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Prevalence of *Staphylococcus aureus* Contamination and Detection of Enterotoxin A Gene in Raw Barbecue Chicken Meat Sold in Babol City, Iran

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Abstract The application *Staphylococcus aureus*, producing enterotoxin, is recognized as the most common cause of staphylococcal food poisoning, leading to gastroenteritis, diarrhea, and vomiting. The presence of enterotoxin-producing genes in this bacterium can be the main reason for these symptoms. The aim of this study was to determine the prevalence of *Staphylococcus aureus* contamination and detection of enterotoxin A gene in raw barbecue chicken meat sold in Babol city, Iran. After collecting 60 samples of raw *barbecue chicken* meat, the samples were examined for the presence of *Staphylococcus aureus* using standard culture techniques. Following DNA extraction, PCR tests were conducted using specific primers to identify the sea gene. According to the results obtained, *Staphylococcus aureus* was isolated from 12 samples (20%). The highest contamination rates were found in chicken shops (31%), barbecue shops (22%), and protein shops (21%). Additionally, PCR analysis revealed that the sea gene was not present in any of the isolates. *Staphylococcus aureus* can easily cause outbreaks of staphylococcal food poisoning by contaminating raw chicken barbecue. Therefore, implementing quality control standards and emphasizing food safety among the public is essential, as is investigating the prevalence of enterotoxin-producing genes *in Staphylococcus aureus* isolates from raw barbecue chicken meat.

Introduction

Foodborne diseases are considered a significant public health hazard worldwide and are one of the major causes of morbidity and mortality. Despite scientific and industrial advancements, even developed countries continue to suffer from its economic, health, and even political repercussions (1). Numerous bacteria are recognized as pathogens responsible for diarrhea, vomiting, and food poisoning, with the most notable being *Escherichia coli,* Shigella spp., Salmonella spp., *Campylobacter jejuni, Staphylococcus aureus,* and *Bacillus cereus* (2). Barbecue chicken meat is a food rich in minerals, fats, proteins, and vitamins, and its consumption is increasing not only in Iran but also in many other countries. However, there has yet to be a documented study on the contamination status of this food product with *Staphylococcus aureus* (2).

According to previous studies conducted, the PCR method can be used as a suitable, safe, and rapid method for detecting *Staphylococcus aureus* and its virulence factors in barbecue chicken meat samples (2). On the other hand, the production process under unsanitary conditions and the lack of hygiene maintained by poultry slaughterhouse workers and vendors can be the primary cause of barbecue chicken meat contamination.

Given the high consumption of ready-to-eat protein products, including chicken barbecue, in the region and the potential for high microbial load, the significance of the foodborne nature of *Staphylococcus aureus* and its high prevalence in food poisoning cases is evident. Furthermore, considering the lack of microbiological, epidemiological, and health studies regarding *Staphylococcus aureus* in barbecue chicken meat s sold in Babol city, this research aims to study the detection of enterotoxin-producing *Staphylococcus aureus* in barbecue chicken meat samples offered in Babol city, Mazandaran, Iran.

Materials and method

Sample collection and preparation

A total of 60 barbecue chicken meat samples were collected randomly from restaurants and kitchens, protein and chicken shops, and supermarkets throughout the city of Babol, Mazandaran, Iran. It was determined based on practical considerations, including time constraints, financial limitations, and laboratory facilities. The collected samples were immediately transported to the food microbiology laboratory in a hygienic manner and under sterile conditions. Upon arrival at the laboratory, based on standard laboratory protocols and previous similar studies, 10 grams of the raw barbecue chicken meat sample were measured and separated using a sterile scalpel next to a flame, with the help of a digital scale. At first, the sample was homogenized, next, thoroughly mixed with 90 mL of physiological saline solution (prepared by dissolving 4.5 g of NaCl in 500 mL of distilled water) in an Erlenmeyer flask and incubated at 37°C for 24 hours.

Microbial culture and bacterial confirmation

To isolate *Staphylococcus aureus*, an enrichment culture was first performed. After completing the enrichment steps, a 50 µL sample was taken using a sampler and inoculated onto Baird-Parker agar plates enriched with potassium tellurite supplement and egg yolk, following the manufacturer's instructions. The inoculation was performed in a linear streak method across four quadrants using a loop, and the plates were incubated at 37°C for 24 hours, this method facilitates the separation of colonies so that suspicious colonies can be easily identified and further tested. After the incubation period, the plates were examined for suspicious colonies with characteristics such as black coloration, a diameter of 1-2 mm, and a smooth, dome-shaped, shiny halo. Suspicious colonies were then confirmed by further diagnostic examinations with catalase test, gram staining culture on mannitol salt agar, and coagulase test (3, 4).

Gene isolation

From the identified coagulase-positive *Staphylococcus aureus* samples, a small amount of bacterial colony, cultured on mannitol salt agar, was inoculated in a linear streak method across four quadrants on mueller hinton agar and incubated for 24 h at a temperature of 35-37 °C. From the cultured bacterial colonies, a microbial suspension equivalent to a mcfarland turbidity of 0.5 was prepared in 1.5 mL microtubes and transferred to the designated laboratory for PCR testing. To confirm the presence of the enterotoxin A gene in *Staphylococcus aureus* isolates from raw barbecue chicken meats, a PCR test was conducted. The bacterial genome was extracted using the Sinnaclon kit's (50T-EX6071) protocol, and the purity of the extracted product was verified at a wavelength of 260 nanometers using a spectrophotometer (Shimadzu, Japan), optical density equivalent to

1.6 to 1.8 nm. PCR was performed to identify the A gene using specific primer oligonucleotide sequences. The PCR reaction was carried out in a total volume of 25 µL, which included 200 mMol of dNTPs, 10 pMol of primers, 1.5 mMol per liter of magnesium chloride, 0.5 units of Taq enzyme, and 50 ng of template genome. Specific primer oligonucleotide sequence, 5'-ATTAACCGAAGGTTCTGT-3, to identify the A gene, length of fragment, 270, and gene encoding virulence factor, reverse primer for sea.

The thermal cycle included an initial denaturation step of 4 minutes at 94 °C, followed by 30 cycles consisting of a denaturation step for 60 seconds at 94 °C, an annealing step for 45 seconds at 50 °C, and an extension step for 45 seconds at 72 °C. Finally, there was a single cycle of 10 minutes at 72 °C. The PCR products were analyzed for the presence of the target genes by performing electrophoresis on a 1% agarose gel, followed by staining with ethidium bromide (5).

Statistical analysis

The study data were subjected to statistical analysis using SPSS software, version 27, to assess the prevalence of contamination. Data visualization, including the generation of graphical representations, was conducted utilizing Microsoft Excel 2016. This methodological approach enabled a clear and effective presentation and interpretation of the study findings.

Results

The microbiological analysis demonstrated that 20% of the total samples examined were contaminated with *Staphylococcus aureus*, whereas 80% exhibited no contamination. Differential analysis by source revealed that 22% of samples collected from barbecue meat shops within the city tested positive for *Staphylococcus aureus*, with the remaining 78% showing no contamination. In contrast, none of the samples obtained from restaurants were positive for *Staphylococcus aureus*, indicating an absence of contamination in this category. Furthermore, samples sourced from protein shops in Babol city exhibited a contamination rate of 21% for *Staphylococcus aureus*, while 79% were uncontaminated. Finally, analysis of samples from chicken shops in Babol city revealed the highest contamination rate, with 31% testing positive for *Staphylococcus aureus* and 69% free from contamination (Figure 1).

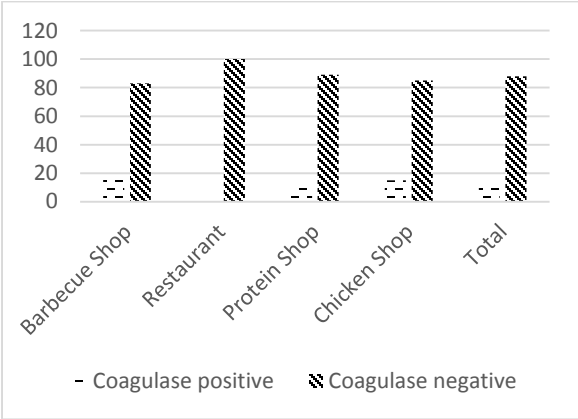

Fig 1. Prevalence (%) of contaminated samples with *Staphylococcus aureus*, from different sources

According to the results, 12% of total *Staphylococcus aureus* isolates from Babol city were coagulase-positive, while 88% were coagulase-negative. In barbecue meat shops, 17% of *Staphylococcus aureus* isolates were coagulase-positive and 83% were coagulase-negative. All *Staphylococcus aureus* isolates from restaurants were coagulase-negative. In protein shops, 11% of isolates were coagulase-positive and 89% were coagulase-negative, whereas in chicken shops, 15% were coagulase-positive and 85% were coagulase-negative (Figure 2).

Among the ten coagulase-positive *Staphylococcus aureus* isolates examined, only one isolate (10%) was found to harbor the sea gene, whereas the remaining isolates did not possess the enterotoxin A gene.

Discussion

Staphylococcal food poisoning is a significant foodborne illness caused by the ingestion of heat-resistant enterotoxins produced by *Staphylococcus aureus*, particularly

enterotoxin group A, even in very small quantities. These toxins are highly stable and can withstand normal cooking temperatures, making them a persistent threat in food safety. The illness is most commonly associated with ready-to-eat foods that become contaminated after preparation, such as salads, sandwiches, and desserts, which are widely consumed in Iran and globally (1).

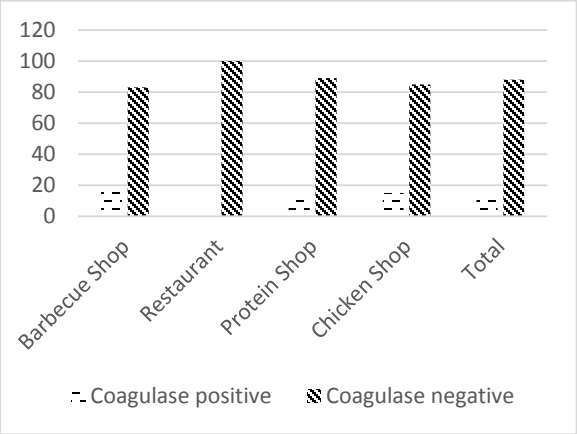

Fig 2. Prevalence (%) of coagulase positive and negative samples of *Staphylococcus aureus*, from different sources

The prevalence of *Staphylococcus aureus* contamination in food products varies widely across studies and regions, with reported rates ranging from 0% to 100% depending on the food type, sampling methodology, and hygienic practices. In the present study conducted in Babol city, Iran, 20% of ready-to-eat barbecue chicken meat samples were found to be contaminated with *Staphylococcus aureus*, a figure consistent with several international reports. For example, Bui et al. found a 22.2% prevalence in ready-to-eat foods in Vietnam. The high percentage of contamination may be attributed to inadequate hygienic controls during food production, secondary contamination during transportation and storage, reuse of contaminated containers, or improper washing of utensils and packaging materials (1). However, other studies have reported higher rates, such as Gencay et al., who found contamination rates as high as 77.7% in certain food categories. In the study by Gencay et al., 65.6% of beef, 55% of poultry, 73.9% of dairy products, 77.7% of ready-to-eat foods, and 77.7% of food ingredients tested positive for contamination with *Staphylococcus aureus,* indicating a higher level of contamination than observed in our study (2). Wu et al. collected 1,850 samples of raw meat and meat products from 39 cities in China and found that 35% of the samples were contaminated with *Staphylococcus aureus.* These results are somewhat similar to those obtained in the present study (3). In a study conducted by Giannatale et al. in 2011 on the isolation and identification of *Staphylococcus aureus* from human food in Italy, they were able to isolate *Staphylococcus aureus* with a prevalence of 19.3% from meat products, which aligns with the findings of the current research (4). Soltan Dallal et al. in 2010 analyzed 1,040 samples of various food items for the presence of coagulase-positive *Staphylococcus aureus*, isolating 100 samples (9.5%) that tested positive. The contamination rates were 17.1% in dairy products, 3.5% in meat products, and 4.5% in other food items (5). In the present study, based on the obtained results, 12% of the total samples collected from Babol city were contaminated with *Staphylococcus aureus*, while 88% were free of contamination. Several studies have been conducted in Iran to investigate the contamination of chicken meat with *Staphylococcus aureus*. Javadi and Safa Mashaei reported that 65% of poultry meat samples tested positive for the presence of *Staphylococcus aureus*. In this context, the results obtained from Feizi et al. indicated an 81.75% contamination rate in chicken meat, while Eshraghi et al. reported a contamination rate of 3.5% (6–8).

The main reasons for the discrepancies in the prevalence of *Staphylococcus aureus* observed in different studies include the type of samples examined, sampling methods, testing procedures, geographical region, climatic conditions, and levels of hygiene maintained. Contamination levels vary across different countries, leading to different prevalence rates. Additionally, in this study, PCR techniques revealed that the sea gene was not present in any of the isolates. In a study conducted on the prevalence of *Staphylococcus aureus* and

enterotoxin A in fried chickens in Italy, researchers could not identify any sea genes among 20 industrial fried chickens (9). In a study conducted by Esnaashari et al. on the prevalence of common enterotoxin genes in *Staphylococcus aureus* isolated from buffalo milk in Tabriz county using PCR, the results showed that out of 75 bacterial samples, one isolate contained enterotoxins SEB and SEC, three isolates contained enterotoxin SEC, and the gene corresponding to enterotoxin A was not identified in any of the isolates(10). These findings are consistent with the current research. In another study examining the enterotoxin gene profiles of *Staphylococcus aureus* and other staphylococci isolated from various foods, out of 70 *Staphylococcus aureus* isolates, the sea gene was identified in two samples of chicken meat (2.9%) (2), which does not align with the present study's findings.

Conclusion

The detection *of Staphylococcus aureus* in 20% of ready-to-eat barbecue chicken meat samples in Babol city, Iran, underscores the ongoing public health risk posed by this pathogen. The absence of the sea gene in these isolates suggests a lower immediate risk of classical staphylococcal enterotoxin A-mediated food poisoning, but the presence of the bacterium itself, particularly antibiotic-resistant strains, remains a concern. These findings highlight the necessity for continuous monitoring, improved hygiene practices during food handling, and robust surveillance of antibiotic resistance in foodborne pathogens to safeguard public health.

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Conflict of interest

The authors declare that there are no conflicts of interest related to the conduct or findings of this study. The study was conducted with full transparency and adherence to ethical guidelines to ensure unbiased and objective outcomes.

Ethical approval

All procedures involving sample collection and laboratory analysis were performed following established ethical guidelines to ensure safety, confidentiality, and integrity. Since the research involved only food samples collected from commercial sources and did not involve human or animal subjects, informed consent was not applicable. Nonetheless, all efforts were made to conduct the study responsibly, maintaining transparency and adherence to relevant regulations and best practices in food safety research.

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