40 samples of pasteurized cheese tested in this research, zero percent contamination was reported. In the study of Dehghani et al. in 2013 regarding the contamination of pasteurized cheeses with *Staphylococcus aureus* bacteria, they showed that the contamination rate of pasteurized cheeses in Sari city is zero percent ²². In the present study, the contamination rate of pasteurized cheeses was reported as 0%. The results of the antibiotic sensitivity investigation in this research showed that the isolated strains of *Staphylococcus aureus* were completely sensitive to the antibiotics Rifampin, Clindamycin, Vancomycin,

Tobramycin and Gentamicin. Only one of the isolated strains of *Staphylococcus aureus* showed resistance to tetracycline and Oxacillin antibiotics, (4.16%), and the highest level of resistance reported in this study was related to the antibiotic methicillin, which four of the isolated bacterial strains *Staphylococcus aureus* showed resistance to this antibiotic. Interstitial resistance, two cases of antibiotics, i.e. ciprofloxacin and tetracycline antibiotics, each showed an interstitial resistance to the isolated strains. In a study conducted by Sultan Delal et al. in 2017 on Tehran food products, the rate of resistance to tetracycline was 26%, ciprofloxacin was 3%, clindamycin was 3%, Tobramycin was 0%, Vancomycin was 0% and Rifampin was 0%, Oxacillin was 3%, methyl Silin is also reported as three percent. In our study, the rate of resistance to Tobramycin, Vancomycin and Rifampin antibiotics was 0% and the rate of Oxacillin resistance was 4.4% ²³ which is almost compatible with the intended study. In another study conducted by Sultan Delal et al. in 2008, titled the isolation of methicillin-resistant *Staphylococcus aureus* strains in Tehran food, they reported that the rate of resistance to methicillin was 2%, clindamycin was 4%, ciprofloxacin was 1.6%, and tetracycline was 22%. 4.4% showed resistance ²⁴. This study is almost consistent with the result of our study that the rate of tetracycline resistance is 4.16. In a study conducted by Mirzaei et al. in 2015, the rate of resistance to ciprofloxacin was 0%, Gentamicin was 0%, and Co-trimoxazole was 3.85% reported²⁵. In our study, the rate of resistance to the antibiotic Co-trimoxazole was reported as 4.16, and the rate of resistance to Gentamicin was reported as 0%. In 2007, Normanne et al. tested 1634 cheese samples, including 641 samples of dairy products and 993 samples of meat products, and reported that most of the samples were infected with *Staphylococcus aureus* and isolated strains of *Staphylococcus aureus* resistant to methicillin ² which is consistent with our study. In a research conducted by Dehghani et al. in Sari city in 2013 on cheese samples, they reported the resistance of isolated strains of *Staphylococcus aureus* to ampicillin, methicillin and cephalothin as 87.5%, 25% and 12.5% respectively. In this study, the lowest level of resistance related to Vancomycin, Gentamicin and Co-trimoxazole antibiotics was observed ²². The rate of resistance to methicillin in our study was 16.64%, which is not much different from this result, and in our study, the rate of resistance to Vancomycin and Gentamicin was not reported at all. In a study conducted by Gündoğan et al. in 2006, *Staphylococcus aureus* strains were highly resistant to penicillin, methicillin, and bacitracin and highly sensitive to Vancomycin and ciprofloxacin ²⁶, which is in agreement with the result of our study of high resistance

to Methicillin. And the high sensitivity to Vancomycin is completely consistent. In a study in Iran conducted by Hoveida et al. in 2004, pathogenic bacteria were isolated from 100 non-pasteurized ice cream samples in 87% of cases and from 300 pasteurized ice cream samples in 3% of cases. The most isolated bacteria in non-pasteurized ice creams were *Staphylococcus aureus* and *E. coli* ²⁷.

Conclusion

The result of the present study showed that no contamination with *Staphylococcus aureus* bacteria was reported in the pasteurized cheeses of the study area, and the rate of contamination of the traditional cheeses of the study area with *Staphylococcus aureus* bacteria was reported to be 62%, which indicates the high contamination of the traditional cheeses of the Maragheh region. The bacterium was *Staphylococcus aureus*.

Highlights

What Is Already Known?

Antibiotic resistance in traditional dairy products has been studied in various parts of the world.

What Does This Study Add?

Antibiotic resistance in traditional dairy products was studied for the first time in the city of Maragheh, Iran.

Authors' Contributions

A.S, Z.H and M.A.S developed the theoretical formalism, performed the analytic calculations, and performed the numerical simulations. The authors contributed to the final version of the manuscript. Z.H : supervised the project.

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The extent of AI use

Not applicable.

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