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Detection of Classical Enterotoxins of Staphylococcus aureus Strains Isolated From Raw Meat in Esfahan, Iran

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Background: *Staphylococcus aureus* is an important pathogen of humans in both community acquired as well as nosocomial infections. It is also among the four most common causes of food-borne illnesses.
Objectives: This study was conducted to determine the prevalence of enterotoxin producing *S. aureus* strains in different raw meat and hamburgers in Isfahan.
Materials and Methods: From August to December 2012, 370 samples of raw beef (n = 160; minced and carcass), lamb (n = 80), goat (n = 80), and camel (n = 50) meat were purchased from randomly selected butcheries in Isfahan, Iran, and analyzed for the presence of *S. aureus*. Isolates were also tested for their ability to produce staphylococcal enterotoxins A, B, C, D, and E by enzyme linked immunosorbent assay (ELISA) test.
Results: Totally, 223 (60.3%) *S. aureus* were isolated. Among the 223*S. aureus* isolates 30 (13.5%) were found to be enterotoxigenic. Twenty-six (86.7%) were positive only for one type of SEs (14 SEA, 1 SEB, 6 SEC and 5 SED) while the remaining (13.3%) were positive for more than one SEs. None of the isolates were positive for SEE.
Conclusions: Only 8.3% of the total meat samples examined in the current study showed this count or above. This low degree of contamination by *S. aureus* is tolerated in most food stuffs and they are not considered a risk for public health. However, we need more epidemiological investigations about enterotoxigenic *S. aureus* isolates and their toxins for better management of food products and to decrease human diseases. The results of this study showed that most *S. aureus* strains isolated from samples produced SEA and SED compared to other SEs.

Keywords: Staphylococcus; Enterotoxins; Meat

1. Background

Staphylococcus aureus is an important pathogen of humans in both community acquired as well as nosocomial infections (1, 2). *S. aureus* is also among the four most common causes of food borne illnesses. Staphylococcal food poisoning (SFP) results from consumption of food contaminated with staphylococcal enterotoxins (SEs) produced by *S. aureus* (3). SFP is widespread and quite frequent. The number of staphyloenterotoxicosis cases is probably underestimated significantly. There may be many reasons behind this, including: not contacting medical services by many ill people due to the short duration of the disease or mild symptoms and improper sample collection and laboratory examination. The food incriminated in SFP is variable from one country to another and mainly depends on nutritional habits. It has been proved that, the main source of staphylococcus in processed food is humans while animals are the main origin of contamination in raw food (3). The pathogenicity of *S. aureus* depends on a number of virulence factors such as the heat stable enterotoxins as well as other enterotoxins (4). *S. aureus* produces 15 enterotoxins (5), and usually produces one or more of these toxins simultaneously (6, 7). The five classic enterotoxins (SEA, SEB, SEC, SED, and SEE) are known to be responsible for 95% of staphylococcal food poisoning cases and SEA and SED are most common enterotoxins recovered from food poisoning out-breaks (8), possibly because they can be produced in a wide range of growth conditions (aw, pH and Eh) (9). According to Balaban and Rasooly (10), 100 ng of the toxin is enough to induce symptoms when the population reaches 105 colony-forming units per gram (CFU/g). The principal symptom of SFP is vomiting within 1 - 6 hours after eating contaminated food, usually followed by diarrhoea, abdominal cramping, and exhaustion. In more severe cases, additional symptoms can include headache, muscle cramping, and changes in blood pressure and pulse rate. Death from SFP is rare, but can occur among certain high risk people such as infants, elderly; and chronically ill individuals (10). Among the foods implicated in SFP, milk, dairy products and meats, especially handled

Implication for health policy/practice/research/medical education
The content and results of the present communication has identified the risk indicators and it is useful for the policy/decision makers to reduce the food intoxication.

foods, play an important role since enterotoxigenic strains of *S. aureus* have frequently been isolated from them (11-13).

2. Objectives

Currently, there is limited information regarding the prevalence and enterotoxin production of *S. aureus* in raw meat in Iran. The purpose of the present study was to determine the prevalence of enterotoxin producing *S. aureus* strains in different raw meat in Isfahan; no similar work has been previously performed in Iran.

3. Materials and Methods

3.1. Sample Collection

Four hundred and ten samples of raw beef (n = 160; mince and carcass), lamb (n = 80), goat (n = 80) and camel (n = 50) were purchased and unpacked from randomly selected butcheries in Isfahan, Iran, From August to December 2012. All samples were placed in separate sterile plastic bags to prevent spilling and cross contamination and were immediately transported to the laboratory and cooled with ice packs.

3.2. Isolation and Identification of S. aureus

The samples were processed immediately upon arrival using aseptic techniques. Of each milk sample, 25 mL was homogenized and transferred to 225 mL of buffered peptone water (BPW). The samples were diluted with BPW, and 0.1 mL of diluted samples were streaked on Baird-Parker (BP) agar (Oxoid) supplemented with egg yolk-tellurite emulsion (Oxoid). After inoculation at 37°C for 24 h, typical colonies of *S. aureus* with similar morphologies, from each selective agar plate were isolated and cultured separately on slants of Brain–Heart Infusion (BHI, Oxoid). The identification was performed using standard microbiological and biochemical procedures including gram staining, production of coagulase, catalase, DNAse and oxidation and fermentation of mannitol (14).

3.3. Detect Staphylococcal Enterotoxins S. aureus

To detect staphylococcal enterotoxins, *S. aureus* isolates were cultured aerobiocally in 10 mL of nutrient broth at 37 °C,overnight.Culture supernatants of the isolated bacteria were then used for detection of *S. aureus* enterotoxins. Staphylococcal entrotoxins SEA, SEB, SEC, SED and SEE were detected byenzyme linked immunosorbent assay (ELISA) detection kit (RIDASCREEN® SET A, B, C, D, E; R-Biopharm AG, Darmstadt, Germany) according to the manufacture's instructions. The detection limit was 0.1 mg/mL and *S. aureus* and *S. epidermis* strains were used as positive and negative controls for each test.

3.4. Statistical Analysis

Data were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), chi-square test and fisher's exact two-tailed test analysis were performed and differences were considered significant at values of $P < 0.05$.

4. Results

Table 1 shows the prevalence of *S. aureus* isolated from beef (minced and carcasses), lamb, goat and camel meat samples in Isfahan, Iran. In this study, 223 of 370 meat samples (60.3%) were contaminated with *S. aureus* . The mean count for meat samples ranged from 2.6 × 10 2 to 4.8 × 10 3 CFU/g. The highest prevalence of *S. aureus* was found in minced beef (76.3%), followed by lamb meat (68.8%), beef meat (57.5%), goat meat (47.5%), and camel meat (46.0%). There were no significant differences ($P > 0.05$) in the level of contamination with *S. aureus* between different meat samples. Among the 223 *S. aureus* isolates, only 30 (13.5%) were found to be enterotoxigenic (> 100 ng). Twenty-six (86.7%) were positive only for one type of SEs (14 SEA, 1SEB, 6 SEC and 5 SED) while the remaining (13.3%) were positive for more than one SEs (Table 1).

Table 1. Prevalence and Enterotoxin Production of Staphylococcus aureus Isolated From Beef, Lamb, Goat and Camel Meat Samples

Samples	Samples, No	Positive Samples for *S. aureus*, No, (%)	Mean of *S. aureus* Count, CFU/g[a]	Positive Samples for Enterotoxigenic *S. aureus*, No	SEs, %[c]					
					SEA[a]	SEB	SEC	SED	SEA + SEC	SEA + SED
Beef meat	80	46 (57.5)	5.6×10^2	8	5	-	-	2	-	1
Minced meat[b]	80	61 (76.3)	4.8×10^3	11	6	-	-	3	-	2
Lamb meat	80	55 (68.8)	7.1×10^2	4	1	-	3	-	-	-
Goat meat	80	38 (47.5)	2.6×10^2	3	1	-	2	-	-	-
Camel meat	50	23 (46.0)	4.3×10^2	4	1	1	1	-	1	-
Total	370	223 (60.3)	8.3×10^2	30	14	1	6	5	1	3

[a] Abbreviations: CFU/g, colony forming units per gram; SEA, staphylococcal enterotoxins A
[c] None of the samples were positive for SEE
[b] Made from raw beef or cow meat

5. Discussion

In this study, 223 of 370 meat samples (60.3%) were contaminated with *S. aureus* (The mean count ranged from 2.6×10^2 to 4.8×10^3 CFU/g). Similar results were also reported by Marthenge and Ombui (15) and Al-Tarazi et al. (16) for meat and meat products; however, lower contamination rates (10.5% to 16.4%) have also been reported (17,18). The contamination of food by *S. aureus* may directly occur due to skin lesions of workers containing bacterin or sneezing and coughing. Approximately 50% of the human population carries *S. aureus* as commensals. Other contamination sources of *S. aureus* are soil, water, dust and air (10,14). From 223 *S. aureus* isolates, 30 (13.3%) were found to be enterotoxigenic (Table 1). The relatively high percentage of classical enterotoxin-forming *S. aureus* strains from meat samples found in our study is confirmed by previous findings (12,16,19). However, these results were not in agreement with some other investigators including that reported by Marthenge and Ombui. They reported that 66% of the studied samples were contaminated. The examined samples contained 200 raw milk, 100 beef carcass swabs, 50 minced meat samples and 50 chicken carcasses. Variable contamination rates ranging from 21% to 66% have been recorded for food stuff including meat, milk, eggs and their derivatives in different countries (20-24). The difference between the results ofour study and other reports in the prevalence and production of enterotoxins among *S. aureus* isolates from meat samples may be a result of different sampling techniques employed, seasonal effects, number and kinds of examined samples, and/or laboratory methodologies employed. Recently, new super antigenic enterotoxins (SEG-SEU) have also been described. Due to technical limitations, we were not able to screen the new SEG-SEU, since commercial ELISA test kits for detecting these new enterotoxins are not available. There are no specific standards for the permissible number of *S. aureus* in fresh or raw meat in Iran; however, 10^3 CFU/g is the highest permissible count of *S. aureus* commonly specified by the international agencies (25). Only 8.3% of the total meat samples examined in the current study showed this count or above. This low degree of contamination by *S. aureus* is tolerated for most foodstuffs and is not considered as a risk for public health (3). This is expected because in fresh or chilled meat, *S. aureus* is not a good competitor with normal microflora (8). However, further molecular based studies are necessary to test enterotoxigenic *S. aureus* isolates or their toxins for improved management of food products and to decrease human diseases.

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Authors' Contribution

Study concept and design: Ebrahim Rahimi, Esmail Ataye Salehi. Analysis and interpretation of data: Fatemeh Nonahal, Ebrahim Rahimi, Manuchehr Momeni. All authors contributed to the literature review and writing of the article.

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