Original Article

Prevalence of *Staphylococcus aureus* in Raw Hamburgers from Kashan in 2017

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Abstract

Aims: *Staphylococcus aureus* is considered a major cause of food poisoning in the world. We aimed to investigate the prevalence of *S. aureus* isolated from hamburgers in Kashan city. **Materials and Methods:** In this study, 71 hamburger samples were collected from supermarkets in Kashan from March to July 2017. The samples were examined after dilution in the Baird–Parker Agar Base medium of inoculation for the bacterial growth of *S. aureus*. Gram staining, catalase tests, mannitol fermentation, coagulase, DNase, and sensitivity to novobiocin were used to detect the bacteria. **Results:** The collected hamburgers were about eight types of products. Of the total samples, 15 (21.13%) were Gram-positive bacteria contaminated, including seven cocci and eight bacilli. Seven samples (9.86%) of hamburgers were contaminated with *S. aureus*. The average counts of *S. aureus* in positive samples were 1.94×10^3 colony-forming unit (CFU)/g. **Conclusion:** The outbreak of *S. aureus* isolates in meat products, particularly hamburger, is of serious threat to public health. To prevent the outbreak of this pathogen in hamburgers, permanent control and monitoring should be performed in food industries.

Keywords: Coagulase, foodborne diseases, public health, *Staphylococcus aureus*

Introduction

Staphylococcus aureus is a Gram-positive and sphere-shaped bacterium. Some strains of this microorganism have pathogenesis capable of causing multiple human and animal diseases which include hospital infections, pneumonia, and food poisoning.[1,2] Bacterial contamination can occur in both humans and animals. It is colonized in several areas of the body including the skin, mouth, and nasal mucus membranes.[3] Carrier animals have an important role in spreading the bacteria in food products. Furthermore, contamination of animals with this pathogen is considered a public health concern. Through contaminated animal products, the bacteria possess the ability to spread in the human population; thus, it is capable of animal-to-human transmission.[4] *S. aureus* has the ability to survive well in several environmental conditions, such as high salt content and low temperature. This pathogen produces enormous amount of toxin during proliferation.[5] The produced toxins are released into the surrounding environment. If the environment around the bacteria is food, it leads to food poisoning in humans.[6] Currently, meat products are the sources and supply of protein for humans.

Hamburger is produced from meat and poultry. In recent decades, hamburger has been a popular product because of its high nutritional value, cost–effectiveness, and ease of preparation. However, despite the benefits of meat products, food poisoning has been extensively reported.[7]

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S. aureus is a pathogen related to food poisoning. Now, this bacterium is a common cause of food poisoning in the world. Annually, 241,000 foodborne diseases caused by the bacteria are reported in the United States.[8] A study conducted in China in 2018 showed that *S. aureus* is present in 35% of the meat products.[9] In another study conducted in West Africa, this contamination rate was reported to be 10%.[10] When meat products are contaminated of animal origin, or during processing by carrier staff, there is a potential for bacteria to grow and produce toxin.[11] On the other hand, in hot climate areas, suitable conditions for the growth of *S. aureus* are present in these products, which can lead to food poisoning.[12] Epidemiological studies are necessary for preventing foodborne *S. aureus* infections.

However, few studies have investigated the association between *S. aureus* contamination and hamburgers. Therefore, we conducted a study to determine the prevalence of *S. aureus* isolated from hamburgers in Kashan.

Materials and Methods

Ethical approval

Ethical approval was obtained from the ethical committee of Kashan University of Medical Sciences (code number: IR.KAUMS.REC.1395.68).

Samples

This is a cross-sectional descriptive study. Here, 71 cases of hamburgers were randomly collected from supermarkets in Kashan from March to July 2017. All the samples were kept in sterile conditions by ice and were immediately transferred to the bacteriology laboratory of Kashan University of Medical Sciences (at 2 h).

Isolation and count bacteria

Initially, 10 g of hamburger was added to 90 ml of phosphate-buffered saline (Zist Mavad Pharmed Tehran, Iran). After making it homogeneous, 1 ml of the suspension was inoculated into Baird–Parker Agar medium (Merck KGaA, Germany). For colony count, tenfold serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) of the suspension were prepared in a sterile medium; each one of these diverse dilutions was then used as inoculum for culture. Inoculation environments were incubated with suspension at 37°C for 24–48 h. After this period of time, the number of grown bacterial colony was counted.[13]

Identification of *Staphylococcus aureus*

For detecting *S. aureus*, Gram staining was used. The positive Gram stain bacteria that were spherical and clustered were selected, and the catalase diagnostic test was performed. Bacteria with catalase positivity were cultured on the Mannitol Salt Agar (MSA) medium (Merck KGaA, Germany), and we found a change in the color of the medium from red to yellow. Yellow pigmented bacterial colonies were selected. These colonies were analyzed for novobiocin susceptibility (Mast Battle, UK), DNase (Merck KGaA, Germany), and Coagulase tube tests.[13, 14] The standard strain of *S. aureus* ATCC 25923 was used as positive control.

Statistical analysis

The correlation between contaminated hamburgers and the diverse brands of samples was analyzed using Chi-square test. Statistical analysis was performed by SPSS software version 19 (IBM NY, USA).

Results

Samples

In 71 collected samples, the hamburgers were produced in eight types by five brands. The brands were anonymously selected X1–X5 to avoid any bias in this work. The number of hamburgers evaluated based on the percentage of meat is listed in Table 1. These products were contaminated with bacteria including seven samples of 60% hamburgers (20%), three samples of 85% hamburgers (25%), and five samples of 90% hamburgers (21%).

Isolation and count bacteria

Overall, out of the 71 specimens, 15 samples of hamburger (21.13%) were contaminated with bacteria, and the other samples (78.87%) did not have any bacterial contamination. All the isolated bacteria were Gram positive.

The overall total bacterial colony counts for seven hamburger samples were between 3×10^2 and 4×10^3 CFU/g, with an average of 1.94×10^3 CFU/g. The details are listed in Table 1.

Identification of *Staphylococcus aureus*

Out of the 15 isolated bacteria, 7 were cocci and 8 were bacilli. The cocci were found black and shiny colonies measuring 1–1.5 mm in diameter, but the bacilli appeared as brown colonies. All the isolates of cocci were catalase positive. These bacteria were cultured in the MSA and their medium changed from red to yellow. All of these isolates were DNase positive, coagulase positive, and also susceptible to novobiocin; therefore, they were detected as *S. aureus*. In other words, out of a total of 71 hamburger samples, 9.86% of them were contaminated with *S. aureus*.

Discussion

In this study, the prevalence of hamburger contamination to

Table 1: Type of hamburgers, prevalence, and mean total counts of *Staphylococcus aureus*

Type of hamburger (percentage of meat)	*n* (%)	Prevalence of *Staphylococcus aureus* (*n*)	Mean bacterial count (CFU/g)
A 60	35 (49.3)	3	3×10^2
B 85	12 (16.9)	3	1.5×10^3
C 90	24 (33.8)	1	4×10^3

CFU: Colony-forming unit

S. aureus was 9.86%. The most common way of transmitting *S. aureus* in the humans is eating meat contaminated with this pathogen; therefore, the consumption of beef, especially in products such as hamburgers which are mostly not well cooked, facilitates transfer of pathogens and causes increased infection rates in the human. In recent years, the ready-to-eat meal consumption, particularly meat products, has grown considerably. According to research reported by Irikura *et al*., Americans consume around 5 billion hamburgers a year.[15] In the last decade, many parts of the world are reported to have had transmission of microbial pathogens by food, showing prevalence rates of its bacterial contamination in varying patterns.[16] The foodborne illness in most developed countries is lower when compared to other countries. Studies showed that the average percentage of raw meat contamination in the world is 29.2%. The highest contamination in Asian countries was 44.6%, and in American and African countries, it was 28.1% and 13.6%, respectively.[17] The least amount of foodborne illness is related to the European countries. It is due to more using of guide food safety policy.[18]

It is possible to estimate the incidence rate of foodborne diseases in different countries according to the reports. In the study of Wu *et al*. in China conducted from 2011 to 2016 on meat and its products, the amount of contamination of these products of *S. aureus* was reported to be 35%.[9] In a study done by Zehra *et al*. in India in 2018, 21.81% of samples were found to be contaminated with bacteria.[19] In a study conducted in the USA, 27.9% of the meat cases were contaminated with the pathogen.[20] In a study performed in Iraq, the pathogen's contamination was reported as 54.2%.[21] In another study conducted in Nigeria on meat samples, one-third of the samples were contaminated with diverse *Staphylococcus* species, of which 9.7% of the total samples have been exposed to *S. aureus*.[22] In various studies, the amount of bacterial infection has been studied, but only in one study, hamburger contamination has been reported to be caused by *S. aureus*. In a study conducted by Shahraz *et al*. (2012) in Tehran, samples of hamburger were collected and explored for these microbes. The results of this study showed that 25% of the samples were contaminated with the pathogen.[13] Comparing the results of this study with those of our study, it can be said that the amount of pollution in the hamburger samples has been two times more than that of our study. The reasons for this difference can be due to different factors. The multiplicity of retail stores at the city level can be a reason for infections. Another cause of high infection rate can be the source material of food product as it has been shown in a study where 68% of the source material was contaminated by *S. aureus*.[23,24] However, during the processing of hamburger, various measures have been taken to reduce microbial load, but in the absence of adequate monitoring and proper control measures, foodborne diseases can emerge. The leaven in the hamburger can cause infection due to multiple processes of transportation. One way to reduce or eliminate microbes in foods is to keep them in −20°C (refrigerator temperature).[25] However, during the study, it has been shown that although this condition does not reduce the number of *S. aureus* in hamburgers, it can inhibit bacterial proliferation and toxin production.[26] In general, food prepared from meat should not be stored at temperatures ranging from 5°C to 60°C because microorganisms multiply quickly in this range. Therefore, the best way to prevent the growth of microbial and toxin production in food is to keep it under 4°C or below in refrigerators.[27,28]

Conclusion

There are very few studies on the current hamburger contamination by *S. aureus* in Iran. Nevertheless, contamination of meat and other product by microorganisms such as *S. aureus* can lead to serious threats to public health. Hence, it is necessary to reduce and eliminate this bacterium in food products. This could lead to a reduction in the foodborne illness. For inhibition of outbreak of these microorganisms in hamburger, constant control and monitoring need to be performed in food industries. Major meat product contamination occurs during handling, processing, and distribution of food. Therefore, it can be used to promote personal health care. Hence, strict control and monitoring programs are suggested which reduce the risk for transfer of animal-associated *S. aureus* to humans.

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Conflicts of interest

There are no conflicts of interest.

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