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Microbiological Quality of Ready - to - Eat Food from Dhaka, Bangladesh

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Abstract

The objective of this research was to assess the microbiological quality of ready-to-eat food available in Dhaka city, Bangladesh, and check the risk factors associated with ingestion of ready-to-eat food from popular public places. This study was conducted in the Center of Excellence in the Department of Microbiology, Primeasia University, Dhaka, Bangladesh during August 2016 to February 2017. Forty-five samples belonging to 18 categories were collected aseptically in triplicates in pre-sterilized zip-lock bags or sterile bottles from Banani area from local street vendors. Samples were transported to and analysed in the Laboratory of Department of Microbiology, Primeasia University according to standard food analysis methods. Total viable count (TVC) and Total coliform count (TCC) were determined by using plate count agar (PCA) and MacConkey agar plates respectively. Antibiogram of the isolated strains were conducted with commercial antibiotics according to Kirby-Bauer disc diffusion method on Mueller-Hinton agar medium. Identification of the coliforms together with antibiotic-resistance profile showed *Escherichia coli, Enterobacter sakazaki, Citrobacter freundii* and *Salmonella typhimurium* were present in various foods. *E. coli* and *S. typhimurium* showed increased sensitivity against Ampicillin 10 mg and Sulfamethoxazole 25 mg. Occurrence of antibiotic-resistance potential pathogens in ready-to-eat food poses a considerable health risk to consumers. Public awareness and timely assessment of food safety are needed to avoid the risks of food-borne infection and intoxication from ready - to - eat food.

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Introduction

Food-borne outbreaks cause wide range of illnesses from bacterial, viral, protozoal and chemical contamination of food.[1] Most deaths in hospital are due to bacterial agents, however viruses cause half of the food borne diseases.[2] Pathogens cause a

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large spectrum of these infections or intoxications, such as enteric complications, abdominal pain, fever, hemorrhagic colitis, bloodstream infection, meningitis, joint infection, kidney failure, paralysis, miscarriage, etc that was reported previously.[3] The common manifestation of food poisoning occurs by diarrheal diseases, which is often caused by a toxin released from microbes.[4,5] WHO estimates that globally food-borne and waterborne diseases together kill about 2.2 million people annually, 1.9 million of whom are children.[4,6] In Bangladesh, diarrhea is responsible for one-third of childhood deaths, the actual number of casualty might be underestimated in absence of a national health database. The insufficient data on food-borne infection shows 501 diarrhoeal cases are reported daily during monsoon seasons.[6,7,8] Due to the absence of regular monitoring, assessment of the public health impact of food-borne illnesses is a tedious task. Ready-to-eat foods are often consumed at the sale points in the ready food conditions and it could be cooked or raw, hot or chilled consumed without any types of further cook treatment.[9,10] Traditional food habits of general Bangladeshi people are changing with passing time due to rapidly modified and urbanization. Changing lifestyle, the involvement of the woman in official jobs and change in the family structure are encouraging more people to consume ready-to-eat food in contrast to the home-cooked food which was the common practice in the last century. In the current economical edge, many local food factories process traditional food items and market them in small packs, commonly known as 'home-made' items. Unfortunately, such food items are sold by groceries, departmental store, vendors and super shops without supervision of authorities. These ready - to - eat foods often substitute homemade foods and are important for the nutritional status of the urban population. The unhygienic condition during production, preparation and selling of food often deteriorates the microbiological quality of food items.[11] At present, the tendency of consuming ready - to - eat food products has increased tremendously. Attractive packages, marketing on a reputed shop and good advertisement strategies encourage people to consume certain products without checking for food safety and nutritional information. Generally, the food manufacturers in Bangladesh need to obtain license from Bangladesh Standard Testing Institute (BSTI) to market their products. BSTI follows the guidelines of the International Organization for Standardization (ISO) to ensure food safety.[12,13] High-risk pathogens like Campylobacter spp., vero-toxigenic *Escherichia coli* (VTEC), Salmonella spp., Shigella spp. and V. cholerae should be absent in ready-made foods according to food quality standards.[14]

This microbiological study showed the prevalence of considerable numbers of aerobic and coliform bacteria in ready-to-eat food. This study also pointed out that multidrug-resistant bacteria might be dispersing over the population through contaminated ready - to - eat foods, an observation shared by similar reports from independent groups.[1] To assess the potential risk to public health this study also suggested conducting a detailed microbial analysis and profiling their drug-resistance patterns. Consequently, the purpose of this study was to appraise the microbial status of food-borne pathogens, spoilage bacterial content, and food processing hygiene different ready - to - eat foods from Dhaka city, Bangladesh.

Materials and Methods

Sample Collection and Enrichment Procedure

Microbiological analysis of the ready-to-eat food samples were collected at random from the local shops and vendors in Banani area in Dhaka city. Sampling sites comprised of the most crowed places. 18 categories of food items were purchased in triplicates from local street vendors and local superstore of Banani, Dhaka city from August 2016 to February 2017. Collected samples were taken to the (Centre of Excellence) Laboratory, Department of Microbiology of Primeasia University, Dhaka, within the earliest time from purchase for further processing and analysis.[12] Solid food samples were collected in pre-sterilized Stomacher bags (165mm x 150mm x 0.55mm). Fresh juice samples were collected in sterile Duran Bottles and transported in the icebox. All samples were analyzed according to the standard microbiological methods for food analysis.[15]

Microbiological Analysis

For determining the microbial load in samples, the total viable count (TVC) and total coliform count (TCC) were determined with spread-plate method. The plate count agar and MacConkey agar (Oxoid Ltd, Hampshire, UK) respectively used to indicate

the TCC and TVC for the collecting samples. 1g of each sample was diluted in 10 - folds in sterile normal saline water (0.85 % NaCl) and properly diluted up 10-1 to 10-6 decimal in normal saline water.[12] 100µL from each dilution of each sample were spread on selected media by spread plate method with sterile glass spreader. Plates were incubated overnight at 37 °C and visible colonies were counted and represented as CFU/g or CFU/mL in log scale. Cell counts (CFU/g and CFU/mL) were derived from average of 3 independent experiments (Fig. 1).[16,17,18]

Isolation and Identification of Micro organism

Twenty-five gram (25 g) of each sample was homogenized in 225 milliliters of buffered peptone water (Oxoid Ltd, Hampshire, England) and incubated overnight at 37 °C. One milliliter pre-enrichment culture was mixed with 10 milliliters of Henja - Tetrathionate Broth (HiMedia Laboratories, Mumbai, India) and was incubated at 37 °C for 24 hours.[1] The culture broths were subsequently streaked onto Salmonella-Shigella Agar (SS) media (Oxoid Ltd, Hampshire, England) and Bismuth-Sulphite Agar (BSA) media (HiMedia Laboratories, Mumbai, India). Presumptive identification of each colony came from biochemical tests and biochemically confirmed isolates were re - confirmed with API 20E kits (BioMerieux, Inc.).[12,19]

Twenty - five (25 g) of each sample was homogenized in 225 milliliters of buffered peptone water (Oxoid Ltd, Hampshire, England) and pre-enriched by incubating for overnight at 37 °C. One milliliter of pre-enrichment culture was mixed with 9 - milliliter lactose broth medium (Oxoid Ltd, Hampshire, England) with Durham fermentation tubes and incubated overnight at 37 °C. After incubation periods, the fecal coliform microorganisms produce gas in the Durham tube.[18] Then one loop-full culture broth was streaked onto Eosine-Methylene Blue Agar (EMB) (Oxoid Ltd,

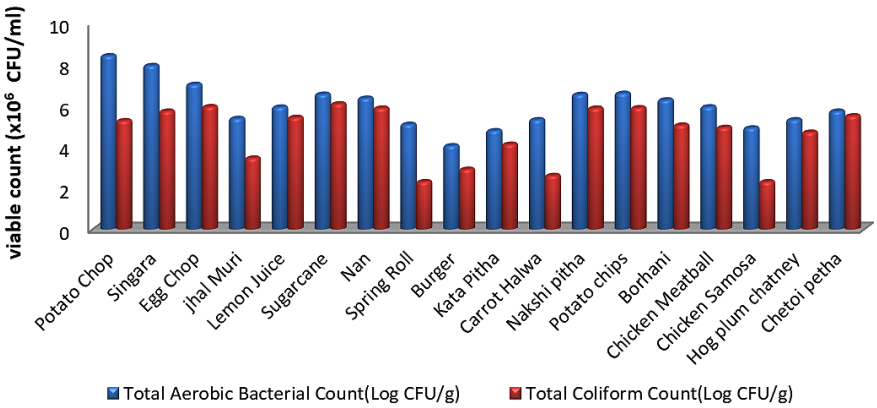

Fig. 1: Total Aerobic Count and Total Coliform Count of ready-to-eat items from Dhaka city

Table 1: Biochemical profile of bacterial isolates from ready-to-eat foods from Banani

Isolated Strains	Kia Slant	Kia Butt	Gas	H_2S	Indole Test	Mr Test	Vp Test	Citrate Test	Oxidase Test	Catalase Test	Motility	Gram Stain
E. coli	Y	Y	+	-	+	+	-	-	-	+	+	Rod, (-)
Salmonella sp.	R	R	-	+	-	+	-	-	-	+	+	Rod, (-)

Note: KIA = Kligler's Iron Agar, Y = Yellow (Acid), R = Red (Alkaline), MR = Methyl red, VP = Voges-Proskauer test, positive = (+), Negative = (-)

Hampshire, England) incubated for 24 hours at 37° C to identify *E. coli*. The identification of the isolates upto genera was confirmed with API20 (BioMerieux, Inc.) (Table 1). The *E. coli* isolates were tested for detection of β- glucouronidase on *Escherichia coli*-methyl umbiliferyl glucuronate (EC-MUG, Oxoid, New Hampshire, England) medium under standard bacteriological conditions (Table 2).

Biochemical Tests for Identification of Isolates

Pure colonies from nutrient agar (NA) plates were taken for biochemical tests such as Kligler Iron Agar (KIA) (HiMedia Laboratories, Mumbai, India), Simmons citrate agar media, MR-VP media for Methyl red and Voges - Proskauer test, SIM media for Indole and motility test (Oxoid Ltd, Hampshire, England), Oxidase and catalase test for super-oxide dismutase and hydrogen peroxide[20] and API 20E kits (BioMerieux, Inc.) for biochemical profiling.[20]

Antibiogram (Antibiotic Susceptibility Profile) Test

Susceptibility of isolated strains was tested in vitro towards different antimicrobial agents through the Kirby-Bauer method using antibiotic disc diffusion on Muller - Hinton agar.[21] It allowed determination of the action of the antibiotic which shows the inhibition of the pathogen to the degree proportional to the diameter of the zone of inhibition that resulted from diffusion of the antimicrobial that surrounding the disc onto the agar medium. Commercial antibiotic discs were used in this experiment; Ampicillin 10µg (AMP), Azithromycin 15µg (AZM), Ciprofloxacin 5µg (CIP), Gentamicin10µg (CN), Kanamycin 30µg (k), Sulfamethoxazole 25µg (SXT), Tetracycline 30µg (TE). Briefly, 5 mL of Mueller–Hinton broth was inoculated with a pure culture of a specific isolate and incubated overnight at 37° C. The 0.5 McFarland standard was used adjust to the turbidity of actively-growing broth cultures.[22] The intensity of antibiotic action was interpret from the National Committee for Clinical and Laboratory Standards.[22,23,24]

Result

In this study, different kinds of "Ready-to-eat" food including sugarcane juice, lemon juice, kata pitha, chetoipitha (traditional rice cookies), carrot halwa, Nakshipitha (sweetened dissert), nan (bread), potato chips, singara, egg chop, potato chop, spring roll, chicken meatball, chicken samusa (fried snacks), burger, jhalmuri (Spicy puffed rice), borhani (spiced yogurt drink), hog plum chatney (pickle) were analyzed. The total viable count and total coliform count from these foods are given in Fig.1. Some specific food like spring roll, sugarcane had both spoilage flora and bacterial pathogens as stated by others before.[25,26,27]

The API-20 test was done on selected isolates of *E. coli* , *Enterobacter sakazaki*, *Citrobacter freundii*

Table 2: API-20 identification tests for bacterial isolates from the ready - to - eat food samples from Dhaka, Bangladesh

Name of food sample	Percentage of API in an isolated microorganism
Singara	*E.coli* - 97.7%
Egg Chop	*E.coli* - 99.7%
Lemon Juice	*E.coli* - 99.7%
Sugarcane	*E.coli* - 98%
Nan	*Entrobacter sakazaki* - 98%
Kata Pitha	*E.coli* - 99.9%
Potato chips	*E.coli* - 95.8%
Nakshipitha	*E.coli* - 99.7%
Chicken Samosa	*Citrobacter freundii* - 99.7%
Hog plum chatney	*E.coli* - 99.5%
Lemon Juice	*Citrobacter freundii* - 96.9%
Singara	*Salmonella typhimurium*-97.5%
Egg Chop	*Salmonella typhimurium*-95.5%
Sugarcane	*Citrobacter freundii* - 99.9%

Table 3: Production of β - glucuronidase from the *E. coli* isolates from ready - to - eat food items from Dhaka

Sample name	β - glucuronidase
Singara	+
Egg Chop	+
Lemon Juice	+
Sugarcane	+
Kata Pitha	+
Nakshipitha	+
Potato chips	+
Hog plum chutney	+

Note: positive = (+), Negative = (-)

and *Salmonella typhimurium* to conform the result of the biochemical test (Table 2). The *E. coli* isolates were further cultured on EC-MUG medium (Oxoid, Hampshire, England) to observe the presence of β-glucuronidase with the probability of finding *E. coli* O157:H7, because the dangerous *E. coli* O157:H7 strains are negative for β-glucuronidase (Table 3).[28,29] Finally, the antibiotic resistance pattern of the isolates indicated Ampicillin and Sulfamethoxazole resistance (Figure 2 and 3).

Discussion

In Bangladesh, food contamination exposes consumers to food-borne hazards. Lack of awareness and lack of adherence to the food laws and regulation together with infrequent implementation of existing regulations are contributing significantly to dissatisfactory food safety circumstances of Bangladesh.[2,6] Street-vended, ready - to - eat food items are becoming a globally growing trend for ease, quickness and convenience.[30,31] As per a report, a range of food-borne infections are the cause of most microbial diseases. In the developing countries a high level of mortality rate is found due to bacterial diarrhea.[32] The frequency of typhoid among the city slum-dwellers is estimated as 3.9 episodes per year when screening in one thousand people. whereas in case of pre-school children who are aged between 2 to 5 years were estimated to be 8.9 times more at a risk of getting infected by

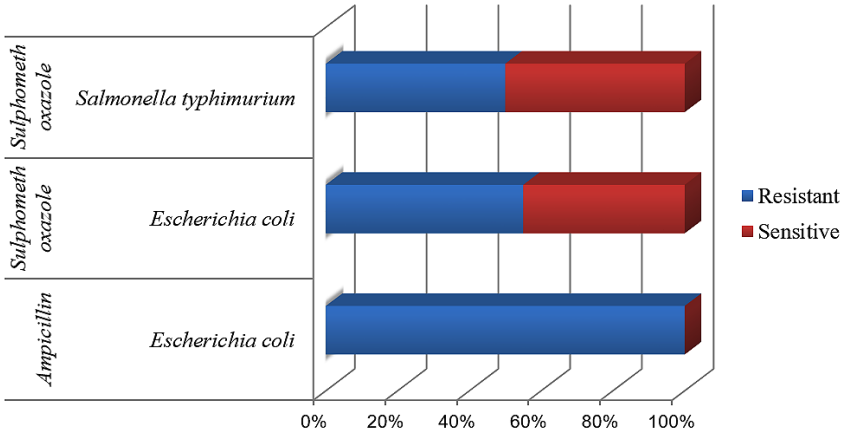

Fig. 2: Antibiotic resistance profile of the most abundant bacteria isolated from ready - to - eat food

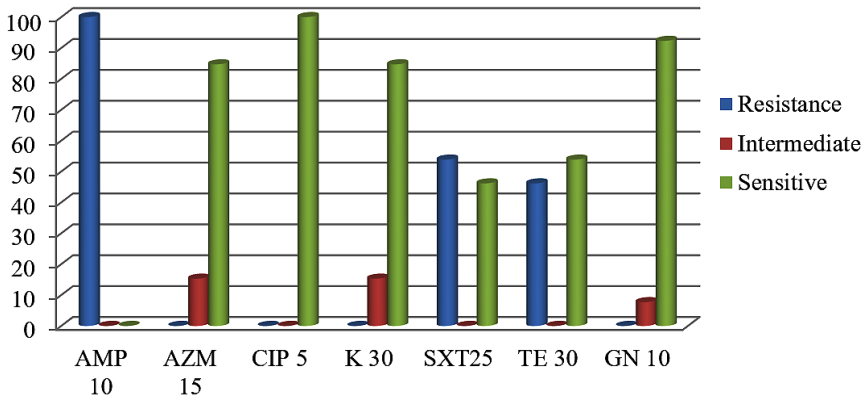

Fig. 3: Antibiotic sensitivity pattern of bacterial isolates from ready - to - eat food samples from Dhaka

typhoid.[29] Among the diarrhea affected people in Dhaka city, Salmonella covered 6.4% of the bacterial isolates.[32] Contaminated water, food and person-to-person contact are the important sources of Salmonella spp. and *E. coli*. This study emphasizes the role of sub-standard food items retailed in the super-shops as a source of Salmonella spp. and *E. coli* infection. Globalization has made foreign items like chicken nuggets and sausages popular to the urban youth. Moreover, the popular traditional items like rice cookies (pitha), mashed sweets (Halwa) and hard caramels (Badam papri, Helen papri) are only marketed by small-scale industries popularly advertised as 'Homemade' products. The name, number and location of these traditional small-scale producers (Home-made brand) are not recorded with the Bangladesh Standard Testing Institute (BSTI). The BSTI approval does not appear on the packages of the homemade brands. The occurrence of high Total Heterotrophic Count (HTC) and relatively high Total Coliform Count (TCC) reflect poor quality of the finished product (Fig. 1). Fried foods like potato chops, egg chop, singara, chicken meat ball, baked items like Nan, sugary foods like sugarcane juice, nakshi pitha have high burden of TAC (6 x 106 CFU /mL)) and TCC (> 4 x 106 CFU / mL) (Fig 1). *E. coli* was found as the dominant species from singara, egg chop, lemon juice, sugarcane juice, kata pitha, potato chips, nakshi pitha and hogplum chutney (Table 2). All isolates of *E. coli* were β- glucuronidse positive (Table 3). *Enterobacter sakazaki* was found in Nan bread. *Citrobacter freundii* was isolated from chicken samosa, lemon juice and sugarcane juice. Potential pathogen *Salmonella typhimurium* was isolated from singara and egg chop. The other important aspect of the identification ssof food pathogens is to assess the burden of multi-drug resistant pathogens. Simultaneous resistance against Ampicillin and Sulfamethoxazole makes it difficult to treat an infection. Successive spread in resistance poses a hazard of an epidemic by resistant *E. coli* with a little therapeutic option. This could be particularly grim in Bangladesh since the infectivity of common, affordable and well-tolerated drugs might mean a hike in mortality. On the contrary to the published reports the *E. coli* isolates were susceptible to the common antibiotics (Fig 3), indicating a reduced risk of therapeutic failure. *E. coli* isolates were resistant against Ampicillin, Sulfamethoxazole and Tetracycline. Occurrence of β-glucuronidase positive strains means lesser risks because the vero-toxigenic *E. coli* are often β-glucuronidase negative. The circumstance is more threatening in young children and the population. Presence of *E. coli* in processed and packaged food indicated poor handling and lack of adherence to good manufacturing practice by the local manufacturers.[33,34,35] Awareness among food producers, consumers, retailers and the inspection authority might improve the situation of food safety in Dhaka city.

Conclusion

The current study exhibited that, the microbiological quality of the ready-to-eat street foods available in Dhaka city, Bangladesh, expose the customers to high risk of acquiring food-borne diseases from multi-drug resistant bacterial pathogens.

Acknowledgment

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Conflict of Interest

Authors have declared that no competing interests exist.

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