Microbial Status and Multidrug Resistance Pattern of Pathogenic Bacteria Isolated from Street Food in Dhaka City, Bangladesh

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Authors’ contributions

This work was carried out in collaboration between all authors. Authors STT, AB and SD designed the study. Authors AB and MA managed the experimental process and analyses of the raw data. Authors STT and AB wrote the protocol and the first draft of the manuscript. Author MA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aims to evaluate the microbiological safety in street foods available in Dhaka city, Bangladesh.

Study Design: Seven categories of street foods were collected aseptically in triplicates from 10 locations of the most populous areas of Dhaka city, transported to the microbiology laboratory of Center of Excellence, Primeasia University, Dhaka. The samples were analyzed for microbiological quality.

Place and Duration of Study: The study was carried out in Dhaka city, Bangladesh, between November 2015 to March 2017. The microbiological analysis was done at the microbiology laboratory of the Center for Excellence Laboratory (CEL), Department of Microbiology, Primeasia University, Dhaka-1213, Bangladesh.

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Methodology: Ninety street food samples belonging to 7 different categories were collected aseptically from ten different places in Dhaka. All samples were tested according to the standard food analysis methods. Total viable count (TVC), total coliform count (TCC), total Salmonella-Shigella count (TSSC) and total S. aureus count (TSAC) were estimated by using Plate Count agar (PCA), MacConkey agar, Salmonella-Shigella agar and Mannitol Salt agar plates respectively. Kirby-Bauer disc diffusion method on Mueller Hinton agar was used to determine the sensitivity of the isolated strains to commonly prescribed antibiotics.

Results: Fried Aubergine, sugarcane juice, potato balls, peanut, rice cake, sweetened coconut, local salty snacks nimki and chanachur, sesame cookies revealed high total viable count ($10^{11}$ CFU/gm or/mL) and high total coliform count ($10^9$ CFU/g or/mL). Some street foods were found to contain potential pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Vibrio* spp. and *Campylobacter* spp. Some of the isolates of *E. coli*, *S. aureus* and *Salmonella* isolates were found to be resistant against azithromycin (15 µg), sulphomethoxazole (25 µg), penicillin (10 µg), nalidixic acid (30 µg), vancomycin (30 µg) and tetracycline (30 µg).

Conclusion: This study reveals the presence of pathogenic bacteria in street foods of Dhaka, Bangladesh. Hence, there is a necessity for strict surveillance on microbial safety of street foods. There should be public engagement projects for public awareness against consumption of low-quality and unhygienic street foods of Dhaka, Bangladesh.

Keywords: Street foods; enteric pathogens; Multi-drug resistance; food poisoning; Dhaka.

1. INTRODUCTION

The food-borne outbreak is a pressing issue for public health and economy [1]. Current modification in food production, processing practices and rapidly-changing food habits of the consumer are important factors for the increasing consumption of street foods. Food-borne disease (FBD) represent an important worldwide health problem and now it's involving a wide range of illness caused by viral, bacterial, parasitic and chemical contamination of food [1]. Many of the food-borne illnesses occur due to viruses and bacterial agents [2]. Among food-borne diseases, diarrhoea is one of the most serious global concerns [3]. Approximately 1.7 billion cases of child deaths caused by diarrhoeal diseases are recorded annually worldwide, and most of these cases are attributed to contaminated food and water [4]. The annual reports from the World Health Organization (WHO) stated in Bangladesh, diarrhoea is responsible for one-third of infant deaths and this is likely to be a gross underestimation of the true burden. Limited data from the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) indicates 501 hospital visits per day for treatment of diarrhoea that were attributable to food and water-borne pathogens [5].

Gradually-developing and rapidly-urbanizing countries like Bangladesh are experiencing change in traditional food habits. Changing lifestyle, involvement of woman in official jobs and change in the family structure forces people to consume street foods in contrast to the home-cooked food which was the common practice [6]. During festivals, weekend and holiday’s people roam and consume street food. Unique flavours, easy availability, cheapest price as well as convenient, street foods are attractive option than home-cooked food, especially among the young and low-income community [6,7,8]. Unhygienic conditions, open yards displays and easy contamination from dust, insects, smoke, hands of vendor, lack of access to basic sanitary facilities such as potable water, sanitation of personnel and equipment, lack of disposal of garbage lead to cross-contamination of street foods [9]. People consuming street food on a regular basis are more vulnerable to food-borne diseases like as diarrhoea, cholera, typhoid fever and food poisoning [10,11]. While these street foods often substitute homemade food for the urban population, the unhygienic conditions in which these foods are prepared, stored and served raise a question regarding their microbiological quality.

Furthermore, previously published reports show that street food consumers are prone to diseases caused by pathogenic bacteria such as *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens* and *Vibrio cholera* [12,13,14,15]. Therefore, this study was carried out to evaluate the microbial status and multidrug resistance pattern of pathogenic bacteria isolated from street foods in Dhaka City, Bangladesh.
2. MATERIALS AND METHODS

2.1 Sample Collection and Enrichment Procedure

A total of 90 street food samples were collected in triplicates (fried salty, spicy boiled, sweet sugary solids, fruits, juice and rice cookies) from different vendors from 10 different areas (Banani, Mohakhali, Agargaon, Baridhara, Nikket, Uttara, Rampura, Farmgate, Dhannondi & Newmarket) around Dhaka city between November 2015 to March 2017. All Samples were collected in pre-sterilized zip-lock bags (165 mm x 150 mm x 0.55 mm) and freshly-extracted juice samples (100 mL each) were collected in sterile bottles, transported to the laboratory in ice-boxes (4°C). All samples were transported to the Centre for Excellence Laboratory (CEL), Department of Microbiology, Primeasia University, Dhaka-1213, Bangladesh within 2 hours for processing and further assessment.

2.2 Sample Preparation

Ten grams of solid food sample was added to 90 mL of normal saline, homogenized and prepared for spread plate technique. For juice samples, 10 mL of samples were properly diluted in 90 mL sterile normal saline (0.85% NaCl). One mL of each homogenate from samples was collected in sterile bottles, transported to the laboratory in ice-boxes (4°C). All samples were transported to the Centre for Excellence Laboratory (CEL), Department of Microbiology, Primeasia University, Dhaka-1213, Bangladesh within 2 hours for processing and further assessment.

2.3 Isolation and Identification of Specific Pathogens

The pre-enrichment technique was used to detect delicate food pathogens E. coli, Salmonella spp, S. aureus, Vibrio spp., and Campylobacter spp. Twenty-five gm of each sample was homogenized in 225 mL of buffered peptone water (Oxoid Ltd, Hampshire, England) and incubated at 37°C for 20 to 24 hours, followed by culture in specific medium as detailed in the next sub-sections.

2.3.1 Presumptive identification of Salmonella spp

One mL of pre-enrichment culture was mixed with 10 mL of Henja Tetrathionate Broth (HiMedia Laboratories, Mumbai, India) and was incubated at 37°C for 20 to 24 hours. The culture broths were subsequently streaked onto Salmonella-Shigella agar (SSA) (Oxoid Ltd, Hampshire, England) and Bismuth Sulphite agar (BSA) (HiMedia Laboratories, Mumbai, India) to identify Salmonella spp.

2.3.2 Presumptive identification of Vibrio spp

One mL of the homogenized food sample was mixed with 9 mL of alkaline peptone broth (Oxoid Ltd, Hampshire, England), incubated at 37°C for 20 to 24 hours at alkaline level pH (8-9) spread onto Thiosulfate Citrate bile salts sucrose (TCBS) agar media (Oxoid Ltd, Hampshire, England) and incubated for 24 hours at 37°C to identify Vibrio spp.

2.3.3 Presumptive identification of Campylobacter spp

One mL of the homogenized food sample was mixed with 10 mL of Preston Campylobacter Enrichment Broth (PCE) (Oxoid Ltd, Hampshire, England) and was incubated at 37°C for 8 hours. Then the culture broth was spread on Charcoal Cefoperazone Deoxycholate agar (CCDA) media (Oxoid Ltd, Hampshire, England) incubated for 24 hours at 37°C in aerobic condition for the presumptive identification of Campylobacter spp.

2.3.4 Presumptive identification of E. coli

The pre-enriched 1 mL cultures were mixed with 9 mL lactose broth medium (Oxoid Ltd, Hampshire, England) with Durham fermentation tubes and incubated at 37°C for 20 to 24 hours. Gas production in the tubes was used to indicate the presence of faecal coliforms. The enrichment culture streaked onto Eosine Methylene Blue agar (EMB) (Oxoid Ltd, Hampshire, England) and MacConkey Agar Media (Oxoid Ltd, Hampshire, England) and incubated for 24 hours at 37°C to identify E. coli.

2.3.5 Presumptive identification of S. aureus

The homogenized food sample was streaked onto Mannitol Salt agar medium and incubated at 37°C for 20 to 24 hours to identify S. aureus.
2.4 Biochemical Tests

All pure discrete colonies from selective media were sub-cultured on nutrient agar and subjected to biochemical tests for confirmation of initial isolation procedure. Kligler Iron agar (KIA) (HiMedia Laboratories, Mumbai, India), Simmons Citrate Agar media (Oxoid Ltd, Hampshire, England), MR-VP media (Oxoid Ltd, Hampshire, England) and SIM media (Oxoid Ltd, Hampshire, England) were used for biochemical tests. In addition, oxidase test, catalase test, coagulase test, Carbon Utilization profiling and extracellular enzyme production tests were also done with appropriate reagents (all from Hi-Media Laboratories, Mumbai, India). All isolates were identified presumptively up to the Genus level according to Bergey’s Manual of Determinative Bacteriology (6th edition) [16,17].

2.5 Antibiotics Susceptibility Test

The isolates of different genera were subjected to antibiotic resistance profiling in vitro through Kirby-Bauer method [18] against azithromycin 15 µg (AZM), ciprofloxacin 5 µg (CIP), sulfamethoxazole 25 µg (SXT), tetracycline 30 µg (TE), imipenem 10 µg (IPM), streptomycin 10 µg (S), meropenem 10 µg (MEM), penicillin 10 µg (P), vancomycin 30 µg (VA), rifampicin 5 µg (RIF), nalidixic acid 30 µg (NA) (all from Oxoid, New Hampshire, England) and SIM media (Oxoid Ltd, Hampshire, England), MR-VP media (Oxoid Ltd, Hampshire, England), and KIA were used for biochemical tests. In addition, oxidase test, catalase test, coagulase test, Carbon Utilization profiling and extracellular enzyme production tests were also done with appropriate reagents (all from Hi-Media Laboratories, Mumbai, India). All isolates were identified presumptively up to the Genus level according to Bergey’s Manual of Determinative Bacteriology (6th edition) [16,17].

2.6 Statistical Analysis

After conducting biochemical analysis of isolates, results were analyzed in STATA 14.1 statistical program for cluster analysis by multivariate analysis inward linkages.

3. RESULTS

The present study was conducted to isolate and identify bacteria in street foods with the status of vendors, vending site and food handling practices along the streets in Dhaka city. A total of 157 isolates were obtained from 90 samples from 10 significant locations. Samples included 7 different types: fried spicy snacks, rice cakes, fruits, juice, sweet snacks, spicy crackers & homemade packet snack are shown in Table 1.

The microbial assay and colony counts were measured. The zone diameter for individual antimicrobial agents was then interpreted into categories of susceptible, intermediate and resistant according to the guidelines from National Committee for Clinical and Laboratory Standards (NCCLS, 2015) [19]. The results from this assay were compared to the findings of other similar studies by Paul et al., Uddin et al., Mahfuza et al., Tabashsum et al., [20, 21, 22, 23].
intestine, resulting in probable disease. The occurrence of overwhelming numbers of *Salmonella* or *E. coli* or both in many food items makes them absolutely unfit for consumption. Potato balls, stuffed dumplings (shingara), egg balls, cucumber and sugarcane juice from Banani showed high TSSC count (10^7 CFU/g), which is potentially harmful for consumption. Fried products such as fried dumplings (samosa), potato balls and fried aubergine from Banani showed the highest range of TSAC (10^5 to 10^7 CFU/gm). The summary of the microbial isolates from the food samples are given in (Fig. 1A-J).

All the 90 samples of street food showed presence of various bacterial pathogens. *E. coli* was found in 28 samples, *Salmonella* spp. in 40 samples, *Campylobacter* spp. in 5 samples, *Vibrio* spp. in 18 samples and *S. aureus* in 66 samples (Fig. 2). Biochemical tests revealed biochemical profile representative of respective genera presented in Bergey’s Manual of Determinative Bacteriology [17].

<table>
<thead>
<tr>
<th>Location in Dhaka City</th>
<th>Food item</th>
<th>Food category</th>
<th>Food content</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Market Area Nilkhet</td>
<td>Hogplum, cucumber, pineapple, local plum Carrot, Guava</td>
<td>Raw fruit</td>
<td>Sour solid fruit</td>
</tr>
<tr>
<td>Dhanmondi Farmgate Agargaon</td>
<td>Sweet pea</td>
<td>Boiled food</td>
<td>Boiled sweet pea, onion, spices</td>
</tr>
<tr>
<td>Rampura Mohakhali Banani Baridhara Uttara</td>
<td>Monakki, Morali, Coconut chips</td>
<td>Sugar-coated crisp</td>
<td>Crispy sticks made of wheat flour coated with sugar</td>
</tr>
<tr>
<td></td>
<td>Kadma, Sesame sticks, Coconut crisp</td>
<td>Sugary solid</td>
<td>Traditional sweet-balls made of sugar</td>
</tr>
<tr>
<td></td>
<td>Onion crisp</td>
<td>Salty fried food</td>
<td>Onion and mashed lentil fried with spices</td>
</tr>
<tr>
<td></td>
<td>Fried peanut</td>
<td></td>
<td>Roasted peanut fried in oil with salt, sweet and spices</td>
</tr>
<tr>
<td></td>
<td>Potato balls</td>
<td></td>
<td>Mashed potato moulded into balls with corn flour, onions and spices</td>
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<tr>
<td></td>
<td>Fried Aubergine</td>
<td></td>
<td>Sliced aubergine coated with flour and spice</td>
</tr>
<tr>
<td></td>
<td>Chanachur</td>
<td></td>
<td>Assortment of wheat flour, lentil flour and nuts</td>
</tr>
<tr>
<td></td>
<td>Stuffed dumplings</td>
<td></td>
<td>Cooked vegetables and meat wrapped with thick layer of wheat flour</td>
</tr>
<tr>
<td></td>
<td>Fried dumplings Potato chips</td>
<td></td>
<td>Meatballs cooked with spice Fried slices of potato</td>
</tr>
<tr>
<td></td>
<td>Nimki</td>
<td></td>
<td>Fried chunks of wheat flour</td>
</tr>
<tr>
<td></td>
<td>Soup</td>
<td>Thick salty liquid</td>
<td>Corn flour, salt, sugar, spice, protein, lipid</td>
</tr>
<tr>
<td></td>
<td>Puffed rice</td>
<td>Salty roasted</td>
<td>Puffed rice roasted in oil and spice</td>
</tr>
<tr>
<td></td>
<td>Popcorn Sweatpea</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sugarcane Juice</td>
<td>Sugary liquid</td>
<td>Fresh pressed juice of sugarcane</td>
</tr>
<tr>
<td></td>
<td>Rice cake</td>
<td>Salty steamed</td>
<td>Soft lumps of rice flour steamed</td>
</tr>
<tr>
<td></td>
<td>Lemon juice</td>
<td>Acidic liquid</td>
<td>Lemon, salt and sweet mixtures.</td>
</tr>
</tbody>
</table>
1A. Microbial counts of food from Banani

1B. Microbial counts of food from Agargaon

1C. Microbial counts of foods from Uttara

1D. Microbial counts of foods from Nilkhet
1E. Microbial counts of foods from New Market Areas

1F. Microbial counts of foods from Rampura

1G. Microbial count of foods from Dhamnmondi

1H. Microbial counts of foods from Baridhara
1. Microbial counts of foods from Farmgate

1J. Microbial counts of foods from Mahakhali

Fig. 1 (A-J). Bacterial count for food samples from 10 different areas in Dhaka city

Note: TVC (Total viable count), TCC (Total coliform count), TSSC (Total Salmonella-Shigella count), TSAC (Total Staphylococcus aureus count)

Food items: fried aubergine=beguni, stuffed dumpling=singara, lemon juice=lebur shorbat, sugarcane juice=akher shorbot, alu crisps=peyaju, sesame sticks=tiler khaja, jhalmuri=puffed rice, local plum=boroi, sweatpea=motor, rice cake=chetoi pitha, coconut crisp=narikel chira, coconut chips=narikel vaja, pineapple=anarosh, Guava=peyara, hogplum=amra, potato chips=alu chips
Results from the antibiogram of the isolated microorganisms are shown in Fig. 3. *Salmonella* spp. showed the highest degree of resistance against tetracycline, vancomycin, penicillin and streptomycin (100%), followed by rifampicin (95%), meropenem (80%) and azithromycin (75%) (Fig. 4). The other isolates, *Campylobacter* spp. and *S. aureus* were found to be sensitive to nalidixic acid having 15% and 10% resistance respectively whereas highest susceptibility was found against imipenem (80%). Most potent faecal coliform *E. coli* showed moderate level of sensitivity against meropenem (60%), azithromycin (45%), streptomycin and ciprofloxacin (both were 40%). Additionally nalidixic acid, vancomycin, penicillin and tetracycline were not effective against *E. coli* as it showed about 100% resistance. *Klebsiella* spp. was resistant to tetracycline (100%) and streptomycin, penicillin, vancomycin (90%). However, *Klebsiella* isolates were sensitive to imipenem (90%) (Fig. 4).

The multi-drug resistance profile of potential food-borne pathogens identified in this study is of concern (Fig 4). Most of the isolates of the *Salmonella* spp. are resistant against streptomycin, nalidixic acid, azithromycin, rifampicin and penicillin. *S. aureus* isolates were found to be resistant against penicillin, meropenem, sulphomethoxazole, nalidixic acid and to a lesser extent, vancomycin. The *Campylobacter* isolates were found to be resistant against sulphomethoxazole, nalidixic acid, ciprofloxacin, azithromycin and penicillin. The *Vibrio* isolates showed resistance to nalidixic acid, ciprofloxacin, azithromycin, rifampicin, vancomycin, tetracycline and meropenem. The *E. coli* isolates were found to be resistant against streptomycin, sulphomethoxazole, nalidixic acid, ciprofloxacin and rifampicin.
4. DISCUSSION

Fried Aubergine, sugarcane juice, potato balls, peanut, rice cake, sweetened coconut, local salty snacks nimki and chanachur, sesame cookies revealed high total viable count (10^11 CFU/gm or /mL) and high total coliform count (10^6 CFU/g or /mL). Some street foods were found to contain potential pathogens such as Escherichia coli, Staphylococcus aureus, Salmonella spp., Vibrio spp. and Campylobacter spp. Some of the isolates of E. coli, S. aureus and Salmonella isolates were found to be resistant against azithromycin (15 µg), sulphomethoxazole (25 µg), penicillin (10 µg), nalidixic acid (30 µg), vancomycin (30 µg) and tetracycline (30 µg).

Bacterial diarrhoea contributes to high-level mortality in the developing world [25]. The incidence of typhoid among the urban slum-dwellers is 3.9 episodes/1000 people/year and pre-school children (2-5 years) are 8.9 times more relative to the risk of contracting typhoid [26]. Salmonella spp. comprises 6.4% of the bacterial isolates from diarrhoeal patients in Dhaka city [25]. Enterotoxigenic E. coli is an endemic in Dhaka population [27]. The presence of high microbial load of pathogens in foods is a good indication of the food quality and the potential health risk they pose to consumers [28]. The total count analyses indicated the poor hygienic level of food handling and sanitary condition of retail stores. The aerobic plate count indicated bacterial isolates (E. coli, Salmonella and S. aureus) presented a potential health hazard to consumers.

According to the microbiological standard of foods in Bangladesh, aerobic plate counts ranging from 10^5 to 10^6 CFU/g can be considered safe, 10^6-10^7 CFU/g acceptable, 10^7-10^8 CFU/g, not acceptable from a public health perspective [29]. The findings of this investigation are similar to those from previous observations from Bangladesh [20,21,22,23,28]. The current study indicated more pathogenic bacteria (E. coli, Salmonella spp., S. aureus, Campylobacter spp., Vibrio spp.) in the street food samples. Tabashsum et al. [23] previously showed the TVC (10^6 CFU/g) and TCC (10^4 CFU/g) in stuffed samples. In this study, local snack stuffed dumpling produced TVC of (10^6 CFU/g) TCC of (10^5 CFU/g). Rice cakes from Banani showed TVC (10^6 CFU/g) and Nilkhet showed similar result (10^5 CFU/g) from a previous report [25]. In hog plums and slice cucumber samples, TVC and TCC are found in the same range as Tabashsum et al. [23]. TVC and TCC from fruit samples were similar to other reports [22]. In New Market area, collected food samples (Pineapple, Guava) showed TCC of (10^5 CFU/g), similar to the report of Mahfuza et al. [22]. Sugarcane juice samples showed high TBVC count in every area. Sugarcane samples in Uttara area showed TVC (10^8 CFU/mL) and TSAC (10^7 CFU/mL) similar to a previous work by Uddin et al. [21]. The microbiological quality of puffed rice from Baridhara, fried aubergine from Banani and fried dumpling from Uttara are...
similar to the results from Hoque [29]. The spread of multi-drug resistant isolates poses hazard of an endemic. This could be particularly grim in Bangladesh since loss-of-activity of affordable and well-tolerated drugs might mean a hike in mortality. The \textit{E. coli} isolates found in this study were susceptible to the common antibiotics, indicating reduced risk of therapeutic failure. However, lack of proper training, awareness and improper personal hygiene leads to contamination of street foods to continue and the public health risk to escalate [28].

5. CONCLUSION AND RECOMMENDATION

\textit{E. coli, Salmonella typhi, Campylobacter spp., Vibrio spp.} are pathogenic bacteria which should be absent in food products. \textit{S. aureus} causes food poisoning. Major reason for these food-borne pathogens to be present in food products are mainly due to unhygienic environment for food preparation, the use of contaminated water and ingredients, absence of awareness, training and practice of food sanitation by producers and handlers, disregard of food safety law in Bangladesh, lack of implementation of international standards from consumables goods (Hazard Analysis and Critical Control Point). Street food vendors should be trained and certified by food safety agencies before being able to start up a shop or food carts. Zones or specific locations should be isolated for street food sellers in Bangladesh around markets or shopping district with planning and permission of City Corporation. Regular inspection of food courts should be conducted by mobile courts consisting food specialist, microbiologist and nutritionist. Street food, food carts, food courts in markets should also come under jurisdiction. Food safety laws should be implemented along with corrective actions and preventive measures. Keeping pace with the changing food habits in Dhaka city, the Bangladesh Food and Drug Authority could implement the Food Safety Law more effectively by instructing the retailers not to sell items without ISO 9001 and BSTI approval. Consumers can be alerted about the poor quality of these products and children should be discouraged to consume these products. Raw fruits should be thoroughly washed well before consumption. A universal hand hygiene and hand washing practice should be encouraged across all walks, ages and levels of society, emphasizing the benefits of simple practices that promote health.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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