## Microbial Status and Identification with Antibiotic Susceptibility Patterns of Enteric Pathogen *Escherichia Coli* and *Vibrio Cholerae* Isolated from Different Street Foods Sold in Dhaka City, Bangladesh

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#### ABSTRACT

There are various benefits offered by street vended foods, but street foods are contaminated with many foodborne pathogens includes enteric pathogenic bacteria like Escherichia coli and Vibrio cholerae has become a potential health hazard problem all over the world. The aim of this study was to evaluate the complete microbial status of common foodborne pathogens including enteric pathogen Escherichia coli and Vibrio cholerae and identify the presence and find out the contamination level of Escherichia coliand Vibrio cholerae with Antibiotic sensitivity status from different street foods in Dhaka City, Bangladesh. For this assessment, 42 street food samples of 6categories were collected from 7 different areas of Dhaka city. In all food samples, Total Viable Bacterial Count (TVBC) was Ranged from 1.3x107 to 8×107cfu/g, Total Coliform Count (TCC) was Ranged from 1x107 to 4.5 x107cfu/g, Total Escherichia coli Count (TEC) was Ranged from 2×10<sup>4</sup> to 7.9x10<sup>6</sup> cfu/g and Total Vibrio cholerae Count (TVC) was ranged from 1x10<sup>2</sup> to 5.3x10<sup>6</sup>cfu/g. In addition, out of the 42 analyzed food samples Escherichia coliwas found in 28 (66.67%) samples and Vibrio cholera was found in 26 (61.90%) samples. The isolated pathogenic Escherichia coli and Vibrio cholerae was identified by Cultural, Gram Staining, and Biochemical tests. The isolated pathogens were then tested for antibiotic sensitivity and the results revealed that isolated *Escherichia coli* were resistant against Streptomycin (85.71%), Ceftriaxone (100%), Erythromycin (100%), Cefixime (100%), Meropenem (100%), and Gentamycin (71.42%) and isolated Vibrio cholerae were resistant against Streptomycin (84.62%), Erythromycin (100%), Cefixime (100%), Meropenem (100%), Amikacin (100%), and Gentamycin (57.69%) areoutrageous. The results revealed that the contamination percentage of pathogenic Escherichia *coli*(22%) and *Vibrio cholerae* (23%) in Gulistanwas high than the other areas.

**KEYWORDS**: Street Foods; Total Viable Bacterial Count; Total Coliforms Count; Escherichia coli; Vibrio cholerae; Antibiogram

#### INTRODUCTION

Food is a shockingly active vehicle for the conveyance of a diversity of pathogens[1]. Nowadays diverse foodborne diseases are becoming a prominent concern connecting a wide range of illnesses caused by bacterial, viral, parasitic, or chemical contamination of food. In addition, the resistance of these microorganisms to multi-drugs made this circumstance more of a concern to public health[2]. The World Health Organization (WHO) reported in the Foodborne Disease Burden Epidemiology Reference Group (FERG) that 31 foodborne pathogens caused 600 million cases of illness leading to 420, 000 deaths globally [3]. Among foodborne diseases, Diarrhoea is one of the foremost genuine worldwide concerns [4]. Around 1.7 billion cases of child deaths caused by Diarrhoeadiseases are documented yearly worldwide, and a maximum of these cases are attributed to contaminated food and water [4]. In developing countries like Bangladesh, foodborne disease outbreaks have emerged as the foremost foodborne health

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hazards[5]. In Bangladesh, roughly 30 million people suffer from foodborne illnesses each year of which diarrheal diseases kill almost 2.2 million people including many children annually[1]. Street foods could be leading vehicles for the transmission of severe foodborne infections and deadly diseases [6]. In developing countries food vended by street hawkers is the key source of foodborne illness due to lack of appropriate sanitary conditions, hygiene practices and appropriate storage and mishandling[6][7]. A combined study by scientists of the ICDDRB and Emory University, USA, unveiled that because of unhygienic street foods, Dhaka is at high threat of contracting enteric diseases like Diarrhoea, Cholera, and Typhoid. Foodborne enteric pathogen Escherichia coli and Vibrio cholerae were found with a high concentration in drinking water and food items of the street. [8] Escherichia coli has become recognized as a serious foodborne pathogen with high prevalence and multidrug resistance may reason health hazards like Diarrhoea, Typhoid, and Dysentery [8][9][10]. Aliya Naheed reported that as Escherichia coliis not safe in humans and if found in food, we can't acceptmore than 20 Escherichiacoliin food acceptable [9]. On the other hand, *Vibrio cholera* (is also resistant against nearly all regularly used antibiotics[11]) has long been well-known to be blamable for the life-threatening secretory Diarrhea termed as Asiatic cholera or epidemic cholera and foods contamination can also be a vital mode for cholera transmission [12]. Many previous cases reported that street foods are hazardous, mainly for enteric bacteria like Escherichia coli and Vibrio cholerae cause foodborne diseases considered as the major public health problem that could be life-threatening[1][8]. For example, In 2015 Between October and November, Chipotle Mexican Grill fast food had an Escherichia coli outbreak trusted source. Around 55 people in 11 states became ill after eating at the restaurant during the preliminary outbreak and they admitted to 22 hospitalizations and eventually no deaths. In a second outbreak for this fast-food chain, five people became ill from a different strain of Escherichia coli. There's no confirmed cause for either outbreak [13]. In 1991, the first report on the incidence of Cholera in the United States associated with the food transported from an area with the epidemic disease was made during March-April [14]. In developing countries like Bangladesh, because of good taste, flavor and image, variety, attractiveness, easy availability, food value, reasonable cost and accessibility people are interested in Street foods [15][16]. Infect in Dhaka city, about 60 lakh people eat street foods daily[8].

Hence, this study was carried out to assess the detailed microbial status of common foodborne pathogens including enteric pathogen*Escherichia coli* and *Vibrio cholerae* and identify the presence and find out the contamination level of *Escherichia coli* and *Vibrio cholerae* with their drug sensitivity status from different street foods consumed by a large number of people in Dhaka City, Bangladesh which can be cause life-threatening foodborne diseases.

## **MATERIALS AND METHODS**

#### A. Study Design and Sampling Areas

This study with a biological exploratory design was successfully executed in Capital Dhaka City, Bangladesh during August-December 2020. The entire study was divided into three steps. The first step was to include the total Bacterial viable, Coliform, *Escherichia coli*, and *Vibrio cholera* counts of the collected samples. Isolation and identification of the bacteria from the sample was embraced in the second step and found through the cultural, morphological, and biochemical test, and eventually, assessment of antibiotics sensitivity against the isolated bacteria was in the final step.

#### **B.** Sample Collection and Processing

A total offourty-two street food Samples of six categories (Alur chop, Fuchka, Singara, Samosa, Beguni, Puri) were purchased from seven important areas around Dhaka city where the number of streets food vendors and their customers are high included Banani, Mahakhali, Badda, Motijheel, Gulistan, Dhanmondi and Hatirjheel. Each sample was collected from seven separate vendors of each area. These street food items and places were selected as per consumer preference and their availability. All samples were transferred in labeled pre-sterilized Stomacher bags (165mm x 150mm x 0.55mm) after purchasing to avoid their interaction with any other source that might contaminate the samples and without delay transported to the laboratory for microbial analysis. The collected street food samples were analyzed twice.

# C. Samples Preparation of Total Viable Bacterial Count (TVBC) and Total Coliform Count (TCC)

Each Sample (10g) was weighed and added into a sterile Durham Bottle containing 90ml sterile Normal Saline and homogenized with sterile Blender (Retsch, GM 200, Australia) at 3000rpm for 5-10 mins to prepare a stock solution. Each stock was serially diluted (1:10) up to 10<sup>-5</sup> by adding 1ml of stock solution to 9ml Normal Saline to prepare for the Microbiological Analysis. [17][7]

#### D. Microbiological Analysis

To evaluate the microbial status and the contamination level, 0.1 ml aliquot from each dilution of each sample were spread on Plate Count Agar (PCA, Himedia, India) plates for Total Viable Bacterial Count, MacConkey Agar (MCA, HiMedia, India) plates for Total Coliform Count, Eosin Methylene Blue Agar (EMB, Oxoid Ltd, Hampshire, England) plates for Total *Escherichia coli* Count and Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS, Oxoid Ltd, Hampshire, England) plates for Total *Vibrio cholera* Count by spread plate method. All agar plates were incubated at 37°C for 24 hours [17][18]. After the incubation time, counts were made using a colony counting device that allows the viewing of individual colonies. The colony count was recorded as colony-forming unit per gram (CFU/g) of the food sample [18].

## E. Isolation of Escherichia coli and Vibrio cholerae

For enrichment, 25 g of each sample were individually added into a sterile Durham Bottle containing 225 ml of sterile Buffered Peptone Water (BPW) (Oxoid Ltd, Hampshire, England) and incubated at 37°C for 24 hours in a Shaking Incubator (Compact, ISSI-100T, ISR KOREA) for homogenized. After being homogenized, 1ml of each sample was transferred to 9 ml Phenol Red Lactose Broth and 9 ml Alkaline Peptone Broth (Oxoid Ltd, Hampshire, England). They were incubated at 37°C for 24 hours. For the isolation of *Escherichia coli*, after an incubation period, the enriched sample was cultured on selective medium Eosin Methylene Blue (EMB) Agar (Oxoid Ltd, Hampshire, England) by the four-way Streak Plate Method and incubated at 37°C for 24 hours. After 24 hours of incubation one isolated colony was picked & again sub-cultured in same media plates to get an isolated pure colony. Morphologically typical colonies (at least 4/plate) produced Green metallic sheen was considered to be presumptive Escherichia coli and were taken into Nutrient Agar platesfor further identification. [19][20] For isolation of *Vibrio cholerae*, after the incubation period, the enriched sample was cultured on selective medium Thiosulfate Citrate Bile Salts Sucrose (TCBS) Agar (Oxoid Ltd, Hampshire, England) by the four-way Streak Plate Method and incubated for 24 hours at 37°C. After 24 hours of incubation, Presumptive colonies from TCBS were picked and sub-cultured on Plate Count Agar (PCA) plates and single pure colonies were maintained. Morphologically typical colonies (at least 4/plate) were cultured on Nutrient Agar plates for further identification. [21]

## F. Identification of Isolates

The bacterial isolates for *Escherichia coli* and *Vibrio cholera* were identified based on their colony characteristics, morphological characteristics by Gram stain of pure culture by microscopic (such as gram reaction, shape, motility etc.) and standard biochemical tests for confirmation. [22]

Pure colonies from Nutrient Agar (NA) plates were subjected for the biochemical characterization of the isolates, different tests including IMViC tests (Indole, Methyl red (MR), Voges-Proskeur (VP) and Citrate), Triple sugar iron (TSI) tests (Slant / Butt, GasProduction and H<sub>2</sub>S Production), Oxidase, Catalase, Motility and Ureasetests were done.

#### G. Antibiotic sensitivity Test

Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Oxoid, Basingstoke, UK) plates were followed to determine antibiotic susceptibility and resistance patterns of isolated Escherichia coli and Vibrio cholerae against Ten (10) commercially purchased Antibiotics Discs (all from Oxoid Ltd, Hampshire, England) which are commonly used as a treatment of bacterial diseases included- Streptomycin (S, 10µg), Cefuroxime (CXM, 30µg), Ceftriaxone(CRO, 30µg), Chloramphenicol (C, 30µg), Erythromycin (E, 15µg), Cefixime (CFM, 5µg), Meropenem (MEM, 10µg), Levofloxacin (LEV, 5µg), Gentamycin (CN, 10µg) and Amikacin (AK, 30µg) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. [23] After incubated for 24hrs at 37°C, the diameter in millimeters (mm) of the zones of inhibition around each of the antimicrobial discs were noted and compared with company commendations. [24]

#### RESULTS

The results of Bacteria count from collected different street le food samples are given in **Table I**.

#### A. Total Viable Bacterial Count (TVBC)

The uppermost total viable bacterial count (8×10<sup>7</sup>cfu/g) was originated in Fuchka, collected from Motijheel and the lowermost total bacterial count was  $1.3 \times 10^7$ cfu/g, which had been collected from Mahakhali was Singara (**Table I**). In this TVBC result, it was observed that every sample retained such a boundless level of bacterial load which disreputably surpassed the boundary of the International Commission on Microbiological Specifications for Foods (ICMSF-1978) standard. According to the ICMSF standard, the total viable bacterial count should stay under  $\leq 10^6$  cfu/g [25].

#### B. Total Coliform Count (TCC)

It was observed that a sample named Samosa  $(4.5 \times 10^7 \text{cfu/g})$  collected from Banani was the highest total coliform count and the nethermost total coliform count was  $1 \times 10^7 \text{cfu/g}$  which was collected from Hatirjil (sample named Singara). None of TCC was detected in the sample of Samosa from Mohakhali and Beguni from Banani and Mohakhali(**Table I**). By comparison to International Commission on Microbiological Specifications for Foods (ICMSF) (standard range for TCC is 11 cfu/g) it was detected that except for threesamples the coliform counts of all examined food samples were beyond the acceptable range [25].

#### C. Total Escherichia coli count

*Escherichia coli* was isolated in 28 out of 42 street food samples. The highest load of *Escherichia coli* was originated in Puri (7.9x10<sup>6</sup>cfu/g) collected from Gulistan and the lowest load of *Escherichia coli* was originated inBeguni (2×10<sup>4</sup>cfu/g) collected from (Mohakhali) (**Table I**). *Escherichia coli* count range was overlapped the acceptable range where the range is 10<sup>2</sup>cfu/g (ICMSF, 2002) [18], which was indicated the presence of high contamination level of *Escherichia coli* and the possibility to persuade infection on investigated street food samples. Our results revealed that 66.67% *Escherichia coli*were isolated from examined street food samples.

#### D. Total Vibrio cholerae count

*Vibrio cholerae*was isolated in 26 out of 42 street food samples. Fuchka (5.3x10<sup>6</sup> cfu/g) samples collected from Gulistan revealed a higher count and Singara (1x 10<sup>2</sup>cfu/g) samples collected from Banani revealed a lower count than the other samples (**Table I**). The results were shown a high quantity of *Vibrio cholerae*whereAccording to the Food Safety Authority of Ireland (FSAI, 2001), if *Vibrio cholerae* detect within 25 g sample actions require is necessary to prevent the unsatisfactory result from reoccurring [26]. The results were indicated the presence of high contamination level of *Vibrio cholerae* and the possibility to persuade the Cholera infection on investigated street food samples. Our results revealed that 61.90% *Vibrio cholerae*was isolated from examined street food samples.

TABLE I Total Viable Bacterial, Coliform, Escherichia coli and Vibrio cholerae Count of Different Street Food

| Samples |                        |                     |                     |                     |                       |  |
|---------|------------------------|---------------------|---------------------|---------------------|-----------------------|--|
| Sl. No. | Street Food Samples    | TVBC (cfu/g)        | TCC (cfu/g)         | TEC (cfu/g)         | TVC (cfu/g)           |  |
| 1       | Alur chop (Banani)     | 6.4x10 <sup>7</sup> | 1.5x10 <sup>7</sup> | 0                   | 0                     |  |
| 2       | Alur chop (Mohakhali)  | 6.4x10 <sup>7</sup> | 3.2x10 <sup>7</sup> | 8x10 <sup>4</sup>   | 0                     |  |
| 3       | Alur chop (Badda)      | 7.4x10 <sup>7</sup> | 3.4x10 <sup>7</sup> | 0                   | 2.7 x 10 <sup>4</sup> |  |
| 4       | Alur chop (Motijheel)  | 3.3x10 <sup>7</sup> | 3.2x10 <sup>7</sup> | 0                   | 1.2×10 <sup>3</sup>   |  |
| 5       | Alur chop (Gulistan)   | 5.1x10 <sup>7</sup> | 1.3x10 <sup>7</sup> | 5.9x10 <sup>6</sup> | $1.6 \times 10^{6}$   |  |
| 6       | Alur chop(Dhanmondi)   | 4.8x10 <sup>7</sup> | 3x10 <sup>7</sup>   | 6.8x10 <sup>4</sup> | 2×10 <sup>3</sup>     |  |
| 7       | Alur chop (Hatirjheel) | 5.5x10 <sup>7</sup> | 2.5x10 <sup>7</sup> | 4.2x10 <sup>4</sup> | 0                     |  |
| 8       | Fuchka (Banani)        | 3.5x10 <sup>7</sup> | 4x10 <sup>7</sup>   | 4.6x10 <sup>4</sup> | $1.1 \times 10^{4}$   |  |
| 9       | Fuchka (Mohakhali)     | 5.8x10 <sup>7</sup> | 3.2x10 <sup>7</sup> | 6.1x10 <sup>4</sup> | 5.4×10 <sup>3</sup>   |  |
| 10      | Fuchka (Badda)         | 4x10 <sup>7</sup>   | 3.8x10 <sup>7</sup> | 4.9x10 <sup>4</sup> | 1.1 x 10 <sup>4</sup> |  |
| 11      | Fuchka (Motijheel)     | 8x10 <sup>7</sup>   | 4x10 <sup>7</sup>   | 6x10 <sup>4</sup>   | 3.8 x 10 <sup>2</sup> |  |
| 12      | Fuchka (Gulistan)      | 3.5x10 <sup>7</sup> | 3.5x10 <sup>7</sup> | 5.2x10 <sup>6</sup> | $5.3 \times 10^{6}$   |  |
| 13      | Fuchka (Dhanmondi)     | 6.2x10 <sup>7</sup> | 4.4x10 <sup>7</sup> | 5.8x10 <sup>4</sup> | $2.2 \times 10^{6}$   |  |
| 14      | Fuchka (Hatirjheel)    | 3.8x10 <sup>7</sup> | 3.2x10 <sup>7</sup> | 5x10 <sup>4</sup>   | 4×10 <sup>3</sup>     |  |
| 15      | Singara (Banani)       | 5.9x10 <sup>7</sup> | 1.7x10 <sup>7</sup> | 0                   | 1x 10 <sup>2</sup>    |  |
| 16      | Singara (Mohakhali)    | 1.3x10 <sup>7</sup> | 1.5x10 <sup>7</sup> | 0                   | $1.5 \times 10^{3}$   |  |
| 17      | Singara (Badda)        | 3.2x10 <sup>7</sup> | 2.2x10 <sup>7</sup> | 9.9x10 <sup>4</sup> | 0                     |  |
| 18      | Singara (Motijheel)    | 3.5x10 <sup>7</sup> | 1.2x10 <sup>7</sup> | 0                   | 2.8×10 <sup>3</sup>   |  |
| 19      | Singara (Gulistan)     | 4.4x10 <sup>7</sup> | 2.8x10 <sup>7</sup> | 5.8x10 <sup>6</sup> | $1.9 \times 10^{6}$   |  |
| 20      | Singara (Dhanmondi)    | 5.9x10 <sup>7</sup> | 2.2x10 <sup>7</sup> | 6.5x10 <sup>4</sup> | $1.1 \times 10^4$     |  |
| 21      | Singara (Hatirjheel)   | 3.1x10 <sup>7</sup> | 1x10 <sup>7</sup>   | 4.2x10 <sup>4</sup> | 0                     |  |

|    |                     |                     | 1                   | - ) e <u></u>       |                       |
|----|---------------------|---------------------|---------------------|---------------------|-----------------------|
| 22 | Samosa (Banani)     | 5.2x10 <sup>7</sup> | 4.5x10 <sup>7</sup> | 7.3x10 <sup>4</sup> | 0                     |
| 23 | Samosa (Mohakhali)  | 6.8x10 <sup>7</sup> | 0                   | 0                   | 2.1×10 <sup>2</sup>   |
| 24 | Samosa (Badda)      | 3.4x10 <sup>7</sup> | 1.5x10 <sup>7</sup> | 3.4x10 <sup>4</sup> | 0                     |
| 25 | Samosa (Motijheel)  | 6x10 <sup>7</sup>   | 1.6x10 <sup>7</sup> | 0                   | 1.7 x 10 <sup>2</sup> |
| 26 | Samosa (Gulistan)   | 3.9x10 <sup>7</sup> | 1.8x10 <sup>7</sup> | 3.9x10 <sup>6</sup> | 1.1×10 <sup>6</sup>   |
| 27 | Samosa (Dhanmondi)  | 5.3x10 <sup>7</sup> | 4x10 <sup>7</sup>   | 3.2x10 <sup>4</sup> | 0                     |
| 28 | Samosa (Hatirjheel) | 4x10 <sup>7</sup>   | 1.2x10 <sup>7</sup> | 0                   | 0                     |
| 29 | Puri (Banani)       | 2.2x10 <sup>7</sup> | 3.5x10 <sup>7</sup> | 2.2x10 <sup>4</sup> | 0                     |
| 30 | Puri (Mohakhali)    | 3.2x10 <sup>7</sup> | 1.4x10 <sup>7</sup> | 0                   | 4.6×10 <sup>3</sup>   |
| 31 | Puri (Badda)        | 4.7x10 <sup>7</sup> | 1.2x10 <sup>7</sup> | 6x10 <sup>4</sup>   | 0                     |
| 32 | Puri (Motijheel)    | 6.8x10 <sup>7</sup> | 1.3x10 <sup>7</sup> | 7.1x10 <sup>4</sup> | 0                     |
| 33 | Puri (Gulistan)     | 6.5x10 <sup>7</sup> | 1.1x10 <sup>7</sup> | 7.9x10 <sup>6</sup> | 4.6×10 <sup>6</sup>   |
| 34 | Puri (Dhanmondi)    | 3x10 <sup>7</sup>   | 3x10 <sup>7</sup>   | 4.6x10 <sup>4</sup> | 1.8 x 10 <sup>2</sup> |
| 35 | Puri (Hatirjheel)   | 2.9x10 <sup>7</sup> | 1.2x10 <sup>7</sup> | 0                   | 0                     |
| 36 | Beguni (Banani)     | 1.5x10 <sup>7</sup> | 0                   | 0                   | 0                     |
| 37 | Beguni (Mohakhali)  | 1.8x10 <sup>7</sup> | 0                   | 2x10 <sup>4</sup>   | 0                     |
| 38 | Beguni (Badda)      | 6.4x10 <sup>7</sup> | 3.8x10 <sup>7</sup> | 0                   | 3.2×10 <sup>4</sup>   |
| 39 | Beguni (Motijheel)  | 6.4x10 <sup>7</sup> | 4.2x10 <sup>7</sup> | 0                   | 2.1×10 <sup>2</sup>   |
| 40 | Beguni (Gulistan)   | 3.4x10 <sup>7</sup> | 1.2x10 <sup>7</sup> | 5.1x10 <sup>6</sup> | 2.3×10 <sup>6</sup>   |
| 41 | Beguni (Dhanmondi)  | 5.4x10 <sup>7</sup> | 4.4x10 <sup>7</sup> | 7.5x10 <sup>4</sup> | 1.2 x 10 <sup>2</sup> |
| 42 | Beguni (Hatirjheel) | 3.6x10 <sup>7</sup> | 3.8x10 <sup>7</sup> | 7x10 <sup>4</sup>   | 0                     |

\* cfu/g = Colony Forming Unit Per Gram; 0= Not Detected, TVBC = Total Viable Bacterial Count; TCC = Total Coliform Count, TEC = Total *Escherichia coli* Count; TVC = Total *Vibrio cholerae* Count.

\* According to the International Commission for Microbiological Specification for Foods (ICMSF) acceptable range of TVBC, TCC & TEC are  $\leq 10^6$ cfu/g, TCC is 11 cfu/g and  $10^2$  cfu/g respectively.

\* According to the Food Safety Authority of Ireland (FSAI, 2001) *Vibrio cholerae* detection within 25g is unsatisfactory.

## E. Identification of Escherichia coli and Vibrio cholera

Among 42 (100%) isolated food samples target pathogenic bacteria *Escherichia coli*were present in 28 (66.67%) samples *and Vibrio cholerae*in 26 (61.90%) samples. The probable isolation was representing in target microorganisms after Cultural Characteristics in selective media (Table 2). Pure colonies from Nutrient Agar (NA) plates were identified and recorded according to their microscopical characteristic by Gram stain for primary identification of *Escherichia coli* and *Vibrio cholerae*(**Table II**).

The identities of isolated *Escherichia coli* and *Vibrio cholera* from different street food samples we reconfirmed by the rapid biochemical tests (**Table III**). These biochemical tests were directed three times individually, and the results were found to be reproducible.

## TABLE II Primary Identification of *Escherichia coli* and *Vibrio cholerae* from Different Street Food samples

| The first of the second of the |   |  |  |  |  |
|--|---|--|--|--|--|
| Suspected Bacteria   | Escherichia coli  | Vibrio cholerae                            |  |  |  |
| Cultural Characteristics   | Shiny, Round, Green Metallic Sheen with bultana Black Centered Colonies           | Large, Smooth, Shiny, Flat Yellow Colonies |  |  |  |
| Gram Staining<br>Characteristics   | Gram Negative, Short Plump Rods Shape,<br>Pink Color, single, Paired/ Short Chain | Gram Negative, Curved Rods Shape, Chain    |  |  |  |

## TABLE III Biochemical Identification Result of Isolated Escherichia coli and Vibrio cholera

| Biochemical Tests   |                             | Isolated Strains |                 |  |  |
|---|-----------------------------|------------------|-----------------|--|--|
|   |                             | Escherichia coli | Vibrio cholerae |  |  |
| IMViC   | Indole                      | +                | +               |  |  |
|   | MR                          | +                | +               |  |  |
|   | VP                          | -                | -               |  |  |
|   | Citrate                     | -                | -               |  |  |
|   | Slant / Butt                | A/A              | A/A             |  |  |
| TSI   | Gas Production              | +                | -               |  |  |
|   | H <sub>2</sub> S Production | -                | -               |  |  |
|   | Oxidase                     | se - +           |                 |  |  |
| Catalase + +  |                             | +                |                 |  |  |
| Motility  |                             | +                | +               |  |  |
| Urease  |                             | -                | -               |  |  |
| * A= Acid Reaction (Yellow Color); + = Positive Reaction; - = Negative Reaction |                             |                  |                 |  |  |

## F. Results of antibiotic sensitivity tests

Resistance against antibiotics by pathogenic bacteria is the foremost concern in the anti-infective therapy of both humans and animals. In this study to determine the percentage of sensitivity or resistance of the isolated bacteria against selected antimicrobial agents, The Kirby-Bauer disk diffusion test was used. The antibiotic sensitivity pattern of isolated *Escherichia coli* 

and *Vibrio cholerae* are shown in **Tables IV**. *Escherichia coli* revealed good sensitivity against Chloramphenicol (60.71%) and Levofloxacin (75%) and were resistant against Ceftriaxone (100%), Erythromycin (100%), Cefixime (100%), Meropenem (100%), Streptomycin (85.71%) and Gentamycin (71.42%). Whereas *Vibrio cholerae*showed good sensitivity against Chloramphenicol (61.54%) & Levofloxacin (53.85%) and were resistant against Streptomycin (84.62%), Erythromycin (100%), Cefixime (100%), Meropenem (100%), Amikacin (100%) and Gentamycin (57.69%). Results were compared with the standard of Clinical and Laboratory Standards Institute (CLSI) [24].

| Name of Antibiotic  | Ec (%)      |              |           | Vc (%)      |              |           |
|---|-------------|--------------|-----------|-------------|--------------|-----------|
| Name of Antibiotic  | Sensitivity | Intermediate | Resistant | Sensitivity | Intermediate | Resistant |
| Streptomycin (S10)  | 0%          | 14.29%       | 85.71%    | 0%          | 15.38%       | 84.62%    |
| Cefuroxime (CXM30)  | 28.57%      | 53.57%       | 17.86%    | 42.31%      | 46.15%       | 11.54%    |
| Ceftriaxone (CRO30)   | 0%          | 0%           | 100.00%   | 15.38%      | 73.08%       | 11.54%    |
| Chloramphenicol (C30)   | 60.71%      | 14.29%       | 25.00%    | 61.54%      | 26.92%       | 11.54%    |
| Erythromycin (E15)  | 0%          | 0%           | 100.00%   | 0%          | 0%           | 100.00%   |
| Cefixime (CFM5)   | 0%          | 0%           | 100.00%   | 0%          | 0%           | 100.00%   |
| Meropenem (MEM10)   | 0%          | 0%           | 100.00%   | 0%          | 0%           | 100.00%   |
| Levofloxacin (LEV5)   | 75.00%      | 25.00%       | 0.00%     | 53.85%      | 34.62%       | 11.53%    |
| Gentamycin (CN10)   | 14.29%      | 14.29%       | 71.42%    | 0%          | 42.31%       | 57.69%    |
| Amikacin (AK30)   | 14.29%      | 42.86%       | 42.85%    | 0%          | 0%           | 100.00%   |
| *Ec= <i>Escherichia coli</i> ; Vc= <i>Vibrio cholerae, %</i> = Percentage |             |              |           |             |              |           |

#### DISCUSSION

Street food which vended predominantly in streets and other public spaces by a hawker not only valued for their unique flavors, reasonable price, convenience but the role which they performed in the cultural and social heritage of societies. Street foods provide a source of reasonable nutrients to the larger part of the people particularly the low-income group in the developing countries [27] but the sale of foods in the streets are very debatable from a health viewpoint. The main health hazard associated with street foods is a high degree of microbial contamination which leads to foodborne diseases considered as the major public health problem that could be life-threatening [6][28]. Contamination of street foods is the result of numerous factors, including an unhygienic environment with flies and dust, poor personal hygiene, dirty utensils, the use of contaminated water and ingredients, multifunctional hands, or personal health status of vendors, inappropriate holding temperature, absence of awareness, training, and practice of food sanitation by producers and handlers and disregard of in developing countries, food safety law like Bangladesh[4][29].

Therefore, the current study was an attempt to check the microbial load of pathogenic microorganisms in terms of total viable and coliform count as well as determine the presence and contamination level of enteric pathogen*Escherichia coli* and *Vibrio cholerae* with their drug sensitivity status in different street foods consumed by a large number of people in Dhaka City, Bangladesh.

After the samples were analyzed it can be stated all collected street food samples were found to contain microbial contamination. During analysis most of the tested food samples showed higher than the standard recommended levels of bacterial populations in Total Viable Count (which are responsible for the rotation of food samples [30]), Total Coliform Count (which indicates an unhygienic condition of food sample or food processing surfaces [31]), Total *Escherichia coli* Count (an indicator of Fecal contamination [21]) and Total *Vibrio cholerae* Count (an indicator of waterborne contamination [21]). These findings demonstrate the poor and pathogenic microbiological status of tested foods. Moreover, 28 (66.67%)enteric pathogen*Escherichia* coliand 26 (61.90%) enteric pathogen*Vibrio* choleraewere isolated and identified by biochemical tests. The presence of *Escherichia* coli(22%) and *Vibrio* cholerae (23%) in Gulistan was high than the other areas (**Fig 1 & 2**).

On top of that Escherichia coli isolates showed drug resistance against Streptomycin (85.71%), Ceftriaxone (100%), Erythromycin (100%), Cefixime (100%), Meropenem (100%) and Gentamycin (71.42%) and for showed Streptomycin (84.62%). Vibrio cholerae Erythromycin (100%), Cefixime (100%), Meropenem (100%), Amikacin (100%), and Gentamycin (57.69%). Only Chloramphenicol (60.71%) and Levofloxacin (75%) showed good sensitivity for *Escherichia coli* and Chloramphenicol (61.54%) & Levofloxacin (53.85%) for Vibrio cholerae (TableIV). The presence of drug resistance enteric bacterial pathogens exposed the high alarming possibilities of acquiring food-borne diseases for the street foods consumers in Dhaka city, Bangladesh.

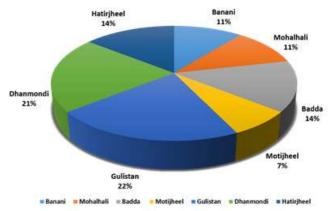
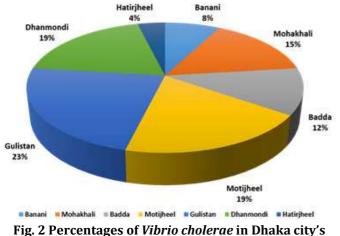


Fig. 1 Percentages of *Escherichia coli* in Dhaka city's selected areas



selected areas

#### CONCLUSION

In conclusion it can be said that the current study shows a total microbiological status of different street foods to guarantee food safety for an exact control over consumer's wellbeing hazard. We investigated the street foods in Dhaka city, Bangladesh were heavily contaminated with many foodborne pathogens include enteric pathogenic bacteria among the street food samples that ought to be absent in food. This study reveals that through the street foods, alarming levels of drug-resistant bacteria could be spreading in the public which is demonstrating the seriousness of consumer's health threats. Overall, the study highlighted that lack of awareness and lack of adherence to the food laws and regulation together with the frequent execution of existing rules are contributing significantly to the dissatisfactory food safety circumstances of Bangladesh. We call upon the monitoring authorities to re-enforce measures on microbiological food safety within the handling of ready-toeat foodstuffs.

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#### **CONFLICT OF INTERESTS**

The authors announce that regarding the publication of this paper there is no conflict of interest.

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