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Microbial characterization of street food collected from Dhaka city, Bangladesh

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Abstract

Ingesting tainted food is one of the methods of microbe transmission to humans. Most people in Bangladesh consume cheap foods prepared by unlicensed vendors. A total of 18 samples, including fuchka, chola, sugarcane juice, and other products, were randomly collected and analyzed for bacterial contamination. Each and every sample examined found to be contaminated with a variety of bacterial species. The total viable count (TVC) in different street food samples ranged from 0.45×10^3 CFU/g to 3.37×10^7CFU/g. Resistance to Imipenem, Meropenem, and Amikacin was quite low in *Salmonella* spp., *Klebsiella* spp., *Vibrio* spp., *Staphylococcus aureus*. Higher resistance to azithromycin and ciprofloxacin have been observed, and *S. aureus* exhibited a 67% resistance to methicillin. According to this study, Dhaka's street food in Bangladesh contains potentially pathogenic microorganisms. This necessitates careful observation of the microbiological safety of street food, and campaigns should be launched to educate the public about the poor and unhygienic quality of street food available in Dhaka, Bangladesh.

Keywords: Street food; Foodborne Pathogens; Antibiotics; Multidrug Resistance

1. Introduction

Street food is defined as foods and beverages that are eatable and ordinarily made and/or sold by sellers in public settings such as streets for immediate consumption or consumption at a later time without further processing or preparation. According to the Food and Agriculture Organization, street food is characterized as 'already cooked foods and refreshments sold on streets or other open places by hawkers' [1].

Foods sold on the street range from meat and seafood to fruits, vegetables, grains, cereals, ready-to-eat meals, ice-cream and beverages [2]. Many low-income groups' economic security and way of life are guaranteed by the street food industry[3]. The majority of street food vendors are often uneducated or ignorant about how to prepare food safely, how to prevent food contamination, the value of cleanliness, and how to maintain good hygiene. Street foods continue to be a substantial source of risk factors for public health because of these reasons. A Food and Agricultural Organization survey estimates that about 2.5 billion people worldwide consume street food each day [4][5]. There is a very high likelihood of breaking food safety and hygiene laws while starting a street food business because there are no formal education or certification requirements. In Bangladesh, there is close to 30 million cases of foodborne illness each year. A substantial portion of this number is caused by consuming street foods. During preparation and processing, water, equipment, utensils, and other aspects can frequently source bacterial and fungal contamination of food [5][6][7][8]. Children who regularly eat these specific items sold on the street and who are in school are particularly at risk for foodborne illness [9]. Many people in underdeveloped nations place a large emphasis on street cuisine in their diet. Though it is impossible to assess the prevalence of food-borne illness worldwide, it has been reported that around 7.69% people suffer from food-borne diseases per annum globally, and around 7.5% of annual deaths (56 million deaths) are due to food-borne diseases worldwide [10].

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The most frequent source of life-threatening and serious foodborne diseases is bacterial contamination of food. More than 90% of foods borne diseases are caused by bacteria, including *Staphylococcus* spp., *Salmonella* spp., *Clostridium* spp., *Campylobacter, Listeria, Vibrio*, *Bacillus* spp., *Escherichia coli, Pseudomonas* spp., *Proteus* spp. and other species. Contamination with such pathogenic bacteria during preparation, packaging, and various handling phases can result in health hazards [11][12]. Moreover, the public health situation regarding food safety became more precarious due to the multi-drug resistance of food-borne bacteria [13]. Antimicrobial resistance (AMR) is recognized as one of the most serious public health issues of the twenty-first century that has developed into a worldwide pandemic that poses a risk to human health and well-being. In 2019, a comprehensive analysis determined that almost 5 million people died worldwide due to bacterial AMR [14]. AMR would result in ten million annual deaths and a cumulative economic loss of GBP 100 trillion by 2050 according to the UK government's Antimicrobial Resistance Review [15].

The organism may naturally possess antibiotic resistance or acquire it. Acquired resistance to an antibiotic is caused by acquisition of genetic elements through horizontal gene transfer or mutations in resistance-related genes in the bacterial genome. These changes lead to express antibiotic inhibitory mechanisms or alteration in antibiotic target sites, transforming the bacteria to resistant [16]. The use of raw foods as ingredients in chutneys is accompanied by the prevalence and growth of all pathogens, particularly in vegetables, fruits, and sprouts [17]. In the Southeast region of Asia, Bangladesh is known for its cheap-to-buy, diverse street food offerings. Vendors make their food by hand and sell it to customers in a variety of crowded locations. Especially the rickshaw pullers, laborers, students, kids, and travelers consume street foods just to satisfy their hunger. In Bangladesh, one can find 128 different types of street food. Chotpoti, Bhelpuri, Shingara, Jhalmuri, Daalpuri, Pakora, and Halim are the most popular among them [18]. Since potable water is inaccessible for food preparation and the majority of food handlers lack even the most fundamental knowledge of adequate personal and environmental hygiene, there are substantial concerns about the sanitation of street meals in developing nations like Bangladesh [19][20]. Regarding this matter, the study's objectives were to determine the bacterial count, the pattern of antibiotic resistance, and the personal hygiene of the vendors in Dhaka, Bangladesh.

2. Methods and materials

2.1. Sample Collection

For this study, street food samples from local vendors & food carts of Dhaka city were selected for their microbial analysis. The most congested spots were selected as sampling locations. From February 2022 to December 2022, a total of 18 street food samples were purchased in triplicate from 3 locations (Banani, Airport, Shahabag) in Dhaka city. The samples were immediately sent to the Department of Microbiology, Primeasia University, Dhaka for further processing using aseptic technique. All samples were analyzed triplicate according to standard microbiological methods.

Table 1 Description of samples collected in this study

Sl. No.	Sample name	Description
1.	Velpuri	Deep-fried papad filled with mashed lentils, spiced chutney and fresh cucumbers.
2.	Chanachur	Snack mix consisting of chickpea flour ganthiya, fried lentils, peanuts and chickpeas
3.	Jhalmuri	Mixture of puffed rice, herbs, spices, veggies, chanachur, and mustard oil.
4.	Shingara	Fried pastries filled with mashed potato curry filling
5.	Chola	Chickpeas cooked with potatoes and served with cucumber and onions
6.	Egg chop	Potato croquet made with boiled egg inside
7.	Beguni	Eggplant slices fried with a gram flour coating
8.	Puri	Snacks made of deep-fried flour dough filled with spiced lentil filling
9.	Peyaju	Deep fried onion fritters
10.	Fuchka	Gram flour disks filled with spiced chickpea filling, tamarind chutney and mixed spices.
11	Cucumber salad	Sliced cucumber mixed with salt
12.	Mixed salad	Sliced cucumber mixed with onion and green chilies.
13.	Sugarcane juice	The liquid obtained from squeezed sugarcane

14.	Basil seeds juice	Basil seeds socked in water then mix with sugar and some fruits
15.	Watermelon juice	Blended watermelon with little bit of sugar and salt
16.	Java plum juice	Blended Juva plum with little bit of salt & sugar
17.	Orange juice	The liquid obtained from squeezed orange with little bit of sugar & salt.
18.	Olive juice	Blended olive with little bit of sugar & salt

2.2. Microbiological analysis

The spread-plate approach was used to compute the total viable count (TVC) and in order to assess the number of microorganisms present in the samples. Immediately following sample collection, they were subjected to serial dilution followed by spreading on Plate count agar (PCA) plate & incubating overnight at 37°c. After an overnight incubation period visible colonies were counted and calculated as CFU/g or CFU/ml.

2.3. Screening and Isolation of Microorganisms

E. coli, *Salmonella* spp., *S. aureus, Pseudomonas* spp. and *Vibrio* spp. are examples of foodborne pathogens that were detected using the pre-enrichment approach. Homogenized samples were inoculated into peptone broth, lactose broth, Henja - Tetrathionate Broth, 8.5% sodium chloride broth and alkaline peptone broth an incubated overnight at 37˚C. Overnight enriched culture was streaked and spread onto Eosine-Methylene Blue Agar (EMB), Salmonella-Shigella (SS) agar, Mannitol Salt agar, Cetrimide agar and Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar for *E. coli, Salmonella, Shigella, S. aureus, Pseudomonas aeruginosa* and *Vibrio* spp. screening, respectively, and incubated at 37˚C for 24 hrs.

2.4. Presumptive Identification of isolates by Biochemical Test

Isolates were presumptively identified using conventional biochemical tests, including KIA test (Kligler Iron Agar), IMViC test and motility test. Additionally, extracellular enzyme production assays including gelatin & casein hydrolysis, oxidase, catalase, and coagulase assays as well as carbohydrate utilization assays were carried out. All reagents ware obtained from Oxoid (UK) & Hi-media (India). Isolates were presumptively identified up to the Genus level according to Bergey's Manual of Systematic Bacteriology [21][22].

2.5. Antibiotic Susceptibility Test

Antimicrobial susceptibility of the foodborne isolates was conducted using Kirby-Bauer disk diffusion assay [23], and the result were interpreted according to Clinical and Laboratory Standards Institute guidelines [24]. In this study, 5 commercially available antibiotics were used, including Meropenem (MEM10µg), Imipenem (IPM10µg), Azithromycin (AZM15µg), Ciprofloxacin (CIP5µg) and Amikacin (Ak30µg). Methicillin (MET5µg) was used to identify Methicillin-Resistant *S. aureus* (MRSA) isolates. All antibiotic disks were sourced from Oxoid (UK).

3. Result and discussion

In this study, eighteen (18) different kinds of street foods were assessed for total aerobic bacterial populations, and the prevalence of some specific foodborne pathogenic bacteria. According to the findings, total aerobic bacterial population was found to be highest in Fuchka, Chola and Sugarcane juice. One of the major findings was that no microbial growth was found in the Egg chop and Beguni samples.

A total of 95 different bacterial isolates were isolated from the street food samples. Among the isolates of aerobic bacterial populations, *Staphylococcus aureus* were found the highest (24%) and the lowest percentage of isolates were *Pseudomonas* spp. (3%). Other isolates found in this investigation included *E. coli* (20%), *Klebsiella* spp. (18%), *Vibrio parahaemolyticus* (15%), *Salmonella* spp. (14%) & *Vibrio Cholera* (6%).

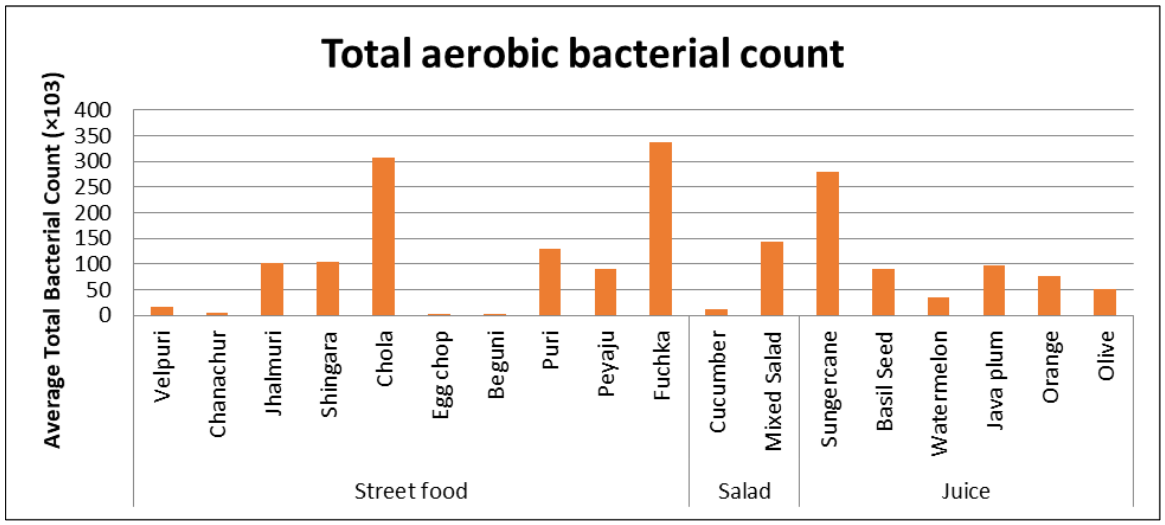

Figure 1 Total aerobic bacterial count in street food samples investigated in this study

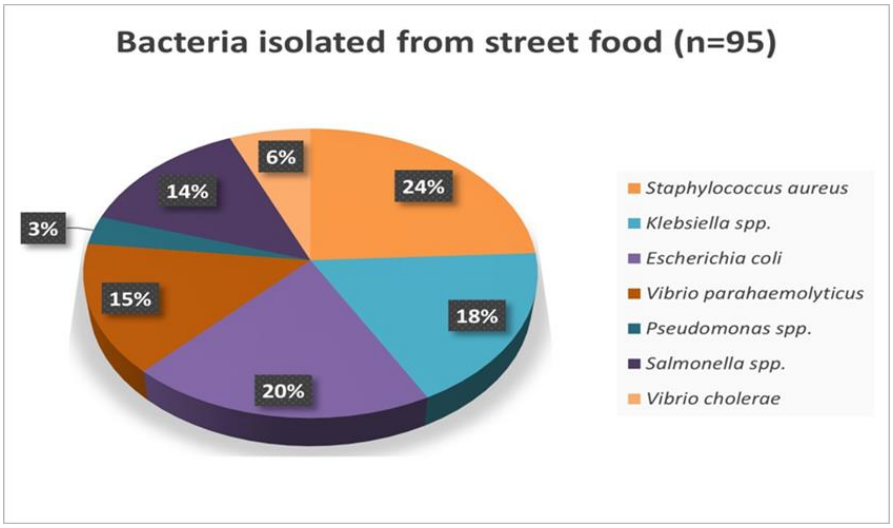

Figure 02 Distribution of bacterial isolates collected from food samples

Five groups of antibiotics such as Aminoglycoside (Amikacin), Macrolides (Azithromycin), Carbapenem (Meropenem, Imipenem), Fluoroquinolones (Ciprofloxacin), and Methicillin (for *S. aureus)* were used in the antibiotic susceptibility assay. The results of this assay revealed that most of the isolates were resistant against Azithromycin and Ciprofloxacin. Almost 68% *Staphylococcus aureus* were resistant against Methicillin, and thus considered to be MRSA. *Pseudomonas* spp. showed 100% resistance against 3 antibiotics (Azithromycin, Imipenem and Ciprofloxacin). But Amikacin, Imipenem and Meropenem could inhibit the growth of most isolates successfully and are considerably better as a current treatment option.

The contamination of street foods with pathogenic microorganisms has been recorded and several outbreaks of diseases have been found due to the consumption of contaminated street foods [8]. The presence of high microbial load of pathogens in foods is a good indicator of the food quality [25]. Street vendors need a satisfactory appreciation of essential food security issues. However, several studies on the sterile practices of street food distribution report that most street food sellers have knowledge of hygienic practices but conclude that most of them don't put this knowledge into their real working practice [26].

Inappropriate food handling and improper waste transfer lead to the transference of pathogens such as *E. coli* and *Salmonella*. Besides, the utilization of dirty utensils leads to cross-contamination of food with *Staphylococcus aureus*, *E. coli*, and *Shigella* due to contaminated water, dishcloths, and handlers. A study on the hygiene practices of street vendors

revealed that 48% of vendors had a dirty stall, 30% wore unwashed clothes, 71% used dirty fruit (they washed the fruit with the same water held in a bucket and reused for hours), 20% had poor waste management, and 45% used unclean work utensils [27].

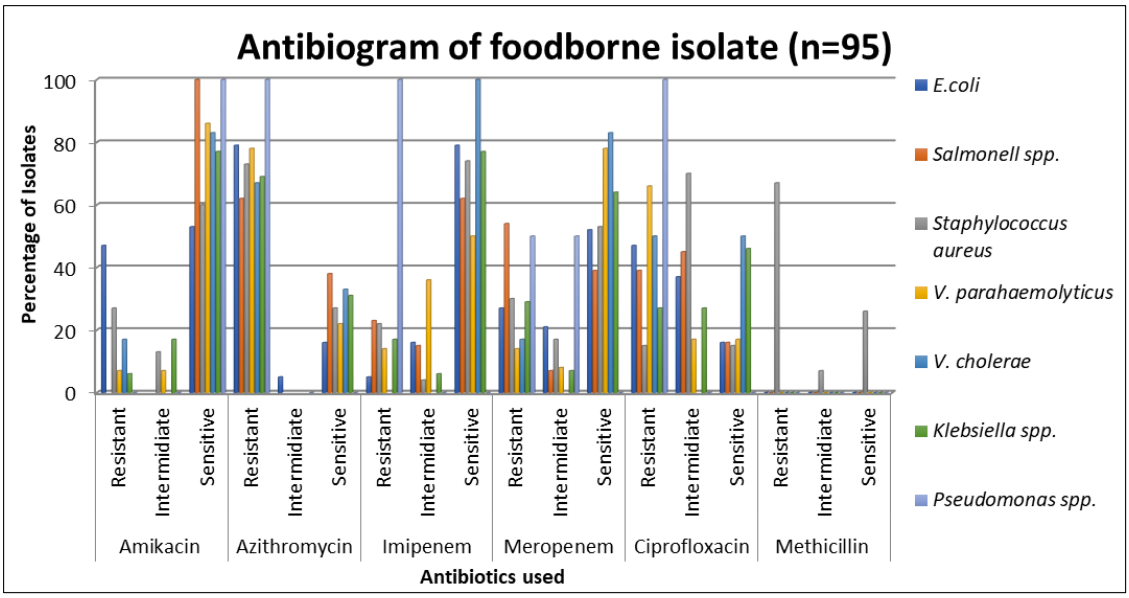

Figure 3 Graphical representation of Antibiogram percentage of bacterial isolates from street food samples against different antibiotics

The aerobic plate count indicates the presence of bacterial isolates like *Salmonella, S. aureus, Vibrio parahaemolyticus, Vibrio cholera* and *Klebsiella* spp. to be a potential health hazard to consumers. According to the microbiological standard of foods in Bangladesh, aerobic plate counts ranging from 10^1 to 10^2 CFU/g can be considered safe, 10^2 -10^4 CFU/g is considered acceptable, and 10^4-10^5 CFU/g is not acceptable from a public health perspective [28]. According to a 2018 study, TVC was recorded 9.7×10^5 CFU/g in fuchka samples [29]. In our study, sugarcane juice was reported with 2.8×10^6 CFU/g TVC, which similar to results showed in previously report on sugarcane juice [30]. A previous report on sugarcane juice in Pakistan found TVC range was 1.5×10^4 to 7.58×10^5 [31]. Several investigations from Bangladesh are in agreement with our study on the alarming amount of viable bacteria present in sugarcane juice [32][33]. *Shanzida et at.* Previously showed beguni was the lowest TVC, which supports the finding of our study [34].

Diarrheal disease caused by enterotoxigenic *E. coli* is endemic for the inhabitants of Dhaka [35]. Maximum foodborne diseases caused by *E. coli, Salmonella, Klebsiella, S. aureus* etc. present a potential health hazard to consumers. Second-generation antibiotics like Azithromycin and Ciprofloxacin are widely used to treat bacterial infections in Bangladesh. However, surge of antibiotic resistance among these bacteria have become an ominous threat for treatment of foodborne infections. In Bangladesh, the unprofessional attitude of street food sellers increases the possibilities of microbial contamination from the environment, which supports the dissemination of multidrug-resistant bacteria through the street food items [36]. Contaminated street food now poses as a seriously potential health hazard as well as risk getting infected with multidrug resistant foodborne pathogens, which could be life-threatening [29].

4. Conclusion

The current investigation was carried out to isolate and identify bacteria recovered from various street foods, and determine their susceptibility to antibiotics. Coliforms may have been present in the samples as a result of low water quality, unclean vendor locations, or unclean vendor behavior. The majorities of street sellers were illiterate and lacked a thorough understanding of food-hygiene. The study's findings indicated that although street foods are inexpensive and delicious, they are unhealthy due to a lack of hygienic practices, filthy equipment, and vendor hygiene. Although many varieties of bacteria are being contributed to these factors, the main pathogens include *E. coli, Salmonella* spp., *Vibrio* spp., *Klebsiella* spp., *Pseudomonas* spp., and *Staphylococcus* spp. Poor hygiene practices are the primary cause of bacterial infections, and this issue can be resolved by enhancing the monitoring of food handling practices and extending consumer education on the hazards associated with food safety. Mobile courts made up of food specialists, microbiologists, and nutritionists have to regularly evaluate food courts. Street food, food carts, and food courts at marketplaces need to fall under this authority. Along with corrective and preventative activities, food safety rules should

be put into effect. The Bangladesh Food and Drug Authority should better enforce the Food Safety Law by telling retailers not to sell products without ISO 9001 and BSTI approval in order to keep up with the changing eating habits in the city of Dhaka.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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