INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 6, No. 5, p. 1-7, 2015

RESEARCH PAPER OPEN ACCESS

Evaluation of bacterial etiology and hygiene status of food-handlers in Jessore city, Bangladesh

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Key words: Food handlers, Food poisoning, Hygiene, *Klebsiella pneumoniae*. *Staphylococcus aureus*.

http://dx.doi.org/10.12692/ijb/6.5.1-7 Article published on March 09, 2015

Abstract

Food handler's hands are common source of food contamination, food spoilage, food poisoning and causation of diseases in consumers. Infected food handlers can transmit both enteric and non-enteric infections via the food that they handled and therefore lead to life threatening effects. Standard hygienic condition should be necessary to ensure food quality and safety. This study aimed to determine the prevalence of bacteria and to evaluate the hygienic status among different types of food handlers in Jessore city, Bangladesh. A total of 21 hand swabs were collected by standard hand swab technique from different restaurant's food handlers and street vendors. Then cultured in bacteriological agar and 77 isolates were identified by standard morphological and biochemical characteristics. Twelve genera of bacteria were found, they were; *Klebsiella pneumoniae* (21%), *Staphylococcus aureus* (16%), *Bacillus* spp. (14%), *Staphylococcus epidermidis* (10%), *Enterobacter aerogenes* (9%), *Micrococcus luteus* (9%), *Escherichia coli* (7%), *Streptococcus pyogenes* (3%), *Shigella flexinary* (2%), *Pseudomonas aeruginosa* (2%), *Serratia marcescens (2%)* and *Streptococcus mutans* (5%). *Staphylococcus aureus* and *Klebsiella pneumoniae* have the highest prevalence in different types of food handlers including indoor, outdoor, dry-food, wet-food handlers and also in food-makers and food-servers. Although *Staphylococcus* spp. and *Streptococcus* spp. are considered as normal commensal on human, but reflect improper hygiene practice such as pocking nose with fingers. Adequate food safety training must be received by the food handlers which may subsequently reduce the food-borne incidences.

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Introduction

Hands are the major source of contamination, which can never be totally free of bacteria. Food handlers are the individuals playing a major role in ensuring food quality and safety throughout the sequence of producing, processing, storage, preparation and service. Consequences of food-borne outbreaks via the food handlers is a common and continual problem worldwide (Zain and Naing, 2002; Scott, 2003; Andargie *et al.*, 2008). Many diseases are considered as communicable and caused by organisms that enter the body via food. The fingernails harbor most of the microorganisms and are difficult to clean (Lin *et al.*, 2003). Outbreaks of gastroenteritis may occur by the consumption of foods obtained from unsafe sources (Lengerich *et al.*, 1994; Hopkins and Juranek, 1991). The centers for disease control and prevention (CDC) have stated that, poor personal hygiene is the third most commonly reported factors that contribute to the food-borne diseases (Lillquist *et al.*, 2005). About 42% of food-borne disease outbreaks, took place from 1975 -1998 in the United States of America, were caused by the hands of food handlers (Sadiq and Abdullahi, 2008). Food handlers having poor personal hygiene could be potential sources of infections of many intestinal pathogens like helminthes, protozoa and enteropathogenic bacteria (Kaferstein and Abdussalam, 1999). Infected food handlers either with or without symptoms are usually regarded as one of the primary source of both enteric and non enteric organisms (Bryan, 1978; Genigeorgis, 1989). Nasal secretions, sneezing, coughing and direct hand contact of infected carriers usually considered as potential reasons of food contamination (Eisenberg *et al.*, 1975; Rooney *et al.*, 2004). Limiting hand contact with all food items is one of the first lines of defense in reducing food-borne illness. Proper hand hygiene practice is essential among food workers as hands have been detected as a possible vehicle for transferring food poisoning bacteria (Gorman *et al.*, 2002; Dharod *et al.*, 2009). In developing country like Bangladesh most of the food handlers live below the poverty line. As a result, they have a very little opportunity to be educated as well as to educate their children. Therefore the food handlers, who do not possess sufficient hygienic condition may lead the production of low quality or contaminated foods and are considered as vital reasons behind various food poisoning and food-borne illnesses.

Material and methods

Collection of Samples and transport

Samples were taken from food handlers of several restaurants (both indoor and outdoor) of Jessore city including Bajpara, Doratana, Chittra more, Railway station, Monihar, Ambottola bazaar and Jessore University of Science and Technology campus area. A detail insight of these food courts are mentioned in Table 1. Swabs from both hands (palm and beneath finger nail) were collected using sterile cotton-tip moistened in physiological saline and then placed into a sterile test tube containing peptone water. Samples were transported immediately to the microbiology Laboratory of Jessore University of Science and Technology and incubated at 37° C for 24 hours for the improvement of bacterial load.

Identification of Bacterial Isolates

After incubation, one loop full of overnight enrichment culture was streaked on nutrient agar, Mannitol salt agar (for the isolation of Gram positive bacteria) and MacConkey agar (for the isolation of Gram negative bacteria) and incubated at 37°C for 24 hours. Colony characteristics of the isolates were observed. Pure culture of the bacterial isolates was subjected to Gram staining and biochemical characterization such as oxidase, catalase, coagulase, citrate utilization, sugar utilization, hemolysis, starch hydrolysis, gelatin hydrolysis, motily, indole production, urease production, methyl red and voges-proskauer tests to identify the isolates. Identification was performed according to the standard protocol of Cappuccino and Sherman (2005).

Results

A total of 77 bacterial colonies were isolated from the hands of food handlers or vendors. Twelve genera of bacteria were identified on the basis of their colony characteristics (Table 2), Gram staining and biochemical tests (Table 3).

Table 1. List of sampling regions and information about the food handlers.

Name of the restaurants		Restaurant's feature	Male/ Female	Age	Type of service	Foods regularly served
Taltola, Bajpara						
1.	Bablu tea stall	Type A	Male	45	food maker	PS
2.	Taltola hotel and restaurant	Type B	Male	26	Food server	SD, RC
3.	Satkhira ghosh dairy	Type B	Male	32	Food maker	SD
4.	Babu hotel and restaurant	Type B	Male	35	Food maker	SD, RC
Railway station						
5.	Karim hotel and restaurant	Type B	Male	23	Food maker	RC
6.	Rupsha hotel and restaurant	Type B	Male	34	Food server	PS, RC
7.	Al-Amin hotel and restaurant	Type B	Male	25	Food server	RC
Doratana						
8.	Shahi Chotpoti	Type C	Male	42	Food server	CF
9.	Jonny hotel and restaurant	Type B	Male	37	Food maker	SD, PS, RC
10.	Kartic sweets	Type B	Male	38	Food maker	SD
11.	Vairab hotel and restaurant	Type B	Male	33	Food maker	SD, PS, RC
Monihar						
12.	Konika sweets	Type B	Male	23	Food maker	SD
Chittra more						
13.	Azad chop house	Type C	Male	34	Food server	CP
14.	Raz kabab house	Type C	Male	28	Food server	KC
15.	Café madina	Type B	Male	35	Food maker	RC
16.	Nirmol chotpoti store	Type C	Male	36	Food server	CF
JUST campus and Ambottola						
17.	JUST café	Type B	Male	26	Food server	SD, PS, RC
18.	Yar Ali hotel	Type B	Male	29	Food maker	RC
19.	Raju hotel	Type B	Male	31	Food maker	RC
20.	Muri mosla	Type C	Male	42	Food server	MM
21.	Ambottola bazaar hotel	Type A	Male	21	Food maker	PS, SD

Type A: Outdoor, tin shaded road side stall, Type B: Indoor, brick built restaurant, Type C: Outdoor, mobile shop, PS: Puri and singara, SD: Sweet and dairy, RC: Rice and curry, CF: Chotpoti and fuska, CP: Chop and piajii, KC: Kabab and chop, MM: Muri-mosla.

They were *Klebsiella pneumoniae* (21%), *Staphylococcus aureus* (16%), *Bacillus* spp. (14%), *Staphylococcus epidermidis* (10%), *Enterobacter aerogenes* (9%), *Micrococcus luteus* (9%), *Escherichia coli* (7%), *Streptococcus mutans* (5%), *Streptococcus pyogenes* (3%), *Shigella flexinary* (2%), *Pseudomonas aeruginosa* (2%) and *Serratia marcescens (2%)* in Figure 1A. Whereas, *Staphylococcus aureus* is 28% among Gram positive isolates *(*Figure 1B*)* and *Klebsiella pneumoniae* is 50% among Gram negative isolates *(*Figure 1C*)*. But, on the other hand, in food handlers of outdoor and indoor restaurants, the most prevalent bacteria were *Staphylococcus aureus* (22%) and *Klebsiella pneumoniae* (33%) respectively (Table 4). These two perhaps are pathogenic that may cause food poisoning.

Table 2. Colony characteristics of bacterial isolates.

Individual pathogens	Colony morphology of the agent
Staphylococcus aureus	White or golden colonies on MSA
Staphylococcus epidermidis	Medium sized circular, pinhead colonies with entire margin on MSA
Bacillus spp.	Dry, flat and irregular colonise with lobate margins on nutrient agar
Micrococcus luteus	Bright yellow with non-diffusable pigment on MSA
Streptococcus pyogenes	Translucent colony on blood agar
Klebsiella pneumoniae	Light pink colonies with raised margin on MacConkey agar
Enterobacter aerogenes	Light pink colonies with transparent margin on MacConkey agar
Escherichia coli	Deep pink to rose-red colonies on MacConkey agar
Shigella flexinary	Slight pink or whitish colonies on MacConkey agar
Pseudomonas aeruginosa	Colorless to pink colonies on MacConkey agar
Serratia marcescens	Slight pink to red colonies on MacConkey agar

On the other hand, *Bacillus* spp. (25%) and *Staphylococcus aureus* (28%) were observed as most prominent bacterial species among dry food and wet food handlers respectively (Table 4). Among the food makers, *Bacillus* spp (21%) was found mostly; whereas, *Staphylococcus aureus* (34%) was observed notably in food servers (Table 4). Though some of these isolates were known to be potential pathogens, still most were commensal skin flora. But the presence of above bacteria indicated that, the hygienic level among food handlers was not satisfactory. In Taltola and Monihar, no significant Gram negative bacteria were found. But, on the other hand, Gram positive bacteria showed its highest frequency in Taltola, Jessore, from where a wide range of food poisoning and skin infection causing organisms were isolated.

Table 3. Gram staining reaction and biochemical tests of bacterial isolates.

Suspected organisms	Gram reaction	Catalase test	Oxidase test	Coagulase test	Hemolysis test	Starch hydrolysis	Gelatine hydrolysis	Citrate utilisation	Gas production	Lactose ferment	Dextrose ferment	Mannitol ferment	Motility test	Indole test	Urease test	MethylRed test	Voges-Proskauer test	MUG
S. aureus	+C	+	-	+	β	-	+	-	-	+	+	+	-	-	-	+	+/-	N
S. epidermidis	+C	+	-	-	γ	-	+	-	-	+	+	-	-	-	-	+	+/-	N
Bacillus spp.	+R	+	-	-	β	+	+	+/-	-	-	+	+/-	+	-	-	-	+/-	N
Micrococcus luteus	+C	+	-	-	γ	-	+	-	-	-	-	+	-	-	+	-	-	N
S. pyogenes	+C	-	-	-	β	-	-	-	-	-	+	-	-	-	-	+	-	N
S. mutans	+C	-	-	-	α	-	-	-	-	-	+	-	-	-	-	+	-	N
Klebsiella pneumoniae	-R	+	-	N	N	N	N	+	+	+	+	N	-	-	+	+/-	+/-	-
Shigella flexinary	-R	+	-	N	N	N	N	-	-	-	-	N	-	+	-	+	-	-
Pseudomonas aeruginosa	-R	+	+	N	N	N	N	+	+	-	-	N	+	-	-	-	+	-
Serratia marcescens	-R	+	-	N	N	N	N	+	-	-	+	N	+	-	-	-	+	-
Enterobacter aerogenes	-R	+	-	N	N	N	N	+	+	+	+	N	+	-	-	-	+	-
Escherichia coli	-R	+	-	N	N	N	N	-	+	+	+	N	+	+	-	+	-	+

C: Cocci, R: Rod, N: Not necessary for identification.

Table 4. Prevalence of individual pathogens among different food handlers.

Individual pathogens	Indoor Food handlers	Outdoor Food handlers	Dry food handlers	Wet food handlers	Food makers	Food servers
Staphylococcus aureus	22%	17%	12%	28%	17%	34%
S. epidermidis	12%	10%	13%	11%	14%	7%
Bacillus spp.	21%	10%	25%	6%	21%	8%
Micrococcus luteus	15%	0%	9%	6%	7%	8%
Streptococcus pyogenes	3%	0%	6%	0%	5%	0%
S. mutans	3%	7%	0%	8%	5%	0%
Klebsiella pneumoniae	9%	33%	13%	26%	19%	15%
Shigella flexinary	3%	0%	0%	3%	0%	4%
Pseudomonas aeruginosa	3%	0%	0%	3%	0%	4%
Serratia marcescens	3%	0%	0%	3%	0%	4%
Enterobacter aerogenes	6%	10%	13%	6%	5%	8%
Escherichia coli	0%	13%	9%	0%	7%	8%

Discussion

Food handlers are becoming a major threat to various food-borne illnesses all over the world especially in the developing countries like Bangladesh. In this study, the high prevalence of *Staphylococcus aureus* and *Klebsiella pneumoniae* indicates the poor personal hygiene among the food handlers. Other findings of both Gram positive and Gram negative bacteria strongly suggested that, they might be affected with various enteric infections or skin infections or had the habit of sneezing, itching the body and pocking nose or teeth by fingers. The presence of bacterial isolates was varied from restaurants to restaurants, handlers to handlers and dry-food to wet-food preparation. Congenial atmosphere for growth, available nutrient supply, reservoirs like infected food handlers and unhygienic condition inside the restaurants may lead to different types of organism's presence. It also largely depends on food handler's life styles, hygienic status and types of food they handled.

The availability of *Staphylococcus aureus, Micrococcus luteus and Streptococcus pyogenes* indicate the existence of skin infections in food handlers which is the consequence of unhygienic status and improper hand washing. However, *Staphylococcus epidermidis* and *Streptococcus mutans* may be present as normal flora in skin and teeth. But, the presence of *Klebsiella pneumonia*, *Staphylococcus aureus, Bacillus* spp., *Enterobacter aerogenes, Escherichia coli, Shigella flexinary, Pseudomonas aeruginosa* and *Serratia marcescens* can lead to significant microbiological threat to the consumers causing food poisoning.

Due to limited water using facilities in outdoor restaurants, most of the time food handlers do not wash their hands properly after using toilet and become contaminated by various enteric bacteria. Besides, dust from road sides may contaminate handlers and the food they handle. In the present study, outdoor food handlers harbored *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Enterobacter aerogenes* and *Escherichia coli* can lead to causing food poisoning. But the food handlers in indoor restaurants, although work in restricted environment but may have potential risk to the consumers similar to the outside restaurants. The food handlers might be contaminated by the utensils or indirectly by their stools or from the raw foods as they handled a range of raw food products every day. It was observed that, there was no hand sanitizer on wash basins for their personnel to wash their hands after using toilet or handling foods or raw ingredients. Asymptomatic carrier and infected handlers also play an important role since they shed the pathogenic organisms. It was reported that, many food workers continue to work while they were suffering from diarrhea, vomiting or pyrexia (Chironna, 2004; Dunn *et al.*, 1995; Hundy and Cameron, 2002; Patterson *et al.*, 1997).

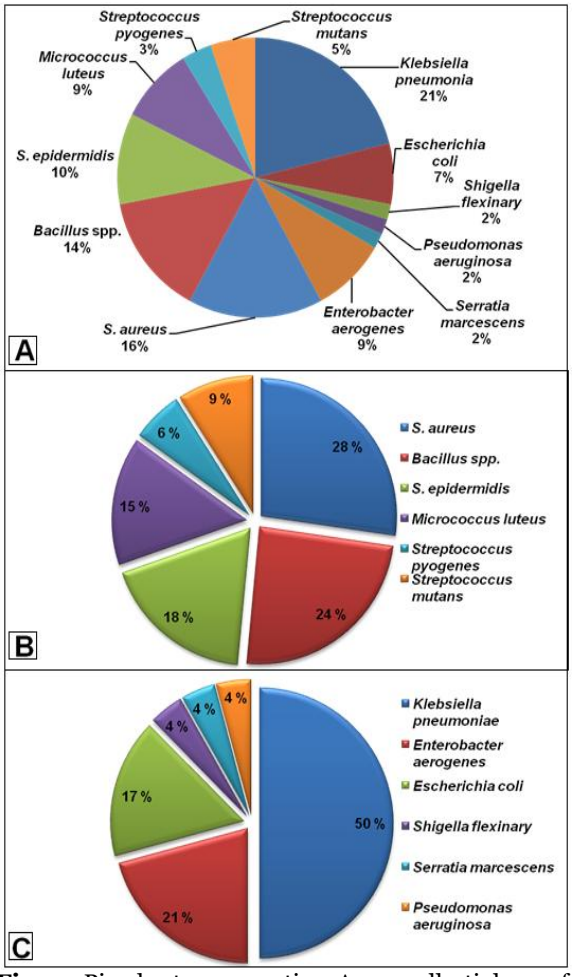

Fig. 1. Pie-chart representing A. overall etiology of bacteria (both Gram positive and Gram negative); B. Prevalence of total Gram positive bacteria; and C. Prevalence of total Gram negative bacteria among food handlers.

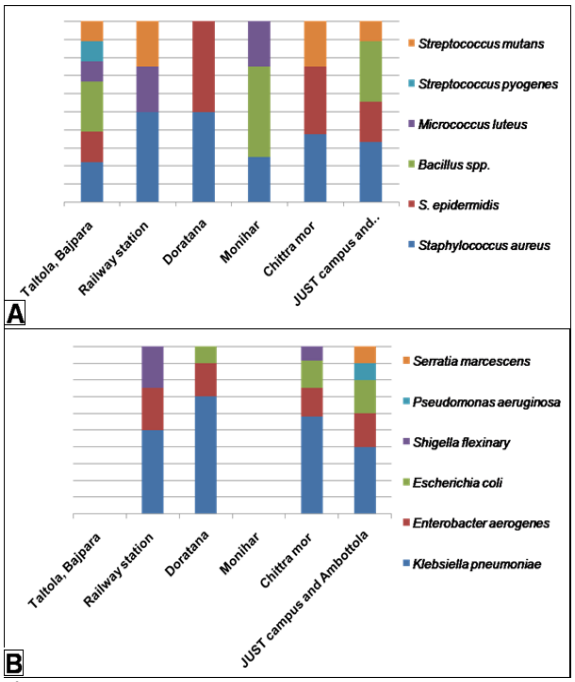

Fig. 2. Pie-chart representing bacterial prevalence among food handlers from different regions of Jessore city A. Gram positive bacteria, B. Gram negative bacteria.

On the other hand, the dry-food handlers although endowed with significant pathogens like *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus* spp., *Enterobacter aerogenes*, *Escherichia coli*, *Streptococcus pyogenes* etc perhaps possess less potential risk to the consumers as most of the organisms died during the frying process or the oil added with the food products like muri-moshla had anti-microbial activities which destroyed most of the contaminating bacteria. Again dry foods have low water activity a_w in which most of the bacteria cannot survive longer. But wet foods have a high water activity a_w which support the growth of most of the bacteria transmitted from food handlers (Frazier and Westhoff, 2011). The presence of significant load of *Klebsiella pneumonia* and *Staphylococcus aureus* in hands of food maker's posses more potential threat to the consumers than the food servers because the formers are directly involved in food preparation. Contamination usually occurred by the food servers are only in the case of miss handlings.

Conclusion

Although food is the substrate that supplies energy to the body and keeps the body fit for work, contaminated food hamper body functions instead. So, everybody should be aware of self hygiene during consuming foods and also be concerned about what we consumed.

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