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Incidence of *Salmonella* serovars and its antimicrobial pattern in barbecued meat and ground beef burgers in Tehran

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ABSRTACT

Background and objective: Shahr-e-Ray , Islamshahr and South Tehran were selected for this investigation. The main reason for the selection of these sites was based on the population density. The main objective of this investigation was to assess the sources and distribution of Salmonella serovars from barbecued meat, ground beef burgers and their antimicrobial resistance.

Materials and Methods: In a one year period, a total of 390 samples of food, consisting of 195 samples of raw barbecuing meat and ground beef burgers and 195 samples of cooked ones were examined for the presence of *Salmonella* contamination.

Results: From a total of 195 raw samples, (n=33 ,16.9%) were *Salmonella* positive. Most detections of *Salmonella* occurred in Shahr-e- Ray (n=15, 45.5%) and then in South Tehran (n=10, 30.3%) and Islamshahr (n=8, 24.2%). The highest rates of detection of *Salmonella* occurred in Summer (n=17,51.5%). In serological evaluations of *Salmonella*, thompson serovar had the highest prevalence in barbecuing meat and ground beef burger samples (n=18,54.5%). *Salmonella* serovars were: (n=31, 93.9%), (n=30 ,90.9 %) and (n=30, 90.9 %) resistant to ampicillin, amoxicillin and nalidixic acid respectively.

Conclusion: The results show that there is no reason for concern in consuming cooked barbecuing meat and ground beef burgers. In case of raw samples, microbes could originate from the vendors. Vendors have to be educated on hygienic practices which could help to reduce risks of food- borne infection. These data indicate that food handlers may contribute to contamination and that there are some handling practices that require more attention.

Keywords: Barbecuing meat, ground beef burger, *Salmonella* serovars.

INTRODUCTION

Food-borne infections still remain as one of the important concerns of public health worldwide. Though data from different countries seem to report increases in the incidence of food-borne diseases, these data may not always represent actual existing facts (1-3).

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Food production, processing and distribution differs from country to country. These practices depend on local consumer preference and the influence of practices in other countries on the local consumer's daily life (4-5).

Demographic changes, globalization, mass population movements, changes in food production and distribution methods, environmental and sanitary conditions, and global warming generate conditions that are highly favorable to the emergence and re-emergence of food-borne infections (6-8).

Disease, caused by the consumption of contaminated food, could be due to the presence

of pathogenic organisms (food-borne infection) or, it could be the result of the presence of toxic chemicals (9-11). There are different types of organisms which are known to cause food- borne infections. The most common bacterial agents are the *Camplyobacter, Salmonellae*, toxigenic /verocytotoxic *E.coli, Shigellae*, toxigenic *Staphylococcus, Listeria monocytogenes* and *Clostridiae* worldwide (12-15). Human cases of botulism have been found to originate from a considerable variety of preserved foods, for example, ham, sausage, canned meats, vegetable products etc. (16).

This study was initiated to assess sources and distribution of *Salmonella* serovars from barbecuing meat, ground beef burger and antimicrobial resistance from three different part of southern Tehran.

MATERIALS AND METHODS

Before the start of this study, visits were paid to selected sites in southern part of Tehran to talk to owners and they were assured of confidentiality.

During the one year period, a total of 390 samples of food, consisting of 195 raw samples (165 barbecuing meat, 30 ground beef burgers) and 195 cooked samples (165 barbecuing meat, 30 ground beef burgers) were examined for presence of *Salmonella* serovars. All samples were aseptically collected by District Health Center (DHC) of Shahr-e-Ray, Islamshahr and South Tehran , all in southern Tehran. After collection, the samples were iced and delivered to Food and Hygiene Control Laboratory (FHCL) within 1-2 hrs of collection.

Microbiological analysis. The samples were analyzed for Salmonella according to ISO 6579 (17) using 25gr of the food samples; and were placed in sterile stomacher bags and 225 ml of buffered peptone water (BPW, Merck) was added in each sample. The sample was homogenized using a stomacher for 2 minutes, followed by incubation for 24 hours at 37˚C. Then, 0.1ml of the pre-enriched broth was transferred into 10ml of Rappaport–Vasisiliadis medium (RV) (Oxoid CM 669) and incubated for another 24 hours at 42˚C. The enrichment samples were then applied onto Hektoen agar (Hi Media , India) plates and incubated for 24 hours at 37˚C. Suspicious colonies were identified with biochemical tests (oxidase reaction, acid production from manitol, 0-nitrophenyl-ß-d-galactopyranoside test (ONPG), H2S and indol production as well as proofs of urease and lysine decarboxylase). Salmonella srains were affirmatively identified and serotyped at Razi vaccine and serum investigation institute in Tehran, with slide agglutination tests as described by Ewing (18) and flagellar antigens were detected by a technique utilizing microtitre plates (19).

Antimicrobial resistance tests. The antimicrobial resistance tests of *Salmonella* serovars were carried out with the agar dilution method as recommended by the Clinical and Laboratories Standards Institue (CLSI) (20). Briefly, the strains were grown to 0.5 McFarland density in Mueller Hinton (MH) Broth (Difco, Detroit, USA) containing antimicrobials in the following concentration: chloramphenicol (chl) 32 µg/ml; gentamicin (gen) 16 µg/ml; tetracycline (tet) 16 µg/ml; trimetoprim (tmp) 16 µg/ml; ciprofloxacin (cip) 0.125 µg/ml; ceftazidim (cef) 30 µg/ml ; amoxicillin (amox) 32 µg/ml; ampicillin (amp) 32 µg/ml; streptomycin (str) 64 µg/ml; nalidixic acid (nal) 32 µg/ml;cefotaxime (cefo) 30µg/ml and imipenem (imip) 10 µg/ml. After incubation at 35^0C for 24 hours, zone size was measured. Standard and reference strains were used and interpretation of the strains as susceptible, intermediate or resistant was made following the recommendations of the CLSI. Reference strains included *Salmonella typhi*. (PTCC 1639).

Statistical analysis. Statistical analysis was done by Stat graphics for Windows software package, V.2.

RESULTS

The cooked specimens did not have contamination of Salmonella serovars. From a total of 195 raw samples, 33 (16.9%) were Salmonella positive. In this study, 18.8% of raw barbecuing meat and 6.7% of raw ground beef burger were contaminated with Salmonella serovars. The highest rates of contamination occurred in summer 17 (49.4%).

In serological evaluations of *Salmonella* serovars, thompson serovar had the highest prevalence in barbecuing meat and ground beef burger samples 18 (54.5%), and 4 (12.1%) of the *Salmonella* strains were not serologically identified (Table 1).The antimicrobial susceptibility of all *Salmonella* serovars to various antibiotics was determined by disk diffusion method using Muller Hinton agar.

Table 1: Sources and distribution of *Salmonella* serovars

Serotype	No. of barbecuing meat sample (%)	No. of ground beef burger sample (%)	Total
S. thompson	16 (51.6)	02(100)	18 (54.5)
S. virginia	01 (3.2)	0.0 (0.0)	01 (3)
S. entertidis	05 (16.1)	0.0 (0.0)	05(15.2)
S. typhimurium	03 (9.7)	0.0 (0.0)	03 (9.1)
S. veyle	02 (6.5)	0.0 (0.0)	02 (6.1)
S. untypable	04 (12.9)	0.0 (0.0)	04 (12.1)
Total	31 (100)	02 (100)	33 (100)

Table 2: Antimicrobial resistance of *Salmonella* serovar*s* isolates from barbecuing meat and ground beef burger samples

Antibiotic	Sensitive	Intermediate	Resistant
Chloramphenicol	33 (100)	0 (0.0)	0.0 (0.0)
Gentamicin	33 (100)	0 (0.0)	0.0 (0.0)
Tetracycline	06 (18.2)	02 (6.1)	25 (75.7)
Trimetroprim	10 (30.3)	01 (3)	22 (66.7)
Ciprofloxacin	33 (100)	0 (0.0)	0.0 (0.0)
Ceftazidime	33 (100)	0 (0.0)	0.0 (0.0)
Amoxicillin	01 (3)	02 (6.1)	30 (90.9)
Ampicillin	01 (3)	01 (3)	31 (93.9)
Streptomycin	05 (15.1)	15 (45.5)	13 (39.4)
Nalidixic acid	02 (6.1)	01 (3)	30 (90.9)
Colistine	33 (100)	0 (0.0)	0.0 (0.0)
Cefotaxime	32 (97)	01 (3)	0.0 (0.0)
Imipenem	33 (100)	0 (0.0)	0.0 (0.0)

Salmonella serovars were 31 (93.9%), 30 (90.9 %) and 30 (90.9 %) resistant to ampicillin, amoxicillin and nalidixic acid respectively (Table 2).

DISCUSSION

In this study, 18.8% of raw barbecuing meat and 6.7% of raw ground beef burger samples were contaminated by Salmonella serovars. The presence of Salmonella spp perhaps is indicative of poor hygiene and a potential danger to consumers. Therefore,Salmonella spp in foods constitutes a significant risk can be used as an indication of cross-contamination as emphasized by other reports (21-23). Considering the high likelihood that raw meats will contain bacterial pathogens, exercise of control measures during final preparation stages are critically important to assure safety. Numerous studies have demonstrated how pathogens can be effectively distributed around the environment and back to food in the absence of strictly observed hygiene guidelines. Values outside of acceptable microbiological limits are indicative of poor hygiene or food handling practices (4, 24- 25).

Serotyping of *Salmonella* isolates from different sources suggests that an association exists between the occurrence of certain *Salmonella* serovars in food

animals, meat products and in man, which could be acquired through ingestion of contaminated food and food products (26-28). Of the 6 different *Salmonella* serovars identified, *S. thompson* and *S. entertidis* were the prevalent ones. Previously other workers have reported some of these and other non-host adapted *Salmonella* serovars in food animals, meat products and man.

The high-risk human population, that is infants, elderly, immuno- compromised and malnourished persons are highly susceptible and the presence of *Salmonella* even in low numbers constitutes a major public health concern (29-30). Particular attention to the hygiene of food handlers and employee training may help tackle this concern.

Antibiotic resistant in food samples may serve as a reservoir, thereby allowing micro-organisms to persist and spread in the community. Antibiotic resistance is increasing to some antibiotics, such as fluoroquinolones and third-generation cephalosporins. These antibiotics are commonly used to treat serious infections caused by bacterial pathogens frequently found in food, such as Salmonella and Campylobacter (31-34). Salmonella serovars identified in indicated food samples were 93.9%, 90.9 % and 90.9 % resistant to ampicillin, amoxicillin and nalidixic acid respectively.

Results from this study indicate that the personal hygiene of food handlers, the use of protective utensils during processing (mask, gloves, hairnets, etc.) and Good Manufacturing Practices (GMP) should be improved.

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