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Article

Detection of enteric bacteria in the popular street food *chotpoti* in Dhaka, Bangladesh

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Abstract: Contaminated street food has been linked to food-borne illness and food-borne outbreak. *Chotpoti* is a popular street food in Bangladesh. This study was conducted to identify enteric bacteria in *chotpoti* sold in different parts of the capital city Dhaka. From July to October, 2012 we purposively selected 18 sites where *chotpoti* was sold in Dhaka city. From each site we randomly selected six *chotpoti* stalls for sample collection. At each stall we aseptically collected approximately 100 gm of a combined sample of *chotpoti*, chili sauce and water used for cleaning utensils. The combined sample was homogenized and was serially diluted in a sterile test tube with normal saline to obtain a dilution up to 10^{-5}. From each test tube 0.1 ml of dilution was plated on to MacConkey and Blood agar plates under aerobic conditions at 37˚C for 24 to 48 hours. Bacterial growth was identified by the colonial morphology, Gram stain, and biochemical tests. Of the 108 samples we analyzed, 84 (78%) were contaminated with bacterial pathogens. Among isolated organisms *Acinetobacter* (66%) was the most dominant followed by *Klebsiella* spp. (54%), *E. coli* (3%) and *Proteus* spp. (0.9%). Two different organisms were present in 47 (44%) samples. In half of the study sites, every sample was contaminated; in only one site bacterial contamination was absent in all samples. Contaminated street food like *chotpoti* may cause food borne illness and pose risk of food borne epidemics. Further studies are required for quantitative assessment of pathogens to detect potential sources of contamination and to develop interventions that can effectively reduce street food contamination.

Keywords: street food; *chotpoti;* microbiological contamination; food safety; Bangladesh

1. Introduction

Food-borne disease (FBD) has been recognized as a growing public health problem worldwide (Bondi *et al*., 2014). In the United states, FBD causes approximately 76 million illnesses, 325000 hospitalizations and 5000 deaths annually (Mead *et al*., 1999). In Bangladesh, about 30 million people suffer from food borne illnesses each year (FAO, 2012). FBD includes a wide range of illness caused by bacterial, viral, parasitic or chemical contamination of food (Cliver *et al*., 2011). Diarrheal diseases are the commonest form of FBD (Keusch *et al*., 2006). Approximately 2.2 million deaths caused by diarrheal diseases are recorded annually worldwide and most of these cases are attributed to contaminated food and water (WHO, 2002; Teplitski *et al*., 2009).

Contamination of street food by chemical and microbiological pathogen is believed to be a significant contributor to FBD (FAO, 1999). People consuming street vended food have been reported to suffer from food-

borne diseases like diarrhea, cholera, typhoid fever and food poisoning (Rane, 2011). Street food has often been associated with travelers' diarrhea (Ericsson, 2003). A study on risk factors for acute and persistent diarrhea in urban Ghana revealed that about 60% of mothers supplemented their children's diet with street food and that there were higher levels of contamination in the street food given to these children than in food cooked at home. The study also showed that children who were feed street food had an increased risk of both acute and persistent diarrhea (Mensah *et al*., 2002). Food handlers can also become carriers of pathogenic food microorganisms such as *Salmonella* spp. (Zaid *et al*., 2011). Vollard *et al.* (2004) identified street food as an independent risk factor for typhoid and paratyphoid fever caused by *Salmonella typhi* and *Salmonella paratyphi* respectively and showed that one in every twenty-five food vendors excreted *Salmonella* spp. in their feces.

Microbiological studies carried out on street-food vending in several developing countries have reported high bacterial counts in food (Das *et al*.; Dawson and Canet, 1991; Ghosh *et al*., 2007a; Mahale *et al*., 2008b; Feglo and Sakyi, 2012). A study on street vended Chilli sauces in Mexico City showed 40% of samples with fecal contamination and 5% harbored sufficient enterotoxigenic *Escherichia coli* to cause disease (Estrada-Garcia *et al*., 2002). A study on various types of ready-to-eat-food samples in Alice, South Africa showed bacterial growth in all the food types tested and found 22% of samples contaminated with *Listeria spp.* 18% with *Enterobacter spp.* 12% with *Aeromonas hydrophila,* 8% with *Klebsiella oxytoca*, 6.3% with *Proteus mirabilis,* 3.2% with *Staphylococcus aureus* and 2.4% with *Pseudomonas luteola* (Nyenje *et al*., 2012). Microbiological analysis of street vended fruit Juices from Mumbai city, India showed a total viable counts of log 6.5 cfu/100ml in all 30 samples with significant load of coliforms, faecal coliforms, *Vibrio* and *Staphylococcal* counts (Mahale *et al*., 2008a). Many of the pathogens isolated from street vended food has been reported to be resistant to commonly used antibiotic (Bello *et al*., 2013). Street vended food has also been implicated in several outbreaks of food borne diseases. In 1981, a cholera epidemic in Pune city, India was attributed to contaminated sugar cane juice with added ice, the ice was found to be contaminated with *Vibrio Cholerae* (Bhat and Waghray, 2000). In Senegal, more than 200 food poisoning cases were reported following consumption of dairy products (Dawson and Canet, 1991). In Cuba, 14 people died and 70 were hospitalized after eating fried foods sold by private vendor (Kubheka *et al*., 2001a). Street foods were responsible for 691 food poisoning outbreaks and 49 deaths from 1983 to 1992 in Shandong province, China (Xingling, 2003).

Like many developing countries, with rapid urbanization the street food industry is growing in Bangladesh .The street food vendors operate their business in bus terminals, industrial areas, market places, streets and railway stations (Suneetha *et al*., 2011). During holidays, festivals, and weekends the floating population highly increase and more people consume street food. The street food industry feeds thousands of people daily with a range of food items. It also plays an important role in providing employment opportunities for millions of men and women with limited education or skills, especially as the initial investment is low (Kubheka *et al*., 2001b; Ghosh *et al*., 2007b). Street foods have unique flavor, are easily accessible and cheap (Tambekar *et al*., 2008; Jouve *et al*., 2010). These foods are also the source of nutrition for the poor urban people. While street foods are an important source of ready-to-eat nutritious, low-cost meals for the urban poor, the health risk posed by such foods may outweigh their benefits (WHO, 2013). Street foods displays on open yards and easily be contaminated by dust, smoke, insects, hands of intending buyers and rains. The places has limited access to basic sanitary facility such as running water, garbage disposal and clean toilets (Feglo and Sakyi, 2012). A study of the socio-economic conditions and determination of the hygienic and sanitary practices of street food vendors in Dhaka city corporation area by FAO in 2010 demonstrated that 25% street food vendors have no formal education. They reportedly work for 13 to 18 hours a day without having toilet facilities. Most of the vending shops (68%) were located on the footpath irrespective of areas surveyed and 30% vending carts were placed near municipal drains and 18% near sewage. A microbiological study on different foods items, drinking water and hand swab samples revealed high numbers of aerobic bacteria and coliform count. The study also suggested the need to conduct a detailed microbial analysis and profile their drug resistance characteristics to assess the potential public health hazards (FAO, 2012).

Chotpoti, a mixture of potatoes, chickpeas, onions, and chilies topped with grated egg, is one of the most popular street foods in Bangladesh. People of all ages and socioeconomic levels frequently consume *chotpoti*. *Chotpoti* stalls are available in most public places such as bus stop, market places, parks, school, colleges and university premises. On an average 50-100 consumers take *chotpoti* from each stall each day. Because it is widely consumed and the popularity is ever increasing and previous studies did not exclusively concentrated on this food item, we designed this study to evaluate the detailed microbial status including food borne pathogen and spoilage bacterial content of *chotpoti* sold in different parts of Dhaka, the capital city, Bangladesh.

2. Materials and Methods

2.1. Study site and study population

Between July 2012 and October, 2012, we purposively selected 18 study sites including marketplaces, bus stops, roadside stalls and amusement parks located within Dhaka metropolitan area, an urban setting with an area of 360 square kilometers and a population of about 15 million (Figure 1).These sites are popular places for *chotpoti* vending and consumption, where many people eat *chotpoti* as an evening snack. Our study participants were *chotpoti* vendors who sell *chotpoti* within Dhaka metropolitan area.

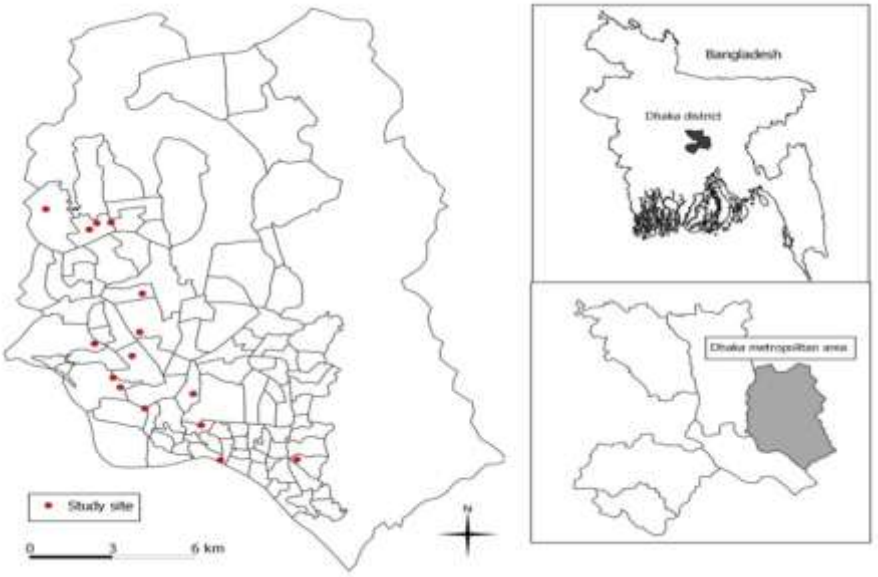

Figure 1. Location of study sites in Dhaka Metropolitan Area.

2.2. Selection of *chotpoti* stalls

Data was not available on the total number of *chotpoti* vending sites and exact number of *chotpoti* vendors in Dhaka metropolitan area, so considering our time and resources we purposively selected 18 study sites for sample collection. At each study site there were usually 15 to 20 *chotpoti* stalls. From each study site we selected six *chotpoti* stalls. First we numbered every stall. Then we randomly selected six stalls using random number table and collected data and samples from 108 vendors.

2.3. Sample collection

From each stall, trained field assistants collected *chotpoti*, chili sauce and water used for cleaning utensils. All three types of samples were combined and collected in one sterile container for each stall. Approximately 100 gm of combined sample was collected from each stall. The container was placed in a cool box maintaining a temperature of between 4°-6°C. The samples were labeled, packaged and sent to the Bangabandhu Sheikh Mujib Medical University (BSMMU) Microbiology laboratory in Dhaka for analysis within four hours of collection.

2.4. Isolation and identification of microorganisms

Laboratory personnel isolated and identified microbes using serial dilution and spread plate technique. One gram of the combined sample was homogenized using a sterile mortar and pestle. One milliliter of the resultant homogenate was added to 9 ml of sterile normal saline in a test tube and diluted serially to obtain 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} up to 10^{-5} dilutions. From each test tube 0.1 ml of the dilution was aseptically pipetted out and plated onto MacConkey agar and blood agar. All the bacterial plates were incubated in an inverted position under aerobic conditions at 37°C for 24 to 48 hours. The inoculated plates were then observed for bacterial growth. Confirmatory identification of the bacterial pathogen was done by gram staining and biochemical tests (Kannan, 2002). Identification of bacterial pathogens was carried out according to Bergey's Manual (Sneath *et al*., 1986).

2.5. Data analysis

We calculated the percentage of contaminated samples. Contamination was defined as presence of one or more pathogens in a sample. We also calculated the percentage of different types of pathogens present in combined

chotpoti samples. Percentage of contaminated samples from each study site was also calculated to identify the sites with the highest level of contamination.

2.6. Ethical considerations

The *chotpoti* vendors provided informed written consent both for the interview and to provide samples for laboratory testing.

3. Results

A total of 108 samples were collected and analyzed and 84 (78%) were contaminated. We isolated *Acinetobacter* in 71 (66%) samples, *Klebsiella* spp. in 58 (54%), *E. coli* in 3 (2.8%), and *Proteus* spp. in 1 (0.9%) samples. Two different pathogens were present in 47 (44%) samples (Table 1).

One hundred percent of the samples were contaminated at half of the study sites. In one site bacterial contamination was absent in all samples (Table 2).

Table 1. Types of isolated bacteria in combined *chotpoti* samples (N=108) in Dhaka, Bangladesh during July-October, 2012.

Types of bacteria	Number (%) of positive samples
Acinetobacter	71 (66)
Klebsiella spp.	58 (54)
E. coli	3 (2.8)
Proteus spp.	1 (0.9)
More than one type of bacteria	47 (44)

Table 2. Percentage of contaminated *chotpoti* samples (N=6) by vending site, Dhaka, Bangladesh.

Vending place	Number (%) of contaminated samples
Shahbag	6 (100)
Dhaka Zoo, Mirpur	6 (100)
Rayer Bazar	6 (100)
Babubazar, Mitford	6 (100)
Donia College	6 (100)
Mohammadpur Bus stop	6 (100)
Shishu Park	6 (100)
Zigatola, Dhanmondi	6 (100)
Mirpur-10 Bus Stop	6 (100)
Mirpur Stadium	5 (83)
Suhrawardi Uddan	5 (83)
Dhanmondi-15	5 (83)
Parliament area	4 (67)
Jatrabari bus stop	4 (67)
Jurain Rail gate	4 (67)
IDB Vhaban	2 (33)
New Market	2 (33)
Benaroshi Polli, Mirpur	0 (0)

4. Discussion

We detected at least one of four types of enteric bacteria in more than three-fourths of the *chotpoti* samples. The predominant bacterium was *Acinetobacter*. All study sites but one sold contaminated *chotpoti*. These results provide evidence for the potential risk of *chotpoti* consumption and useful directions for further research to develop intervention strategy.

Acinetobacter, *Klebsiella*, *E. coli*, and *Proteus* are transmitted via contaminated food and water (Leclerc *et al*., 2002). In our setting, contamination might be primarily due to the water used for food preparation, since *chotpoti* vendors use municipal tap water which is often contaminated (Faruque *et al*., 2010; Nahar *et al*., 2011). Contamination might also occur due to poor food handling practices of the vendors as most vendors lack awareness and knowledge of food safety and have not been formally trained in food safety (Fang *et al*., 2003; Duff SP, 2003; Barro *et al*., 2006b; Chukuezi, 2010; Al Mamun *et al*., 2013). Bacterial transmission via flies might also occur (De Jesús *et al*., 2004; Barro *et al*., 2006a).

The isolated pathogens can cause mild to severe disease including food poisoning (Leclerc *et al*., 2002). *Acinetobacter* was the predominant pathogen in this study whereas *E.coli* was found in a small proportion of samples. In many other studies, *E. coli* was detected frequently in street food samples (Bhaskar *et al*., 2004; Estrada-Garcia *et al*., 2004; Hanashiro *et al*., 2005; Lewis *et al*., 2006). *Acinetobacter* is transmitted via contaminated hands and environmental surfaces (CDC, 2014a) indicating poor hand hygiene of the vendors. This aerobic gram-negative bacteria is widely distributed in the environment (soil, water, food sources, environmental surfaces and people's skin) (CDC, 2014a). *Acinetobacter* is harmless in most healthy people but some species may cause life-threatening hospital acquired infection including pneumonia, blood stream infection and urinary tract infection (Fournier *et al*., 2006; Visca *et al*., 2011). We should be concerned about the presence of this increasingly antibiotic resistant emerging pathogen in food samples because it might cause widespread outbreaks both in hospital setting and community setting (Barbolla *et al*., 2003; Perez *et al*., 2007).
Presence of *E.coli* implies fecal contamination of water and ingredients used for *chotpoti* preparation or hand contamination of the vendors. The infectious dose of *E. coli* is large (10^6 - 10^8 of organisms) and pathogenicity depends on strains (CDC, 2014b). Some strains cause diarrhea, others causes urinary tract infection, respiratory illness and other illnesses (Todar, 2016). Further studies could characterize the pathogen at species level and quantify risks associated with different species. *Proteus* is also transmitted through contaminated food and water and is commonly responsible for urinary and septic infection (Coker *et al*., 2000; Ronald, 2002).The presence of respiratory pathogens such as *Klebsiella* might be attributed to the bacterial aerosols generated from sneezing and coughing in public places (Fiegel *et al*., 2006). *Klebsiella* can lead to a wide range of diseases including pneumonia, urinary tract infections, septicemia, and soft tissue infections (Podschun and Ullmann, 1998).
We detected bacterial presence at genus level and did not quantify or use any bacterial parameters to detect microbial quality of food samples. Therefore it was not possible to assess the actual risks associated with *chotpoti* consumption, as the presence of a bacterial pathogen does not always carry a risk if the total count is low. However, all the identified bacteria are pathogenic and can cause a range of food borne illness, which is suggestive of a potential risk of *chotpoti* consumption. We collected combined *chotpoti* samples *(chotpoti*, chili sauces and water used for cleaning utensil) in one container so it was not possible to identify which of these three components was contaminated. Further study could specifically test each component to obtain more precise information about the source of contamination to design specific interventions. Without a complete list of all available *chotpoti* vending sites in the Dhaka metropolitan area, we selected some of the popular locations purposively as study sites. Therefore the results may not be generalizable to all *chotpoti* vendors within the Dhaka metropolitan area or other urban settings in Bangladesh or other low-income countries. However, these results might be generalizable to some of the other food items sold by street vendors in the study sites.

5. Conclusions

Consumption of contaminated street vended food like *chotpoti* may pose risk of foodborne illness and even food borne epidemics. Frequent occurrence of nosocomial pathogens like *Acinetobacter* highlights the need to increase awareness among vendors and consumers about food safety. Education and training of street food vendors on personal and food hygiene could improve their food handling practices and might reduce food contamination. Further studies are required for quantitative enumeration of pathogens and to identify potential sources of contamination to develop effective intervention strategies that will improve the safety of street food.

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Conflict of interest

None to declare.

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