

# Isolation and Identification of enteric bacteria in different street vended foods collected from different private universities in Dhaka city

A research paper is submitted to the Department of Pharmacy, East West University in conformity with the requirements for the degree of Bachelor of Pharmacy.

Submitted by

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## **Declaration by the Candidate**

I, Shampa Mutsuddy, hereby declare that the dissertation entitled “Isolation and Identification of enteric bacteria in different street vended foods collected from different private universities in Dhaka city” submitted by me to the Department of Pharmacy, East West University and in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, work carried out by me during the period 2016 of my research in the Department of Pharmacy, East West University, under the supervision and guidance of Ms. Nafisa Tanjia, Lecturer, Department of Pharmacy, East West University. The thesis paper has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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This is to certify that the thesis entitled “Isolation and Identification of enteric bacteria in different street vended foods collected from different private universities in Dhaka city” submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by Shampa Mutsuddy, ID: 2012-3-70-004, during the period 2016 of her research in the Department of Pharmacy, East West University, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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## Table of Content

List of Content	Page No.
List of Table	I
List of Figures	I-II
List of Abbreviations	II
Abstract	III

Serial No.	Title	Page No.
CHAPTER 01	INTRODUCTION AND LITERATURE REVIEW	1-19
1.1	Street Vended Foods:	1
1.2	Types of Street Vended Foods:	1
1.3	Food Borne Illnesses:	2
1.4	Who Gets Food Borne Illnesses	2
1.5	Causes of Food Borne Illnesses:	3
1.6	Major sources contributing to microbial contamination	4
1.6.1	Vending Location: Food Handling and Waste Disposal	4
1.6.1.1	Food Handling	4
1.6.1.2	Waste Disposal	4
1.6.2	Quality of Raw Materials: Water and Other Material	4
1.6.2.1	Water	4
1.6.2.2	Other Raw Materials	5
1.6.3	Utensils and Equipments: Chemical and Microbial Contaminants	5
1.6.3.1	Chemical Contaminants	5
1.6.3.2	Microbial Contaminants	5
1.6.4	Food Preparation: Storage and Reheating	5
1.6.4.1	Storage	6
1.6.4.2	Reheating	6
1.6.5	Personal Hygiene of the Vendors or Food Handlers	6
1.7	Parameters of Foods That Affect Microbial Growth	6
1.7.1	Intrinsic Parameters	7
1.7.2	Extrinsic Parameter	7
1.8	Microorganisms Causing Food Borne Illnesses:	7
1.8.1	<i>E.coli</i>	7
1.8.1.1	Pathogenesis	8
1.8.1.2	Source of contamination	9
1.8.2	<i>Salmonella spp.</i>	9
1.8.2.1	Pathogenesis	10

Serial No.	Title	Page No.
1.8.2.2	Source of contamination	11
1.8.3	<i>Shigella spp.</i>	11
1.8.3.1	Pathogenesis	12
1.8.3.2	Source of contamination	13
1.8.4	<i>Vibrio spp.</i>	13
1.8.4.1	Pathogenesis	14
1.8.4.2	Source of contamination	15
1.8.5	<i>Klebsiella spp</i>	15
1.8.5.1	Pathogenesis	16
1.8.5.2	Source of contamination	17
1.9	Frequency of Food Borne Illness in Different Countries	17
1.9.1	Food borne Illness Outbreaks in the United States	18
1.9.2	Food Borne Illness Outbreaks in Australia	18
1.9.3	Food Borne Illness Outbreaks in Korea and Japan	18
1.10	Street Foods Condition in Dhaka city	19
CHAPTER 02	RESEARCH OBJECTIVE	20
CHAPTER 04	METHODOLOGY	21-28
3.1	Bacteriological Subculture	21
3.1.1	Sample Collection	21
3.1.1.1	Sample Category	21
3.1.2	Sample Processing	21
3.1.3	Enrichment of the Organisms	21
3.1.3.1	Enrichment of <i>E.coli</i> and <i>Klebsiella spp</i>	21
3.1.3.2	Enrichment of <i>Salmonella spp</i> and <i>Shigella spp</i>	21
3.1.3.3	Enrichment of <i>Vibrio spp</i>	21
3.1.4	Selective Growth of the Organisms	22
3.1.4.1	Selective Growth of <i>E.coli</i> and <i>Klebsiella spp</i>	22
3.1.4.2	Selective Growth <i>Salmonella spp</i> and <i>Shigella spp</i>	22
3.1.4.3	Selective Growth of <i>Vibrio spp</i>	22
3.1.5	Sterilization Procedure	23
3.1.6	Preparation of Petri dishes	23
3.1.7	Incubation	23
3.1.8	Apparatus & reagent used for isolation and identification of specific organism	24
3.2	Biochemical Tests	25
3.2.1	Kliglar Iron Agar Test (KIA Test)	25
3.2.1.1	Test Tube Preparation for KIA Test	25



<b>Serial No.</b>	<b>Title</b>	<b>Page No.</b>
3.2.1.2	Inoculation for KIA Test	25
3.2.2	MIO Test	26
3.2.2.1	Test Tube Preparation for MIO Test	26
3.2.2.2	Inoculation for MIO Test	26
3.2.3	Citrate Test	26
3.2.3.1	Test Tube Preparation for Citrate Test	26
3.2.3.2	Inoculation for Citrate Test	26
3.2.4	Urease Test	27
3.2.4.1	Test Tube Preparation for Urease Test	27
3.2.4.2	Inoculation for Urease Test	27
3.2.5	Oxidase test	27
3.2.6	Apparatus & reagent used for Biochemical Tests	27
CHAPTER 04	RESULT	29-35
4.1	Bacteriological colony morphology	29
4.2	Suspected organisms from different biochemical tests	32
CHAPTER 05	DISCUSSION	36-38
5.1	Discussion	36
5.2	Conclusion	38
CHAPTER 06	REFERENCE	39-41

## List of Tables

<b>Table no</b>	<b>Name of the table</b>	<b>Page no</b>
Table 1.5	Groups of Food borne Pathogens	3
Table 3.1	Standard Colony Morphology of Suspected Organisms	24
Table 3.2	Standard Biochemical Test Results of Suspected Organisms	28
Table 4.1	Bacterial colony morphology isolated from different street vended food samples	29
Table 4.2	Bacterial colony morphology isolated from different street vended food samples	30
Table 4.3	Number of food samples with growth of suspected organisms determined by colony morphology (n=30)	31
Table 4.4	Identification of the suspected organism ( <i>Klebsiella spp.</i> ) from different biochemical test	32
Table 4.5	Identification of the suspected organism ( <i>E.coli, Vibrio spp. and Shigella spp.</i> ) from different biochemical test	33
Table 4.6	Presence of suspected organisms in no of food samples from different university (n=13)	34
Table 4,7	Incidence of food borne pathogens in various street vended food samples	35

## List of Figures

<b>Figure No.</b>	<b>Name of the figure</b>	<b>Page No.</b>
Fig. 1.1	Street Vended Foods	1
Fig. 1.2	<i>Escherichia coli (E. coli)</i>	8
Fig. 1.3	<i>Salmonella spp</i>	10
Fig. 1.4	<i>Shigella spp</i>	12
Fig. 1.5	<i>Vibrio spp</i>	14
Fig. 1.6	<i>Klebsiella spp</i>	16
Fig. 3.1	Enrichment of the Organisms	22
Fig. 3.2	Autoclave and Hot air Oven	23
Fig. 3.3	Laminer Air Flow Cabinet	23
Fig. 3.4	Petri dishes preparation	23

<b>Figure No.</b>	<b>Name of the figure</b>	<b>Page No.</b>
Fig. 3.5	Incubator	24
Fig. 3.6	Preparation of test tubes for KIA test	25
Fig. 3.7	Preparation of test tubes for MIO test	26
Fig. 3.8	Preparation of test tubes for Citrate test	26
Fig. 3.9	Preparation of test tubes for Urease test	27
Fig. 4.1	Bacterial colony ( pink) on MacConkey agar plate	31
Fig. 4.2	Bacterial colony (blue) on TBX agar plate	31
Fig. 4.3	Different Biochemical test	33

### **List of Abbreviations**

ETEC	<i>Enterotoxigenic E. coli</i>
EIEC	<i>Enteroinvasive E. coli</i>
EPEC	<i>Enteropathogenic E. coli</i>
EHEC	<i>Enterohaemorrhagic E. coli</i>
VTEC	Verotoxin-producing <i>E. coli</i>
HUS	Haemolytic Uraemic Syndrome
CFU	Colony Forming Unit
TSB	Trypticase Soy Broth
YE	Yeast Extract
BPW	Buffered Peptone Water
APW	Alkaline Peptone Water
TBX	Tryptone Bile X-glucuronide
BGA	Brilliant Green Agar
XLD	Xylose lysine deoxycholate
TCBS	Thiosulfate citrate-bile salts sucrose
KIA	Kliglar Iron Agar

## **Abstract**

The objective of this study was to isolate and identify the presence of enteric bacteria (*Escherichia coli*, *Klebsiella spp*, *Shigella spp*, *Salmonella spp* and *Vibrio spp*) in different street vended foods collected from different private universities in Dhaka city. Thirty food samples were collected from fixed and mobile vendors from area around 10 private universities in Dhaka city. The tested samples were jhal-muri, fuchka, vhel-puri, panipuri, bun, cake, danish, chola, peaju, sweet, sheek-kabab, laddu, singara, somucha etc. Sterile polythene bags were used to collect 3 different samples from each university. They were tested for the presence of microorganisms following conventional microbiological processes. Biochemical tests were done for the confirmation of *Escherichia coli*, *Klebsiella spp*, *Shigella spp*, *Salmonella* and *Vibrio spp*. Out of 30 food samples, eight (43%) were suspected to contain *Klebsiella spp*, three (27%) were suspected to contain *Escherichia coli*, one (3%) was suspected to contain *Shigella spp* and one (3%) was suspected to contain *Vibrio spp*. All these enteric pathogens could be the potential cause for food-borne illnesses and provision of education to the vendors would improve quality of street foods.

**Key Words:** Street foods, *Escherichia coli*, *Shigella spp*, *Vibrio spp*, Dhaka city, Private University.



**CHAPTER 01**  
**INTRODUCTION AND LITERATURE**  
**REVIEW**

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## **1.1 Street Vended Foods:**

Street vended foods mean ready to eat foods and beverages that are prepared and sold especially in streets or similar public places by the street vendors or merchants for consumption at the location or later without any further preparation. The street vended foods are prepared under unhygienic conditions and displayed openly to a high degree of contamination. Street foods are sometimes stored at improper temperatures and sold from vending sites which includes kiosks, make-shift accommodation, and push carts as well as other temporary structures. In most cases running water is not available at vending sites, washing of hands and crockery are done in bowls or buckets and sometimes without soap. Thus from the health point of view, selling foods in the street is very controversial (Bereda et al., 2016). The street food industry plays an important role in developing countries particularly following the food demands of urban dwellers. Street foods feed millions of people daily with a wide variety of foods that are relatively cheap and easily accessible. Street foods are sources of nutrition for many low-income groups at affordable prices in large urban areas. These street foods could be main vehicles for the transmission of severe food borne infections and fatal disease that could be life-threatening (Rane, 2011).



**Fig. 1.1: Street Vended Foods**

## **1.2 Types of Street Vended Foods:**

There are different types of street vended foods are available everywhere. The types of street vended foods vary from country to country according to their culture and geographical environment. Some street foods are common in every country such as salads, beef, chicken and gravy etc. Bangladesh has a huge number of street food vendors

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especially in Dhaka city. These vendors prefer to take their products to their customers in the central business areas and at key points of transport such as train and bus terminals, market places, as well as in front of schools. Such locations usually do not meet food and safety requirements. The most popular and traditional street-vended foods in Bangladesh include jhal-muri, fuchka, vhel-puri, panipuri, bun, cake, danish, betel-leaf, chola, peaju, sweet, sheek-kabab, laddu, singara, somucha etc (Rahman, Rahman & Ansary, 2014).

### **1.3 Food Borne Illnesses:**

Food borne illnesses are globally important, as they result in considerable morbidity, mortality, and economic costs. Food borne illnesses (also called food poisoning) are infections or irritations of the gastrointestinal (GI) tract caused by eating contaminated food or beverages. Infectious organisms including bacteria, viruses and parasites or their toxins are the most common causes of food poisoning. Infectious organisms or their toxins can contaminate food at any point of processing, production, growing, harvesting, storing, shipping or preparing (Kirk et al., 2015). Natural and manufactured chemicals in food products also can make people sick. The potential for the contamination of street foods with pathogenic microorganisms has been well documented and several disease outbreaks have been traced to consumption of contaminated street foods. There are more than 250 known food borne diseases. Many microbes can spread in more than one way, so it may not be immediately evident that a disease is food borne. The distinction matters should be identified because public health authorities need to know how a particular disease is spreading and the appropriate steps should be taken to stop it (Sharma & Mazumdar, 2014).

### **1.4 Who Gets Food Borne Illnesses**

Whether anyone become ill after eating contaminated food depends on the organism, the amount of exposure, age and health. Anyone can get a food borne illness. However, some people are more likely to develop food borne illnesses than others. High-risk groups include:

- ***Older adults:*** As anyone gets older, immune system may not respond as quickly and as effectively to infectious organisms as when younger.
- ***Pregnant women:*** During pregnancy, changes in metabolism and circulation may increase the risk of food poisoning. The reaction may be more severe during pregnancy. Rarely, the baby may get sick, too.

- **Infants and young children:** Their immune systems haven't fully developed.
- **People with chronic disease:** Having a chronic condition such as diabetes, liver disease or AIDS or receiving chemotherapy or radiation therapy for cancer reduces immune response (Niddk.nih.gov, 2016).

## **1.5 Causes of Food Borne Illnesses:**

Although a number of different infectious pathogen may be contracted from foods under certain circumstances, there are those that are contracted exclusively or predominantly from the consumption of food products. The recognized food borne pathogens include multicellular animal parasites, protozoa, fungi, bacteria, viruses, and possibly prions. Some harmful microorganisms may already be present in foods when they are purchased. Raw foods including meat, poultry, fish and shellfish, eggs, unpasteurized milk and dairy products often contain microorganisms that cause food borne illnesses.

**Table 1.5 Groups of Food borne Pathogens**

<p><b>Flatworms</b></p> <p>Flukes- <i>Fasciola</i>, <i>Fasciolopsis</i>, <i>Paragonimus</i>, <i>Clonorchis</i></p> <p>Tapeworms- <i>Diphyllobothrium</i>, <i>Taenia</i></p> <p><b>Roundworms</b></p> <p><i>Trichinella</i>, <i>Ascaris</i>, <i>Anisakis</i>, <i>Toxocara</i>, <i>Pseudoterranova</i></p> <p><b>Protozoa</b></p> <p><i>Giardia</i>, <i>Entamoeba</i>, <i>Toxoplasma</i>, <i>Sarcocystis</i>, <i>Cryptosporium</i>, <i>Cyclospora</i></p> <p><b>Fungi—mycotoxin producers</b></p> <p>Aflatoxins, Fumonisin, Alternaria toxins, Ochratoxins.</p>	<p><b>Bacteria</b></p> <p>Gram positive- <i>Staphylococcus</i>, <i>Bacillus cereus</i>, <i>Clostridium botulinum</i>, <i>C. perfringens</i>, <i>Listeria</i> <i>monocytogenes</i>, <i>Mycobacterium paratuberculosis</i></p> <p>Gram negative- <i>Salmonella</i>, <i>Shigella</i>, <i>Escherichia</i>, <i>Yersinia</i>, <i>Vibrio</i>, <i>Campylobacter</i>, <i>Aeromonas</i>, <i>Brucella</i>, <i>Plesiomonas</i></p> <p><b>Viruses</b></p> <p>Hepatitis A, Small round structured viruses (SRSVs), Rotaviruses.</p> <p><b>Prions</b></p> <p>Creutzfeldt-Jakob disease (newvariant form)</p> <p><b>Toxigenic phytoplanktons</b></p> <p>Paralytic shellfish poison, Domoic acid, <i>Pfiesteria piscicida</i>, Ciguatoxin</p>
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(Jay, Loessner & Golden, 2005)



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## **1.6 Major sources contributing to microbial contamination**

### **1.6.1 Vending Location: Food Handling and Waste Disposal**

The conditions under which some street vendors operate are reported to be unsuitable for the preparation and selling of food. The two major sources from where the contaminants can enter the preparation area are: Improper food handling and waste disposal.

#### **1.6.1.1 Food Handling**

Unsanitary handling of street foods by the some of the vendor has been commonly found to be the source of contamination. The vendors can be carriers of pathogens like *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter* and *S. aureus* who eventually transfer these food borne hazards to the consumers. The hands of the food handlers are the most important vehicle for the transfer of organisms from faeces, nose, and skin to the food (Rane, 2011).

#### **1.6.1.2 Waste Disposal**

Few vendors congregate in overcrowded areas where there are high numbers of potential customers, which usually provide limited access to basic sanitary facilities. Hence, the contamination of street foods is often linked to the waste generated by food processing, that is usually dumped near the vending site. The lack of facilities for liquid drainage and wastewater and garbage disposal encourages wastes to be thrown into nearby streets and gutters. Such areas act as habitats for rodents, breeding points for flies and media for growth of microorganisms (Rane, 2011).

### **1.6.2 Quality of Raw Materials: Water and Other Material**

The quality of raw materials used in the preparation of street foods is very important as their contamination can persist through preparation and or cooking.

#### **1.6.2.1 Water**

Water is a critical raw material in many street-vended operations. Contaminated water can create a public health risk when it is used for drinking, washing of foods, incorporated in the food as an ingredient and used in the processing of food or used for washing equipment, utensils and hands. It is a well known vehicle for enteropathogens such as *E. coli*, *Salmonella* spp. and *Campylobacter* spp. amongst others. Studies carried out in different regions of Asia, Africa and South America has frequently pointed the

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unavailability of potable water for various activities at the vending site as a major concern. Due to the shortage of clean potable water, many vendors tend to re-use the water, especially for cleaning utensils and used dishes (Rane, 2011).

### **1.6.2.2 Other Raw Materials**

Besides water, other raw materials are also important to the safety of the street vended foods because of the biological, chemical and physical hazards that they might introduce. In order to keep prices down, some vendors purchase cheap or adulterated ingredients containing unpermitted chemical additives from unauthorized suppliers which may further increase the risks associated with the food so prepared. Raw meat, poultry and vegetables are commonly contaminated with large numbers of bacteria (Rane, 2011).

### **1.6.3 Utensils and Equipments: Chemical and Microbial Contaminants**

Use of proper utensils for cooking and storage of prepared food is often critical to the safety of street vended foods. Poor quality of material coupled with improper practices may lead to toxin formation, pathogen growth or recontamination.

#### **1.6.3.1 Chemical Contaminants**

As some containers will leach hazardous chemicals like copper, lead and cadmium into food, use of equipment and utensils incompatible with the food being handled, should be avoided. This has been observed particularly with acidic food and beverages (Ohiokpehai, 2003).

#### **1.6.3.2 Microbial Contaminants**

The serving utensils used at the vending site are often contaminated with *Micrococcus* spp. and *Staphylococcus* spp. which may have originated from the vendors hands when they touched the food preparation areas, dishcloths, or the water during dish washing or hand washing which indicates cross contamination between dishwater, food preparation surfaces, and the food itself (Cardinale et al., 2005).

### **1.6.4 Food Preparation: Storage and Reheating**

An important issue influencing food contamination and contributing to further increase in contamination is food storage temperature. The preparation of food long before its consumption, storage at ambient temperature, inadequate cooling and reheating, contaminated processed food and under cooking are identified as the key factors.

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#### **1.6.4.1 Storage**

Foods are often held for several hours after cooking and this includes overnight holding at ambient temperatures, until sold, and thus can harbor high microbial populations. Besides, some of the foods are held in the pans in which they are cooked, until sold or reheated, which results in longer holding time, hence creating favorable conditions for the growth of food borne pathogens (Rane, 2011).

#### **1.6.4.2 Reheating**

Time–temperature exposures during reheating need to be sufficiently high or long to inactivate large quantities of infectious microorganisms that could develop during the lengthy holding process. Some food vendors often partially or fully cook some products ahead of time, store them and then reheat them when requested by customers. However, this reheating is often inadequate to destroy bacteria that may be present as this would allow the food borne pathogens that germinate from spores which survived cooking or that contaminate the food after cooking, to survive and proliferate (Omemu & Aderoju, 2008).

#### **1.6.5 Personal Hygiene of the Vendors or Food Handlers**

According to World Health Organization, food handling personnel play an important role in ensuring food safety throughout the chain of food production, processing, storage and preparation. Mishandling and disregard of hygienic measures on the part of the food vendors may enable pathogens to come into contact with food and in some cases to survive and multiply in sufficient numbers to cause illness in the consumer. Some food handlers may introduce biological hazards by cross contamination after handling raw materials when they suffer from specific diseases and physical hazards by careless food handling practices. Most of the vendors pack the food in polythene bags for their customers. When packing these foods, they blow air into the polythene bags to open them, in this process a number of pathogens can be passed on to the consumer (Ohiokpehai, 2003).

### **1.7 Parameters of Foods That Affect Microbial Growth**

As foods are of plant and animal origin, it is worthwhile to consider those characteristics of plant and animal tissues that affect the growth of microorganisms. The plants and animals that serve as food sources have all evolved mechanisms of defense against the

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invasion and proliferation of microorganisms, and some of these remain in effect in fresh foods. By taking these natural phenomena into account, one can make effective use of each or all in preventing or retarding the microbial spoilage of the products that are derived from them (Jay, Loessner & Golden, 2005).

### **1.7.1 Intrinsic Parameters**

The parameters of plant and animal tissues that are an inherent part of the tissues are referred to as intrinsic parameters. These parameters are as follows:

- pH
- Moisture content
- Oxidation-reduction potential (Eh)
- Nutrient content
- Antimicrobial constituents
- Biological structures (Jay, Loessner & Golden, 2005).

### **1.7.2 Extrinsic Parameter**

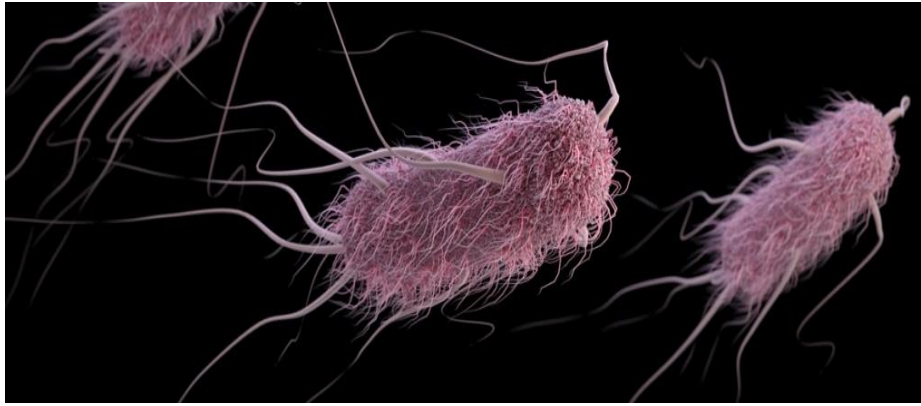
The extrinsic parameters of foods are those properties of the storage environment that affect both the foods and their microorganisms. Those of greatest importance to the welfare of food borne organisms are as follows:

- Temperature of storage
- Relative humidity of environment
- Presence and concentration of gases
- Presence and activities of other microorganisms (Jay, Loessner & Golden, 2005).

## **1.8 Microorganisms Causing Food Borne Illnesses:**

### **1.8.1 *E.coli***

*Escherichia coli* (or *E. coli*) are the most prevalent infecting organisms in the family of gram-negative bacteria known as enterobacteriaceae. *E. coli* bacteria normally live in the intestines of healthy people and animals. The bacteria are rod shaped, non-spore forming, motile with peritrichous flagella or nonmotile. They can grow under aerobic and anaerobic conditions and do not produce enterotoxins (Adams & Moss, 2008).



**Fig. 1.2: *Escherichia coli* (*E. coli*)**

### **1.8.1.1 Pathogenesis**

There are four major categories of diarrhoeagenic *E. coli* based on distinct, virulence properties.

- *Enterotoxigenic E. coli* (ETEC) - Illness caused by ETEC usually occurs between 12 and 36 h after ingestion of the organism. Symptoms can range from a mild febrile diarrhea to a severe cholera like syndrome of watery stools without blood or mucus, stomach pains and vomiting. The illness is usually self-limiting, persisting for 2–3 days, although in developing countries it is a common cause of infantile diarrhea where it can cause serious dehydration (Adams & Moss, 2008).
- *Enteroinvasive E. coli* (EIEC) - Infection by EIEC results in the classical symptoms of an invasive bacillary dysentery normally associated with *Shigella*. Like *Shigella*, EIEC invades and multiplies within the epithelial cells of the colon causing ulceration and inflammation, though EIEC strains do not produce Shiga toxin. Clinical features are fever, severe abdominal pains, malaise and often watery diarrhoea which precede the passage of stools containing blood, mucus, and faecal leukocytes (Adams & Moss, 2008).
- *Enteropathogenic E. coli* (EPEC) - When the properties of ETEC and EIEC were established it was noted that these strains were rarely of the same serotypes first associated with *E. coli* diarrhoea in the 1950s. Symptoms of EPEC infection, malaise, vomiting and diarrhoea with stools containing mucus but rarely blood, appear 12–36 h after ingestion of the organism. In infants, the illness is more severe than many other diarrhoeal infections and can persist for longer than two weeks in some cases (Adams & Moss, 2008).

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- *Enterohaemorrhagic E. coli* (EHEC) - EHEC, sometimes also known as Verotoxin-producing *E. coli* (VTEC), was first described in Canada where in some areas it rivals *Campylobacter* and *Salmonella* as the most frequent cause of diarrhoea. *E. coli* O157:H7 is the most common EHEC serotype reported, although others do occur. EHEC has attracted attention not only because food-borne transmission is more common than with other diarrhoeagenic *E. coli*, but because the illness it causes can range from a non-bloody diarrhoea, through haemorrhagic colitis, to the life threatening conditions haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Adams & Moss, 2008).

### **1.8.1.2 Source of contamination**

Among the many strains of *E. coli*, only a few trigger diarrhea. One group of *E. coli* which includes O157:H7 produces a powerful toxin that damages the lining of the small intestine, which can cause bloody diarrhea. An *E. coli* infection develops when anyone ingest this strain of bacteria. Potential sources of exposure include-

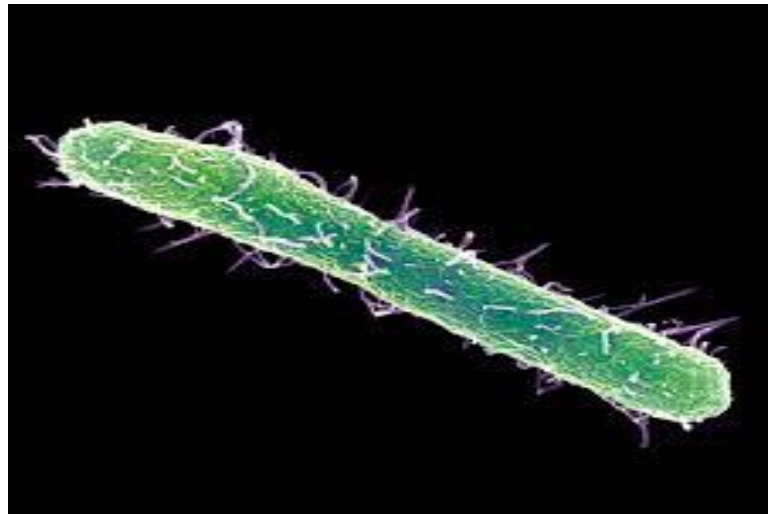
- Contaminated food, especially undercooked ground beef, unpasteurized (raw) milk and juice, soft cheeses made from raw milk, and raw fruits and vegetables (such as sprouts)
- Contaminated water, including drinking untreated water and swimming in contaminated water
- Animals and their environment: particularly cows, sheep, and goats. If you don't wash your hands carefully after touching an animal or its environment, you could get an *E. coli* infection
- Feces of infected people (Mayoclinic.org, 2016).

### **1.8.2 *Salmonella* spp.**

*Salmonella* is a gram-negative bacterium belongs to the family *Enterobacteriaceae* that can cause diarrheal illness in humans. The bacteria live in the gut of infected humans and animals. *Salmonella* is a bacterium shaped like a rod with a cell wall composed of peptidoglycan. It is a motile, facultative anaerobe that is susceptible to various antibiotics. Currently, 107 strains of this organism have been isolated; many containing varying metabolic characteristics, levels of virulence, and multi-drug resistance genes that complicate treatment in areas that resistance are prevalent. The *Salmonella* family

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includes over 2,500 serotypes of bacteria - they are microscopic one-celled organisms (WHO, 2016).



**Fig. 1.3: *Salmonella* spp.**

### **1.8.2.1 Pathogenesis**

*Salmonella* infection, or salmonellosis, is a bacterial disease of the intestinal tract. *Salmonella* is a group of bacteria that causes typhoid fever, food poisoning, gastroenteritis, enteric fever and other illnesses.

***Typical Symptoms of Salmonella infection:*** Appear 6 to 72 hours after eating contaminated food and last for 3 to 7 days without treatment.

- Generalized systemic enteric fever
- Headache
- Malaise
- Anorexia
- Enlarged spleen and
- Constipation followed by more severe abdominal symptoms
- Rose spots on trunk in 25% of Caucasian patients

Complications include ulceration of Peyer's patches in ileum, can produce hemorrhage or perforation. Common enterocolitis may result without enteric fever characterized by headache, abdominal pain, nausea, vomiting, diarrhea, dehydration (Adams & Moss, 2008).

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***Typhoid Fever Symptoms:*** Symptoms of typhoid fever appear between 8 and 14 days after eating contaminated food and last anywhere from 3 to 60 days. They include a fever of 104 F, weakness, lethargy, abdominal pain, coughing, nosebleeds, delirium, and enlarged organs. Typhoid fever is a serious illness that can result in death (Adams & Moss, 2008).

### **1.8.2.2 Source of contamination**

*Salmonella* lives in the intestines of birds, animals and humans. Most human infections are caused by eating food or drinking water that has been contaminated by feces (excrement). Foods that are most commonly infected are:

- **Uncooked meat, egg and poultry** - Meat, milk, poultry, and eggs are primary vehicles; they may be undercooked, allowing the *salmonellas* to survive, or they may cross-contaminate other foods that are consumed without further cooking (Adams & Moss, 2008).
- **Fruits and vegetables** - if fruit and vegetables have been watered or washed in contaminated water there is a much higher chance they will be contaminated. Some kitchen practices may contaminate fruits and vegetables - if the person preparing the food handles raw meat and then touches the fruit without washing his/her hands, for example (Adams & Moss, 2008).
- **Lacks of hygiene** - Human carriers are generally less important than animals in the transmission of salmonellosis. Human transmission can occur if the faecally contaminated hands of an infected food handler touch a food which is then consumed without adequate cooking, often after an intervening period in which microbial growth occurs. A person with contaminated hands can pass the infection on to other people by touching them, or touching surfaces which others then touch (Adams & Moss, 2008).

### **1.8.3 *Shigella spp.***

*Shigella* is a species of enteric bacteria that causes disease in humans and other primates. The disease caused by the ingestion of *Shigella* bacteria is referred to as shigellosis, which is most typically associated with diarrhea and other gastrointestinal symptoms. *Shigella spp.* of the Enterobacteriaceae family, are gram-negative rod-shaped pathogenic bacteria. They are non-motile, non-encapsulated, and facultative anaerobes that do not ferment lactose, or do so slowly. Different serogroups, considered as species, can be



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differentiated by their biochemical properties, phage or colicin susceptibility, and polyvalent antiserum can detect specific polysaccharide antigens. There are 4 species of *Shigella*: *Shigella dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei* (also referred to as group A, B, C, and D, respectively). Several distinct serotypes are recognized within the first 3 species. *S. dysenteriae* is considered the most virulent, and can produce a potent cytotoxin known as Shigatoxin (Adams & Moss, 2008).



**Fig. 1.4:** *Shigella spp.*

### **1.8.3.1 Pathogenesis**

*Shigella spp.* cause bacillary dysentery in humans and other higher primates. The incubation period can vary between 7 h and 7 days although food-borne outbreaks are commonly characterized by shorter incubation periods of up to 36 h.

Symptoms of *Shigella* poisoning most commonly develop lasts from 3 days up to 14 days in some cases and a carrier state may develop which can persist for several months. It is also possible to get *Shigella* but experience no symptoms, and still be contagious to others, a condition known as being asymptomatic. Common *Shigella* food poisoning symptoms are:

- Abdominal pain
- Vomiting
- Diarrhea: Diarrhea ranges from mild to severe. It is bloody in 25 to 50 percent of cases and usually contains mucus

- 
- Fever
  - Stomach cramps
  - Rectal spasms

Milder forms of the illness are self-limiting and require no treatment but *Sh. dysenteriae* infections often require fluid and electrolyte replacement and antibiotic therapy. Shigellosis is an invasive infection where the organism's invasive property is encoded on a large plasmid (Adams & Moss, 2008).

### **1.8.3.2 Source of contamination**

Shigellosis is caused by *Shigella* bacteria. Outbreaks of shigellosis frequently occur in tropical or temperate climates, especially in areas with severe crowding or poor hygiene. Outbreaks sometimes occur in daycare and institutional settings. Organisms are spread through the fecal-oral route, and transmission is typically through one of three mechanisms:

- Ingestion of contaminated foods (washed with fecally contaminated water, or handled with poor hygiene, commonly in tossed salads, chicken, and shellfish)
- Drinking contaminated water (or in swimming pools) or
- By person-to-person contact by anal sexual contact. Spread of infection linked to flies has also been recorded (Adams & Moss, 2008).

### **1.8.4 *Vibrio spp.***

*Vibrio spp.* of Vibronaceae family are gram negative, non-spore forming, curved rod which are oxidase positive. They are very motile and have a single polar flagellum. The bacterium are 1- 3  $\mu\text{m}$  by 0.5-0.8  $\mu\text{m}$ , are a facultative anaerobe. The genus *Vibrio* consists of at least 28 species, and 3 that are often associated with *V. parahaemolyticus* in aquatic environments and seafood are *V. vulnificus*, *V. alginolyticus*, and *V. cholerae*. Although most other known food-poisoning syndromes may be contracted from a variety of foods, *V. parahaemolyticus* gastroenteritis is contracted almost solely from seafood. When other foods are involved, they represent cross-contamination from seafood products. Another unique feature of this syndrome is the natural habitat of the etiological agent—the sea. In addition to its role in gastroenteritis, *V. parahaemolyticus* is known to cause extraintestinal infections in humans (Jay, Loessner & Golden, 2005).



**Fig. 1.5: *Vibrio* spp.**

#### **1.8.4.1 Pathogenesis**

*Vibrio vulnificus* and *Vibrio parahaemolyticus* are bacteria that occur naturally in warm coastal areas, such as the Gulf of Mexico. These bacteria are found in higher concentrations in the summer months when water gets warmer. *Vibrios* typically cause disease in people who eat contaminated seafood.

- *V. parahaemolyticus* typically causes non-bloody diarrhea.
- In persons with liver disease, cancer, or another immune-compromising condition, *V. vulnificus* typically infects the bloodstream, causing a life-threatening illness. About half of *V. vulnificus* bloodstream infections are fatal, and death can occur within two days. In addition to transmission by raw shellfish, *V. vulnificus* can enter the body via a wound that is exposed to warm seawater.
- In healthy individuals: Diarrhea, vomiting, abdominal pain
- In high-risk individuals: Sudden chills, fever, shock, skin lesions (Foodsafety.gov, 2016).

Cholera is a bacterial disease caused by *V. cholerae*. Symptoms of cholera infection may include:

- Diarrhea
- Nausea and vomiting
- Dehydration- signs and symptoms of dehydration includes
  - ✓ Irritability
  - ✓ Lethargy
  - ✓ Sunken eyes

- 
- ✓ A dry mouth
  - ✓ Extreme thirst
  - ✓ Dry and shriveled skin that's slow to bounce back when pinched into a fold
  - ✓ Little or no urine output
  - ✓ Low blood pressure and
  - ✓ An irregular heartbeat (arrhythmia).
  - ✓ Electrolyte imbalance (Mayoclinic.org, 2016).

#### **1.8.4.2 Source of contamination**

Persons who are immunocompromised, especially those with chronic liver disease, are at risk for *V. vulnificus* when they eat raw seafood, particularly oysters. Since it is naturally found in warm marine waters, people with open wounds can be exposed to *V. vulnificus* through direct contact with seawater. There is no evidence for person-to-person transmission of *V. vulnificus* (FDA, 2016).

*Vibrio cholerae*, the bacterium that causes cholera, is usually found in food or water contaminated by feces from a person with the infection. Common sources include:

- Municipal water supplies
- Ice made from municipal water
- Foods and drinks sold by street vendors
- Vegetables grown with water containing human wastes
- Raw or undercooked fish and seafood caught in waters polluted with sewage

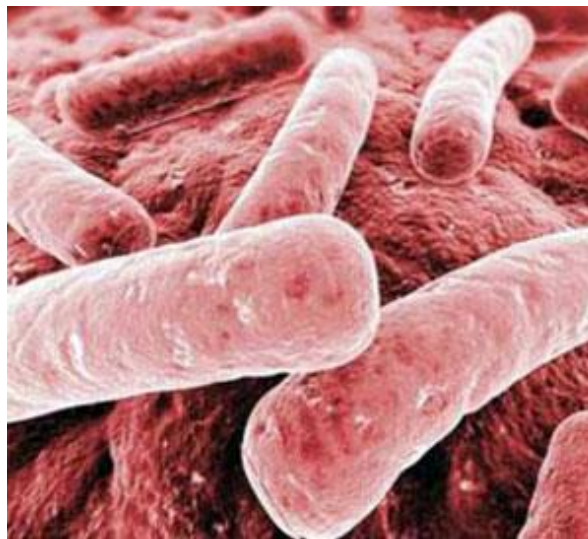
When a person consumes the contaminated food or water, the bacteria release a toxin in the intestines that produces severe diarrhea (Mayoclinic.org, 2016).

#### **1.8.5 *Klebsiella* spp.**

*Klebsiella* is a gram-negative, non-motile, lactose fermenting, rod-shape organism. *K. pneumoniae* is able to grow either with or without free oxygen, deeming it a facultative anerobe which is usually found in the normal flora of skin, mouth, and intestines. This organism is also surrounded by a capsule, which increases its virulence by acting as a physical barrier to evade the host's immune response (Puspanadan et al., 2012). Increasingly, *Klebsiella* bacteria have developed antimicrobial resistance, most recently to the class of antibiotics known as carbapenems. In healthcare settings, *Klebsiella*

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infections commonly occur among sick patients who are receiving treatment for other conditions. Patients whose care requires devices like ventilators (breathing machines) or intravenous (vein) catheters, and patients who are taking long courses of certain antibiotics are most at risk for *Klebsiella* infections. Healthy people usually do not get *Klebsiella* infections. *Klebsiella* is a type of bacteria that can cause different types of healthcare-associated infections, including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis (CDC, 2016).



**Fig. 1.6: *Klebsiella* spp**

### **1.8.5.1 Pathogenesis**

The symptoms of a *K. pneumoniae* infection differ depending on where the infection is located, and are similar to symptoms of the same diseases caused by other microbes.

For instance, meningitis from *K. pneumoniae* produces the hallmark symptoms of bacterial meningitis, including

- Fever,
- Confusion,
- Neck stiffness, and
- Sensitivity to bright lights.

Bloodstream infections (bacteremia and sepsis) from *Klebsiella* cause

- Fever,
- Chills,

- 
- Rash,
  - Light-headedness, and
  - Altered mental states.

Pneumonia from *K. pneumoniae* can result in:

- Fevers and chills
- flu-like symptoms
- Cough, which may produce mucus that's yellow, green, or bloody
- Breathing issues (Bennington-Castro, 2016).

### **1.8.5.2 Source of contamination**

To get a *Klebsiella* infection, a person must be exposed to the bacteria. For example, *Klebsiella* must enter the respiratory (breathing) tract to cause pneumoniae, or the blood to cause a bloodstream infection. In healthcare settings, *Klebsiella* bacteria can be spread through person-to-person contact (for example, from patient to patient via the contaminated hands of healthcare personnel, or other persons) or, less commonly, by contamination of the environment. The bacteria are not spread through the air. Patients in healthcare settings also may be exposed to *Klebsiella* when they are on ventilators (breathing machines), or have intravenous (vein) catheters or wounds (caused by injury or surgery). Unfortunately, these medical tools and conditions may allow *Klebsiella* to enter the body and cause infection (CDC, 2016).

## **1.9 Frequency of Food Borne Illness in Different Countries**

Acute food borne disease, infections and intoxications are much more of a concern to governments and the food industry today than a few decades ago. However, to meaningfully monitor increases or decreases in food borne disease requires an effective surveillance system at the local, national and international levels. To date, resources have been limited for most countries and regions to do this, and current knowledge is based, for the most part, on passive reporting mechanisms. Unfortunately, the agent/ food combination leading to illness in many of the reported incidents were not predicted from existing databases, and no doubt food borne agents will continue to surprise food control agencies in the foreseeable future. Nevertheless, data from around the world do show some common elements (Käferstein, 2003).

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### **1.9.1 Food borne Illness Outbreaks in the United States**

Food borne diseases are a major cause of illness and death in the United States. Each year, 31 major known pathogens acquired in the United States caused an estimated 9.4 million episodes of food borne illness, resulting in 55,961 hospitalizations and 1,351 deaths. 5.5 million (59%) food borne illnesses were caused by viruses, 3.6 million (39%) by bacteria, and 0.2 million (2%) by parasites. The pathogens that caused the most illnesses were norovirus (5.5 million, 58%), nontyphoidal *Salmonella spp.* (1.0 million, 11%), *C. perfringens* (1.0 million, 10%), and *Campylobacter spp.* (0.8 million, 9%). Although the number of illnesses caused by these pathogens is substantial, these illnesses represent only a subset of the total illnesses (Scallan et al., 2011).

### **1.9.2 Food Borne Illness Outbreaks in Australia**

In Australia from 1995 to 2000, 293 outbreaks were identified, with 214 being of food borne origin. There were 20 deaths attributed to food borne illness. Of the 214 outbreaks, bacterial disease was responsible for 61 per cent of outbreaks, 64 per cent of cases and 95 per cent of deaths. The most frequent etiology of outbreaks was *Salmonella* in 75 (35%) outbreaks, *Clostridium perfringens* in 30 (14%), ciguatera toxin in 23 (11%), scombrotoxin in 7 (3%) and norovirus in 6 (3%). Salmonellosis was responsible for eight of the 20 (40%) deaths, as was *Listeria monocytogenes*. Restaurants and commercial caterers were associated with the highest number of outbreak reports and cases. Outbreaks in hospitals and aged care facilities were responsible for 35 per cent of deaths. The most frequently implicated vehicles in the 173 outbreaks with known vehicles were meats 64 (30%), fish 34 (16%), seafood 13 (6%), salad 12 (6%), sandwiches 11 (5%) and eggs 9 (4%). Chicken, the most frequently implicated meat, was associated with 27 (13%) outbreaks (Gould et al., 2016).

### **1.9.3 Food Borne Illness Outbreaks in Korea and Japan**

The average prevalence of reported food borne illness from 1981 to 1995 was 2.44 per 100,000 population in Korea, and 28.01 in Japan. The mean case fatality rate in Korea was 0.74% and in Japan, 0.03%. When both prevalence and case fatality rates in Korea and Japan were compared during the same period, the prevalence in Japan was much higher than that in Korea. However, the case fatality rate of patients in Korea was much higher than that in Japan. Comparison study indicates that Food borne illness outbreaks in Korea most frequently involved homemade foods (47% of the total cases); in Japan,

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restaurants accounted for 31.3%. Food borne illness cases of bacterial origin in Korea were 59.3% of the total and included *Salmonella* spp. (20.7%), *Vibrio* (17.4%), *Staphylococcus* (9.7%), pathogenic *Escherichia coli* (2.4%), and other species (9.1%); in Japan, 72.8% of the total cases and the majority of the bacterial food borne illness were caused by *Vibrio* (32.3%), *Staphylococcus* (15.9%), *Salmonella* (14.2%), pathogenic *E. coli* (3.0%), and other species (7.2%) (Won-Chang et al., 2001).

### **1.10 Street Foods Condition in Dhaka city**

In Dhaka streets, food vending is everywhere; however there is a lacking of information regarding food borne diseases related to street-vended foods. The vendors in Bangladesh lack education regarding the basic food safety issues. Vendors generally use carts and stands, where they do not have easy access to running water, furthermore dish and hand washing is done using the same bucket, sometimes even without soap. Garbage and waste water is typically discarded in the streets nearby and thus attracting and providing food for rodents and insects. Toilets are not available nearby in several cases thus forcing the vendors to eliminate their body wastes in nearby areas and return to their vending sites without washing their hands. Environmental condition and practices like this often lead to contamination of cooked food. Vendors may purchase raw materials from doubtful sources which may either be contaminated with food borne pathogens or be unfit for consumption due to other reasons (Rahman, Rahman & Ansary, 2011).

Foods sold by street vendors in Dhaka city are contaminated with pathogenic bacterial organisms, which are likely to pose a potential hazard to consumers, an issue that needs to be addressed. Provision of health education to the street food vendors on personal hygiene, safe food handling practice and proper disposal of waste would improve food quality and thereby reduce the risk of contamination of street-sold food. Infrastructure development for access to potable water, public toilet, washing and waste disposal facilities also would reduce the health hazards to consumers. Although there is a growing demand for these food products, enough information is not available regarding the microbiological quality of these products in Dhaka city, Bangladesh; there are some limitations in the isolation and confirmation of the presence of other microorganisms present in the food samples. Therefore, future studies will be needed to determine the presence of various microorganisms responsible for food-borne illnesses and their confirmation in the laboratory (Islam et al., 2015).



A decorative graphic consisting of two overlapping horizontal bars. The top bar is orange and the bottom bar is green. The green bar is positioned to the right of the orange bar, creating a stepped effect.

## **CHAPTER 02**

## **RESEARCH OBJECTIVE**

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## Objective of this study

The objective of this research work was therefore focused on the following point:

- To isolate and identify the presence of enteric bacteria (*Escherichia coli*, *Klebsiella spp*, *Shigella spp*, *Salmonella* and *Vibrio spp*) in different street vended foods collected from different private universities.



**CHAPTER 03**  
**METHODOLGY**

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## **3.1 Bacteriological Subculture**

### **3.1.1 Sample Collection**

About 30 solid food samples were randomly chosen and collected from street vendors in the area around top 10 private universities of Bangladesh. These samples were collected in different sealed poly bags to prevent their contact with any other source that can contaminate the samples.

#### **3.1.1.1 Sample Category**

Five different categories of food samples were collected. They were deep fried and fried items (Singara, aluchop, egg chop, pakora, nargis kabab, shik kabab, kathi kabab), spicy items (Panifuchka, chhola), noodles, baked items (Cake, danish, biscuit, nimkey) and sweet items (Laddu, goja).

### **3.1.2 Sample Processing**

Solid samples were crushed by mortar and pestle. Then 5 gm of sample were weighed for each broth.

### **3.1.3 Enrichment of the Organisms**

#### **3.1.3.1 Enrichment of *E. coli* and *Klebsiella spp***

5 gm solid sample were mixed well with 45 ml of Trypticase Soy Broth (TSB) + 0.3% yeast extract (YE) and then transferred them to conical flasks. The open mouths of the flasks were covered with foil paper and incubated at 37°C for 18-24 h.

#### **3.1.3.2 Enrichment of *Salmonella spp* and *Shigella spp***

5 gm solid sample were mixed well with 45 ml of BPW (Buffered Peptone Water) broth and incubated at 37 °C for 18-24 h.

#### **3.1.3.3 Enrichment of *Vibrio spp***

5 gm solid sample were mixed well with 45 ml of APW (Alkaline Peptone Water) broth, then transferred them to conical flasks. The open mouths of the flasks were covered with foil paper and incubated at 37°C for 18-24 h.



**Fig. 3.1: Enrichment of the Organisms**

### **3.1.4 Selective Growth of the Organisms**

#### **3.1.4.1 Selective Growth *E.coli* and *Klebsiella* spp**

Cotton buds were dipped into the enrichment broths and swabbed onto MacConkey and TBX( Tryptone Bile X-glucuronide) agar plates, then streaked with sterile loops and incubated in aerobic condition at 37 °C for 18-24 h for selective growth of the organism.

#### **3.1.4.2 Selective Growth of *Salmonella* spp and *Shigella* spp**

Cotton buds were dipped into the enrichment broths and swabbed onto BGA (Brilliant Green Agar) and XLD (Xylose lysine deoxycholate) agar plates, then streaked with sterile loops and incubated in aerobic condition at 37 °C for 18-24 h for selective growth of the organism.

#### **3.1.4.3 Selective Growth of *Vibrio* spp**

Cotton buds were dipped into the enrichment broths and swabbed onto TCBS (Thiosulfate citrate-bile salts sucrose) agar plates, then streaked with sterile loops and incubated in aerobic condition at 37 °C for 18-24 h for selective growth of the organism.

### **3.1.5 Sterilization Procedure**

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glassware were sterilized by autoclaving at a temperature of 121°C and a pressure of 15-lbs. /sq. inch for 20 minutes. Screw cap test tubes, conical flasks, prepared media etc. were also sterilized.



**Fig. 3.2: Autoclave and Hot air Oven**



**Fig. 3.3: Laminar Air Flow Cabinet**

### **3.1.6 Preparation of Petri dishes**

The different types of prepared Agar solution were poured into each of the five Petri dishes in a way so that each Petri dish gets 12-15 ml agar medium. Agar medium was dispensed into each Petri dish to get 3-4 mm depth of agar media in each Petri dish. After pouring the agar medium, all Petri dishes were kept in room temperature so that agar medium can become properly solidified. Then enrichment broths were inoculated in the Petri dishes with the help of cotton buds and loops.



**Fig. 3.4: Petri dishes preparation**

### **3.1.7 Incubation**

Then all the prepared agar plates with respective samples were placed inside a bacteriological incubator at 36°C temperatures for 24 hours for obtaining growth of specific organism in specified plates.



**Fig. 3.5: Incubator**

**Table 3.1: Standard Colony Morphology of Suspected Organisms**

After overnight incubation of the specific media, organisms were selected based on the following criteria

<b>Organism</b>	<b>Media</b>	<b>Appearance</b>
<i>E. coli</i>	MacConkey	Lactose fermenting pink colonies Non-lactose fermenting colorless colonies
	TBX	Blue colonies
<i>Salmonella</i>	BGA	Typical red colonies
	XLD	Red or clear colonies with black centers
<i>Vibrio</i>	TCBS	Large yellow colonies
<i>Shigella</i>	XLD	Typical red colonies
Klebsiella	MacConkey	Pink colonies

**3.1.8 Apparatus & reagent used for isolation and identification of specific organism**

- Laminar air flow cabinet (ESCO, Singapore)
- Petri dishes
- Autoclave (HIRAYAMA, Japan)
- Hot air oven (FN-500, Niive)
- Agar
  - MacConkey agar
  - XLD agar
  - TBX agar
  - BGA agar

- 
- TCBS agar
  - Enrichment Broth
    - Trypticase Soy Broth (TSB)
    - 0.3% yeast extract (YE)
    - BPW (Buffered Peptone Water) broth
    - APW (Alkaline Peptone Water) broth
  - Inoculating loop
  - Spirit burner
  - Hand gloves
  - Mortar and pestle
  - Incubator
  - Measuring Cylinder (100ml)
  - Distilled water
  - Analytical balance
  - Media preparation bottle

## **3.2 Biochemical Tests**

### **3.2.1 Kliglar Iron Agar Test (KIA Test)**

#### **3.2.1.1 Test Tube Preparation for KIA Test**

Freshly prepared Kliglar's Iron Agar poured into the screw cap test tubes in such a amount so that slant with a deep butt( 1 inch) is produced.

#### **3.2.1.2 Inoculation for KIA Test**

With a sterile straight wire suspected colony was stabbed into the butt to inoculate and the slant was streaked and incubated at 37°C for up to 24 hours.



**Fig. 3.6: Preparation of test tubes for KIA test**



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### 3.2.2 MIO Test

#### 3.2.2.1 Test Tube Preparation for MIO Test

For motility test, about 5 ml of MIO agar medium was poured into screw cap test tubes and kept straight. 100  $\mu$ l of Kovac's reagent was added for indole test.

#### 3.2.2.2 Inoculation for MIO Test

Suspected colonies were inoculated by stabbing the medium with the help of sterile straight wire. The tubes were incubated at 37°C for 24 hours.



**Fig. 3.7: Preparation of test tubes for MIO test**

### 3.2.3 Citrate Test

#### 3.2.3.1 Test Tube Preparation for Citrate Test

For citrate test, about 4.0 to 5.0 ml of Simmons citrate medium was poured into 16-mm tubes and cooled in slanted position (long slant, shallow butt).

#### 3.2.3.2 Inoculation for Citrate Test

Suspected colonies were inoculated by stabbing the medium with the help of sterile straight wire. The tubes were incubated at 37°C for 24 hours.



**Fig. 3.8: Preparation of test tubes for Citrate test**

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### 3.2.4 Urease Test

#### 3.2.4.1 Test Tube Preparation for Urease Test

About 2-3 ml of Christensen's Urea Agar was poured into 5mm screw cap tubes and kept straight.

#### 3.2.4.2 Inoculation for Urease Test

Suspected colonies were inoculated by stabbing the medium with the help of sterile straight wire. The tubes were incubated at 37°C for 24 hours.



**Fig. 3.9: Preparation of test tubes for Urease test**

### 3.2.5 Oxidase test

A piece of filter paper was soaked in oxidase reagent and let dry. A well-isolated colony from a fresh (18- to 24-hour culture) bacterial plate was picked by sterile loop and rubbed onto treated filter.

### 3.2.6 Apparatus & reagent used for Biochemical Tests

- Laminar air flow cabinet (ESCO, Singapore)
- Screw cap test tubes
- Autoclave (HIRAYAMA, Japan)
- Hot air oven (FN-500, Niive)
- Straight wire
- Spirit burner
- Hand gloves
- Incubator
- Measuring Cylinder (100ml)
- Distilled water
- Oxidase Reagents

- Kovac's reagent
- Agar
  - Kliglar's Iron Agar
  - MIO agar
  - Christensen's Urea Agar
  - Simmons citrate medium
- Analytical balance
- Media preparation bottle

**Table 3.2: Standard Biochemical Test Results of Suspected Organisms**

Biochemical Test		Observation After Incubation	
		Positive	Negative
MIO	Motility	Turbidity or haziness	No turbidity or haziness
	Indole	Red colored ring in surface	Yellow colored ring in surface
	Ornithine	Retention of purple color	Change in color
SCA (Simmon's Citrate agar) test		Blue color	No change in color of media (green color)
Urease Test		Pink or purple color	No change in color (light orange)
Oxidase Test		Blue color of colony ( avoid blue color after 10 seconds)	No color change of colony
KIA	H <sub>2</sub> S	Black color	No Black color
	Gas production	Bubble production	No bubble in test tube

For KIA test, slant and butt portion of test tube is also observed to identify acid and alkali. K indicates acid and A indicates alkali. It can be K/A, A/K, K/K or even A/A for slant/butt.



**CHAPTER 04**

**RESULT**

#### 4.1 Bacteriological Colony Morphology

**Table 4.1: Bacterial colony morphology isolated from different street vended food samples**

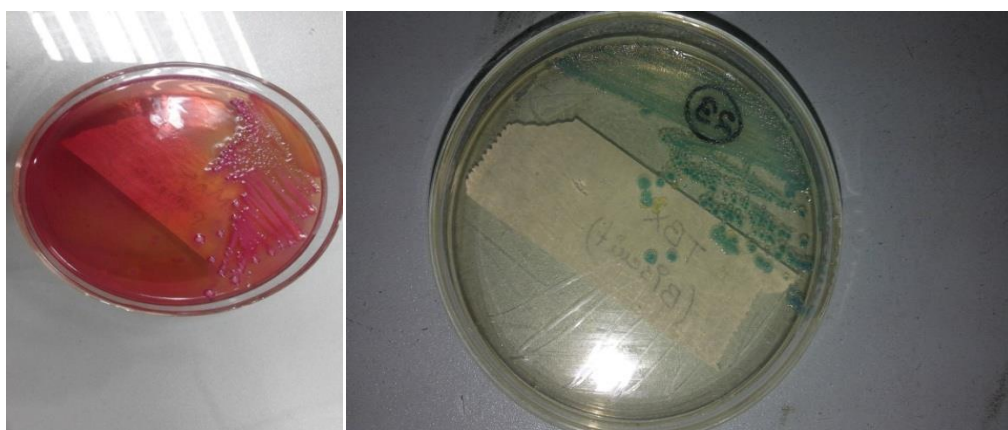
Name of University	Sample	Agar Plates				
		MacConkey	TBX	BGA	XLD	TCBS
North South University (NSU)	Singara 1	Colorless	No growth	No growth	No growth	No growth
	Jilapi 1	No growth	No growth	White	Red	Yellow
	Butter bun	Mucoid pink	Blue	White	No growth	No growth
BRAC University (BU)	Velpuri	Mucoid pink	Blue	No growth	No growth	No growth
	Danish	No growth	No growth	No growth	No growth	No growth
	Singara 2	No growth	White	Yellow	Colorless	No growth
East West University (EWU)	Aluchop	No growth	White	No growth	Yellow	No growth
	Jilapi 2	Pink	No growth	No growth	No growth	Yellow
	Beguni 1	Pink	No growth	No growth	No growth	Yellow
South East University (SEU)	Laddu 1	Mucoid pink	No growth	No growth	No growth	Yellow
	Fuchka 1	Colorless	Blue	No growth	Yellow	No growth
	Jilapi 3	No growth	White	Yellow	Yellow	No growth
United International University (UIU)	Singara 2	No growth	No growth	No growth	No growth	No growth
	Beguni 2	Mucoid pink	No growth	White	No growth	Yellow
	Nargis kabab	Mucoid pink	Blue	White	Yellow	No growth

Table 4.1 shows bacterial colony morphology isolated from different street vended food samples. 15 food samples were collected from the area around five different private universities in Dhaka city. In total 13 samples show growth of different pathogenic or non pathogenic microorganisms. Of which 10 samples show positive growth of our suspected organisms (*E.coli*, *Klebsiella spp.*, *Vibrio spp.*, *Shigella spp.* and *Salmonella spp.*) and 2 samples show no growth in these agar media.

**Table 4.2: Bacterial colony morphology isolated from different street vended food samples**

Name of University	Sample	Agar Plates				
		MacConkey	TBX	BGA	XLD	TCBS
Independent University Bangladesh (IUB)	Patishapta	No growth	No growth	No growth	No growth	Yellow
	Fuchka 2	Pink	No growth	No growth	No growth	No growth
	Cake 1	No growth	No growth	Yellow	Yellow	No growth
University of Asia Pacific (UAP)	Roll	No growth	No growth	No growth	No growth	No growth
	Samucha	Pink	Blue	White	No growth	Yellow
	Patis	No growth	No growth	White	Yellow	No growth
University of Liberal Arts of Bangladesh (ULAB)	Chola	Flat pink dot	Blue	White	Yellow	Yellow
	Laddu 2	Mucoid pink	No growth	No growth	No growth	Yellow
	Chicken chop	Mucoid pink	No growth	White	No growth	No growth
Stamford University (SU)	Noodles	Mucoid pink	No growth	No growth	No growth	Yellow
	Vegetable roll	No growth	Colorless	Yellow	Yellow dot	No growth
	Biscuit	Flat pink	Blue	No growth	No growth	Yellow
American International university (AIUB)	Beguni 3	Pink	Blue	No growth	No growth	No growth
	Jali kabab	Mucoid pink	No growth	No growth	No growth	No growth
	Cake 2	Flat pink dot	Blue	No growth	No growth	Yellow

Table 4.2 shows bacterial colony morphology isolated from different street vended food samples. 15 food samples were collected from the area around five different private universities in Dhaka city. In total 14 samples show growth of different pathogenic or non pathogenic microorganisms. Of which 11 samples show positive growth of our suspected organisms (*E.coli*, *Klebsiella spp.*, *Vibio spp.*, *Shigella spp.* and *Salmonella spp.*) and 1 sample shows no growth in these agar media.



**Fig. 4.1: Bacterial colony on agar plates**

**Table 4.3: Number of food samples with growth of suspected organisms determined by colony morphology (n=30)**

<b>Name of University</b>	<b>No. of samples with +ve growth by <i>E.coli</i></b>	<b>No. of samples with +ve growth by <i>Klebsiella spp</i></b>	<b>No. of samples with +ve growth by <i>Vibrio spp</i></b>	<b>No. of samples with +ve growth by <i>Shigella spp</i></b>	<b>No. of samples with +ve growth by <i>Salmonella spp</i></b>
NSU	2	2	1	1	0
BU	1	1	0	0	0
EWU	2	2	2	0	0
SEU	2	2	1	0	0
UIU	2	2	1	0	0
IUB	1	1	1	0	0
UAP	1	1	2	0	0
ULAB	3	3	2	0	0
SU	2	2	2	0	0
AIUB	3	3	1	0	0

Among 30 samples were collected from street vendors in the area around 10 private universities of Dhaka city. About 27 (90%) food samples were contaminated with pathogenic or non pathogenic microorganisms (Table 5.1 and Table 5.2). Of which 21 (70%) samples were suspected to be contaminated with our targeted organisms (*E.coli*, *Klebsiella spp.*, *Vibrio spp.*, *Shigella spp.* and *Salmonella spp.*)

Table 4.3 shows the number of food samples contaminated with the targeted organisms. In total 19 samples were suspected to be contaminated with either *E.coli* or *Klebsiella spp.*, 13 samples were suspected to be contaminated with *Vibio spp.* And 1 sample was suspected to be contaminated with *Shigella spp.*

#### 4.2 Suspected Organisms from Biochemical Tests

**Table 4.4: Identification of the suspected organism (*Klebsiella spp.*) from different biochemical test**

Sample	Plates	Colony morphology	M	I	O	Cit-rate	Ure-ase	Oxi-dase	KIA			Organism
									Slunt /butt	H <sub>2</sub> S	Gas	
Jilapi 2	Mac Conkey	Pink	-	+	-	+	-	-				<i>Klebsiella spp.</i>
Noodles	Mac Conkey	Mucoid pink	-	+	-	+	-	-				
Chicken chop	Mac Conkey	Mucoid pink	-	+	-	+	-	-				
Laddu 1	Mac Conkey	Mucoid pink	-	+	-	+	-	-				
Laddu 2	Mac Conkey	Mucoid pink	-	+	-	+	-	-	K/A	-	+	
Butter bun	Mac Conkey	Mucoid pink	-	+	-	+	-	-	K/A	-	+	
Beguni 2	Mac Conkey	Mucoid pink	-	+	-	+	-	-	K/A	-	+	
Beguni 3	Mac Conkey	Pink	-	+	-	+	-	-	K/A	-	+	

Among 21 (70%) food samples were subjected for different biochemical test to identify our targeted organisms. Biochemical test results of about 13 (43%) food samples show similarities with the standard biochemical test results of our targeted organisms (*E.coli*, *Klebsiella spp.*, *Vibio spp* and *Shigella spp.* except *Salmonella spp.*) as compared.

Table 4.4 shows identification of the suspected organism (*Klebsiella spp.*) from different biochemical test. In total 8 (27%) food samples were identified to be contaminated with our suspected organism *Klebsiella spp.* from these biochemical tests.



**Table 4.5: Identification of the suspected organism (*E.coli*, *Vibrio spp.* and *Shigella spp.*) from different biochemical test**

Sample	Plates	Colony morphology	M	I	O	Citrate	Urease	Oxidase	KIA			Organism
									Slant /butt	H <sub>2</sub> S	Gas	
Cake 2	Mac Conkey	Flat Pink Dot	+	+	-	-	-	-				<i>E.coli</i>
Biscuit	Mac Conkey	Flat pink	+	+	-	-	-	-	A/A	-	+	
Fuchka 2	Mac Conkey	Pink	+	+	-	-	-	-	K/A	-	+	
Jilapi 1	XLD	Red	-	+	-	+	-	-	K/A	-	+	<i>Shigella spp.</i>
Beguni 1	TCBS	Yellow	+	+	+	+	-	-	K/A	-	+	<i>Vibrio spp.</i>

Among 21 (70%) food samples were subjected for different biochemical test to identify our targeted organisms. Biochemical test results of about 13 (43%) food samples show similarities with the standard biochemical test results of our targeted organisms (*E.coli*, *Klebsiella spp.*, *Vibrio spp.* and *Shigella spp.* except *Salmonella spp.*) as compared.

Table 4.5 shows identification of the suspected organism (*E.coli*, *Vibrio spp.* and *Shigella spp.*) from different biochemical test. In total 3 (10%) food samples were identified to be contaminated with *E.coli*, 1 (3%) food sample was identified to be contaminated with *Shigella spp.* and 1 (3%) food sample was identified to be contaminated with *Vibrio spp.* from these biochemical tests.



**Fig. 4.3: Different Biochemical test**

**Table 4.6: Presence of suspected organisms in no of food samples from different university (n=13)**

Name of University	<i>E.coli</i>	<i>Klebsiella spp.</i>	<i>Vibrio spp.</i>	<i>Shigella spp.</i>	<i>Salmonella spp.</i>
NSU	0	1	0	1	0
EWU	0	1	1	0	0
SEU	0	1	0	0	0
UIU	0	1	0	0	0
IUB	1	0	0	0	0
ULAB	0	2	0	0	0
SU	1	1	0	0	0
AIUB	1	1	0	0	0

Table 4.6 shows presence of suspected organisms in no of food samples from different university. In total 13 (43%) food samples from different university were suspected to be contaminated with our targeted organisms *E.coli*, *Klebsiella spp.*, *Vibrio spp.* and *Shigella spp.* except *Salmonella spp.*

In NSU, 2 food samples were suspected to be contaminated with *Klebsiella spp.* and *Shigella spp.* respectively. In EWU, 2 food samples were suspected to be contaminated with *Klebsiella spp.* and *Vibrio spp.* respectively. In SEU, 1 food sample was suspected to be contaminated with *Klebsiella spp.* In UIU, 1 food sample was suspected to be contaminated with *Klebsiella spp.* In IUB, 1 food sample was suspected to be contaminated with *E.coli*. In ULAB, 2 food samples were suspected to be contaminated with *Klebsiella spp.* In SU, 2 food samples were suspected to be contaminated with *E.coli* and *Klebsiella spp.* respectively. In AIUB, 2 food samples were suspected to be contaminated with *E.coli* and *Klebsiella spp.* respectively.

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**Table 4.7: Incidence of food borne pathogens in various street vended food samples**

Pathogen	Food Categories					
	Deep fried and fried items (n=16)	Spicy items (n=4)	Noodles (n=1)	Baked items (n=6)	Sweet items (n=3)	Total (n=30)
<i>E.coli</i>	Nd	1 (25%)	Nd	2 (33%)	Nd	3 (10%)
<i>Klebsiella spp.</i>	4 (25%)	Nd	1 (100%)	1 (25%)	2 (67%)	8 (27%)
<i>Vibrio spp.</i>	1 (6%)	Nd	Nd	Nd	Nd	1 (3%)
<i>Shigella spp.</i>	1 (6%)	Nd	Nd	Nd	Nd	1 (3%)
<i>Salmonella spp.</i>	Nd	Nd	Nd	Nd	Nd	Nd

Table 4.7 shows the incidence of food borne pathogens in various street vended food samples. Among 16 deep fried and fried items, 4 (25%) samples were suspected to contain *Klebsiella spp.*, 1 (6%) sample was suspected to contain *Vibrio spp.* and 1 (6%) sample was suspected to contain *Shigella spp.* Among 4 spicy items, 1 (25%) sample was suspected to contain *E.coli*. 1 (100%) noodles was suspected to contain *Klebsiella spp.* Among 6 baked items, 2 (33%) samples were suspected to contain *E.coli* and 1 (25%) sample was suspected to contain *Klebsiella spp.* Among 3 sweet items, 2 (67%) samples were suspected to contain *Klebsiella spp.*

A decorative graphic consisting of two overlapping horizontal bars. The top bar is orange and the bottom bar is green. The green bar is positioned to the right of the orange bar, creating a stepped effect.

## **CHAPTER 05**

## **DICUSSION**

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## 5.1 Discussion

Today, food borne illness has become one of the major concerns of public health. The rate of food borne illness is increasing day by day, mostly in developing countries like Asian region and Bangladesh. In developing countries vendors have formed an integral part of the food supply chain. The rate is increasing due to inadequate supervision and monitoring by food safety officers. There is also lack of awareness, training in food safety and good hygiene practices among food handlers. Vendors as well as the customers should be aware to reduce the frequency of these diseases.

The main objective of this study was to isolate and identify enteric bacteria specially *E.coli*, *Klebsiella spp.*, *Vibio spp.*, *Shigella spp.* and *Salmonella spp.* in street foods sold by the vendors in the area around top 10 private university in Dhaka city. A total of 30 samples were collected from the street vended shops beside the 10 university in Dhaka city. Five different agar media were used for isolation and identification of our suspected organisms from the samples. The agar media were MacConkey, TBX (Tryptone Bile X-glucuronide Agar), BGA (Brilliant Green Agar), XLD (Xylose lysine deoxycholate) and TCBS (Thiosulfate citrate-bile salts sucrose). Microbial growth was observed in different media plates.

A study was conducted on 132 samples of beef, chicken, salad and gravy from two street vendors in Johannesburg, South Africa. The predominant populations isolated from the aerobic plate counts were *Bacillus spp.*, *Staphylococcus spp.*, Enterobacteriaceae and *Alcaligenes spp.* *Bacillus cereus* was detected in 17%, *Clostridium perfringens* in 1%, *Staphylococcus aureus* in 3% and *Vibrio metchnikovii* in 2% of the food samples. *Campylobacter jejuni*, *Listeria monocytogenes*, and *Escherichia coli* 0157:H7 were not detected. Non-pathogenic *E. coli* was detected in 13% of food samples, in 86 and 36% of dish water samples collected from vendors 1 and 2, respectively, and in 36% of surface swab samples from vendor 2. Improper food handling practices contributed to the development of this outbreak (Christison, Lindsay & Holy, 2008).

In India, a study was conducted on some raw street vended Indian foods. *Staphylococcus aureus* were detected in 91 (60%) samples of coriander sauce, 87 (58%) samples of coconut slices and 129 (86%) samples of ready-to-eat salads. Twenty-three (15%) samples of coconut slices contained *Shigella* (18 *Sh. dysenteriae* type 1 and 5 *Sh. flexneri* 2a), 13 (8%) samples of ready-to-eat salads and 10 (6%) samples of coriander sauce

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contained *Sh. flexneri* 2a. Spread of bacteria may have been facilitated by lack of access to potable water, toilet facilities and operated under poor hygiene conditions (Ghosh et al., 2007).

Another study was undertaken to investigate the microbiological quality of panipuri sold in Amravati, India. Analysis of the food samples revealed that 93% of panipuri water samples had high loads of bacterial pathogens such as *Escherichia coli* (41%), *Staphylococcus aureus* (31%), and *Klebsiella sp.* (20%), *Pseudomonas sp.* (5%) and yeast (3%), which was caused by mishandling of food during manufacture (Tambekar et al., 2011).

In our present study, 4 suspected organisms *E.coli*, *Klebsiella spp.*, *Vibrio spp.* and *Shigella spp* were found from 13 (43%) samples. From the biochemical test results of the colonies of MacConkey, XLD and TCBS agar media, 3 (10%) food samples were suspected to be contaminated with *E.coli*, 8 (27%) food samples were suspected to be contaminated with *Klebsiella spp.*, 1 (3%) food sample was suspected to be contaminated with *Vibrio spp.* and 1 (3%) food sample was suspected to be contaminated with *Shigella spp* from different university. No *Salmonella spp.* was found from any food sample. Seven biochemical tests were performed for characterizing the organisms but PCR test was not done. Therefore it cannot be said confidently that colonies of the media plates are the claimed ones. In this research, due to limitations of facilities other tests were not performed.

Street vended foods have become major source of serious health problem due to microbial contamination. So, more focuses should be given in this sector and more research work should be carried out. It is also suggested that regular monitoring of the quality of street foods must be practiced to avoid any food-borne infection in future.

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## **5.2 Conclusion**

Incidences of food borne illness are increasing day by day in Bangladesh. The present study revealed that street vended foods in Dhaka city constitute an important potential hazard to human health which needs to be addressed. There were some limitations in this study. Only five organisms were to be identified due to lack of methods and facilities. New method of identification of organisms and assessment of food borne hazards should be implemented. There is a reasonable gap on food safety knowledge among street vendors. Due attention should be given by the government to improve knowledge about food safety and quality standards of street foods sold in the country. Most importantly, relevant agencies such as consumer protection rights and others need to ensure and enforce strict compliance to hazard analysis and critical control points in all food production sectors in Bangladesh.

A decorative graphic consisting of two overlapping horizontal bars. The top bar is orange and the bottom bar is green. The green bar is positioned to the right of the orange bar, creating a stepped effect.

## **CHAPTER 06**

## **REFERENCE**



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