Screening of Food Handlers for Salmonella and Staphylococcus aureus Carriers at University Cafeterias in Khartoum

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Abstract

Background: Food borne diseases are major health problems in developing countries. Food handlers with poor personal hygiene working in food establishments could be potential sources of disease due to carriage of pathogenic organisms.

Objective: To screen food handlers for Salmonella and Staphylococcus aureus carriers at university cafeterias in Khartoum.

Materials and methods: A cross-sectional study was conducted among food handlers working at Al Neelain University cafeterias in Khartoum. A pretested structured questionnaire was used for collecting data. Nasal swabs and stool were investigated for Staphylococcus aureus (S. aureus) and Salmonella species as per the standard of the laboratory methods. Isolated organisms were tested for antimicrobial susceptibility using the Kirby-Bauer technique.

Results: 80 food-handlers were investigated. The majority of food-handlers (73.75%) were young adults aged 20-40 years. 16 food-handlers (20%) were S. aureus nasal carriers. 11 of the S. aureus isolates (68.8%) were resistant to amoxyclov, two (12.5%) were resistant to tetracycline, and all S. aureus isolates were sensitive to methicillin. On the other hand, five food-handlers (6.25%) were Salmonella species stool carriers. Four of these species (5%) were Salmonella paratyphi B (S. paratyphi B) which were all sensitive to ciprofloxacin, cefoperazone, and gentamycin; and resistant to tetracycline and cefepime. Likewise one Salmonella species (1.25%) was Salmonella paratyphi A (S. paratyphi A); and this species was found sensitive to all antibiotics tested.

Conclusion: Unhygienic conditions were detected among food handlers and food processors in the cafeterias investigated. Presence of resistant food pathogens; particularly Staphylococcus aureus isolates was confirmed.

Key words: Food handlers, Salmonella and Staphylococcus carriers, University cafeterias.

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Introduction

Foodborne diseases are among the most wide-spread global public health problems of recent times. These diseases are attributable to a wide range of pathogens and toxins. *Salmonella* is a leading cause of foodborne illnesses. Salmonellosis has an impact on communities in both developing and developed world. Transmission is facilitated by fecal-oral route. According to World Health Organization (WHO) 1995 reports, 88% of all foodborne diseases were caused by *Salmonella* species\(^1\).

Food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main source of food contamination, via manual contact or through respiratory secretions. Staphylococcal food poisoning (SFP) is an intoxication that results from consumption of foods containing sufficient amounts of one (or more) preformed enterotoxin. Symptoms of SFP include: nausea, violent vomiting, and abdominal cramps with or without diarrhea. Laboratory diagnosis of *S. aureus* among food handlers is conducted by culturing nasal swabs in mannitol salt agar, which has a sensitivity of 84.3% and specificity of 100%\(^2\).

Food borne diseases are major health problems in developed and developing countries. The WHO estimated in developed countries up to 30% of the populations suffer from food borne diseases each year, whereas in developing countries up to 2 million deaths are estimated per year. The spread of food borne diseases via food handlers are a common and persistent problem worldwide. Many diseases are communicable and caused by microorganisms that enter into the body via food. Numerous outbreaks of gastroenteritis have been associated with ingestion of raw foods, foods incorporating raw ingredients or foods obtained from unsafe sources\(^3\).

Food poisoning has been reported to be due to infection by enterotoxigenic strains of *Staphylococcus aureus* which produces enterotoxin A, B, C1, C2, C3, D and E. It accounts for 14–20% of outbreaks involving contaminated food in USA. In the United Kingdom restaurants are the second most important place for acquiring staphylococcal food poisoning. This organism may exist on food handler’s nose or skin, from which it may be transmitted to cooked moist protein-rich foods, and become intoxication agents, if these foods are then kept for several hours without refrigeration or stored in containers. Food handlers have also been incriminated as vehicles of *Salmonella*. Clinical syndromes caused by *Salmonella* infection in humans are divided into typhoid fever, caused by *Salmonella typhi* and *Salmonella paratyphi*, and a range of clinical syndromes, including diarrheal diseases, caused by a large number of non-typhoidal *Salmonella* serovars. Borne-gastroenteritis in humans remains a health problem worldwide. The WHO estimates 16 million new cases and 600,000 deaths of typhoid fever annually\(^4\).

This study aimed at investigating the microbial quality of *Salmonella* and *Staphylococcus aureus* carriers; and determining susceptibility pattern of bacterial isolates. The study may assist with control of food contamination at Al Neelain University cafeterias (Khartoum). It may advise on the appropriate antimicrobials needed for treatment. It may also assist in establishing proper food sanitation and advice on practical measures to limit spread contamination and enhance elimination of food poisoning. The study may also monitor and reflect the magnitude of methicillin-resistant *Staphylococcus aureus* (MRSA) strains at Al Neelain University cafeterias.

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Materials and methods

This was a case finding laboratory-based, descriptive, cross-sectional, qualitative study. The study was carried out during the period from May to June, 2013, including literature review, samples collection, laboratory work, data analysis, and writing. It was conducted at Al Neelain University cafeterias (Khartoum). The target population was food-handlers and food-processors. Sampling was a probability, purposive, convenience type. The software used for the analysis of data was the Statistical Package for Social Sciences (SPSS) program (version 16). The means and medians of the continuous variables are compared by Student’s t test program depending on the sample distribution. Confidentiality of information obtained from subjects investigated was maintained. Verbal consent of the subjects was obtained. Results of samples collected were handed over to all patients included in the study and some sample results were dispatched to physicians for treatment. Permission to conduct the study was taken from the Environmental Sanitation Office at Al Neelain University (Khartoum).

A total of 80 samples from food-handlers and food-processors were collected and investigated for the presence of Salmonella typhi (S. typhi) and Staphylococcus aureus as potential food pathogens. Each volunteer subject was given a concise explanation of the need for the microbiological investigation. Nasal swabs were collected using dry, sterile polyester swabs. The swab was inserted into nostrils of subjects parallel to the palate, and left in place for a few seconds. It was then slowly withdrawn in a rotating motion. Specimens from both nostrils were obtained with the same swab. The tip of the swab was put into a plastic vial containing 2-3 ml of transport medium and the applicator stick was broken off.

To collect stool samples, subjects were instructed to submit a fresh stool sample. Subjects were able to deliver the stool sample directly to the university laboratory. Stool samples were received in clean, dry, leak-proof, and wide-mouth containers. Stool samples accepted were without urine contamination.

Nasal swabs were stained directly by Gram stain, and were directly cultured on differential and selective standard media. Stool samples were inoculated in tubes containing selenite-F broth using a sterile wire loop. After an overnight incubation the tubes were held at an angle and a wire loop was rubbed against the side of the tube below the level of the broth and subcultured on deoxycholate citrate agar (DCA) for the isolation of Salmonella typhi; and mannitol salt agar (MSA) for the isolation of Staphylococcus aureus. Inoculation of media was performed using sterile loops, and the inoculum was spread to ensure single colony growth. The plates were incubated aerobically at 37°C overnight.

Biochemical tests used to identify S. aureus were: catalase test, slide coagulase test, tube coagulase confirmatory test, and DNA-ase test.

Biochemical tests used to identify Salmonella species were: cytochrome oxidase test, Kligler-iron agar test, Christensen’s urease test, and citrate utilization test.

Antimicrobial susceptibility tests were performed by the modified Kirby-Bauer susceptibility testing technique. The turbidity of the suspension was matched in a good light with the standard equivalent of McFarland turbidity standard. Using a sterile swab, a plate of Mueller Hinton agar
was streaked. Using a sterile forceps, the appropriate antimicrobial discs were placed, evenly distributed on the inoculated plate. A template was used to ensure correct positions of discs. Control strains of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) were incorporated. After overnight incubation, the control and test plates were examined to ensure that the growth was confluent or near confluent. The diameter of each zone of inhibition was measured in mm. The endpoint of inhibition was where growth started. Antibiotic discs used in this study were: cefepime, ciprofloxacin, gentamycin, tetracycline, cefoperazone, methicillin, vancomycin, amoxyclav, penicillin, and erythromycin. The *Staphylococcus aureus* susceptibility to methicillin was also performed by Kirby-Bauer disc diffusion method; and inhibition zone sizes were measured according to standard methods.

**Results**

Nasal swabs and stool specimens were collected from 80 food-handlers and food-processors working in Al-Neelain University cafeterias. The majority of them (73.75%) were young adults aged 20–40 years. Five food-handlers (6.25%) were found stool positive for *Salmonella* species. Four food-handlers (5%) were carriers of *S. paratyphi B*, and one food-handler (1.25%) was a carrier of *S. paratyphi A* (Table I).

<table>
<thead>
<tr>
<th>Species</th>
<th>No.</th>
<th>Per cent</th>
<th>Valid per cent</th>
<th>Cumulative per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. paratyphi A</em></td>
<td>1</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td><em>S. paratyphi B</em></td>
<td>4</td>
<td>5.0</td>
<td>5.0</td>
<td>6.2</td>
</tr>
<tr>
<td>No growth</td>
<td>75</td>
<td>93.8</td>
<td>93.8</td>
<td>92.6</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

On the other hand, 16 (20%) food handlers were found positive for nasal carriage of *S. aureus*; and 64 food-handlers (80%) were not carriers (Table II).

As regard amoxyclav sensitivity, 5 *Staphylococcus aureus* strains (31.2%) were sensitive, and 11 *Staphylococcus aureus* strains (68.8%) were resistant to this antibiotic. Furthermore, sensitivity of *Staphylococcus aureus* to tetracycline was different, i.e. 14 strains (87.5%) were sensitive and only two strains (12.5%) were resistant to this antibiotic.

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All four *S. paratyphi B* strains were found sensitive to ciprofloxacin, cefoperazone, and gentamycin; and resistant to tetracycline and cefepime. The only *S. paratyphi A* strain detected was sensitive to all antibiotics tested. While the 16 *S. aureus* strains isolated were found sensitive to methicillin, vancomycin, and erythromycin. Two *S. aureus* isolates were found resistant to tetracycline, and 11 *S. aureus* strains were found resistant to amoxyclav. All *S. aureus* isolates were found resistant to penicillin. None of the *S. aureus* strains isolated was found methicillin-resistant (Table III).

### Table III: Antimicrobial susceptibility pattern for *Salmonella* species and *S. aureus* strains isolated

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sensitive <em>S. paratyphi A</em></th>
<th>Sensitive <em>S. paratyphi B</em></th>
<th>Sensitive <em>S. aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefepime</td>
<td>1 (20%)</td>
<td>0 (0%)</td>
<td>Not Tested</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1 (20%)</td>
<td>4 (80%)</td>
<td>Not Tested</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>1 (20%)</td>
<td>4 (80%)</td>
<td>Not Tested</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1 (20%)</td>
<td>0 (0%)</td>
<td>Not Tested</td>
</tr>
<tr>
<td>Methicillin</td>
<td>Not Tested</td>
<td>Not Tested</td>
<td>16 (100%)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Not Tested</td>
<td>Not Tested</td>
<td>16 (100%)</td>
</tr>
<tr>
<td>Amoxyclav</td>
<td>Not Tested</td>
<td>Not Tested</td>
<td>5 (31.2%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Not Tested</td>
<td>Not Tested</td>
<td>14 (87.5%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Not Tested</td>
<td>Not Tested</td>
<td>16 (100%)</td>
</tr>
<tr>
<td>Penicillin</td>
<td>Not Tested</td>
<td>Not Tested</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

### Discussion

Food-borne pathogens are the leading cause of illness and death in developing countries. Changes in eating habits, mass catering, unsafe food storage conditions and poor hygiene practices are major contributing factors to food associated illnesses.

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The widespread habit of semi-cooked beef consumption is a potential cause for food borne illnesses. Foods that are frequently incriminated include: meat and meat products, poultry and egg products, salads (containing eggs, tuna, chicken, potato, and macaroni), bakery products (cream-filled pastries, cream pies, chocolate, sandwich fillings), and milk and dairy products. Food-borne disease is an infectious illness contracted through consumption of food or drinks contaminated with pathogenic bacteria, toxins, viruses, prions or parasites. Food handlers are defined as people employed directly in the production and preparation of foodstuffs, including those in the manufacturing, catering and retail industries people undertaking maintenance or repairing of equipment in food handling areas, whether permanent staff, workers on contract or visitors to food handling areas. Food handling involves all aspects of treating and storing food from receipt of raw materials to the delivery of the final prepared product. An outbreak strain was isolated in the stool of one asymptomatic food handler (in stool collected 3 weeks after first patron onset) who had handled implicated food. The food handler had initiated or propagated the outbreak. The outbreak resolved after the restaurant closed. In each of the following shorter outbreaks the attribution of transmission to asymptomatic food handlers is open to question. An asymptomatic food worker was the likely source at restaurant outbreak. The staff member had commenced work immediately prior to the outbreak and had prepared epidemiologically implicated food items. First stool sample was collected at least 13 days after ill patrons had eaten and no additional restaurant cases were identified. A fellow worker also had the outbreak strain isolated from stool and was presumably infected. Eleven kitchen staff was positive for an outbreak strain. The outbreak was probably due to one of these food-handlers who had prepared a linked food and had been asymptomatic. In this study 16 food-handlers (20%) were S. aureus nasal carriers. This finding was similar to that of other workers who reported a 20.5% nasal carriage of S. aureus. It was also similar to the finding of some authors who reported that 54 subjects (20.8%) were nasal carriers; and similar to that reported by other sources who detected S. aureus in nostrils of 15 food-handlers (23.4%). However our study finding was higher than that reported in some developing countries (1.3%). On the other hand, all S. aureus isolates in this study were sensitive to methicillin; and this finding was different from that documented by some workers who found that four S. aureus isolates (9.8%) were resistant to methicillin. Regarding the frequency of Salmonella species, this study reported five food-handlers (6.25%) were stool carriers; and this was higher than that reported in few developed countries (0.2%) and developing countries (1.2%). Furthermore in developing countries 74.4% and 5.1% S. aureus isolates were resistant to penicillin and erythromycin respectively. However in this study all S. aureus strains were sensitive to erythromycin, and resistant to penicillin. From the results of this study health authorities in Sudan should take proper measures to combat microbes transmitted from infected food handlers; and to take measures to investigate food handlers prior to employment in restaurants and cafeterias. Food-handlers should be educated on the adverse effects of lack of proper personal, environmental hygiene and sanitation. Food consumers should be

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made aware of the risk of consuming food supplied by cafeterias.

Conclusion: The results of this study confirmed the unhygienic conditions of food handling and processing in the cafeterias investigated. The study data also proved the presence of resistant food pathogens; particularly Staphylococcus aureus isolates that emphasized the need for strict supervision of antibiotics abuse.

References

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