Study on Bacteriological Quality of Street-Vended and Expired Food Items Collected from Different Areas in Dhaka City, Bangladesh

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, Md. Noor Islam, hereby declare that the dissertation entitled "Study on Bacteriological Quality of Street-vended Foods Collected from Different Private Universities in Dhaka City and of Expired Foods Collected from Different Shops of Dhaka City, Bangladesh" 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 2016-2017 of my research in the Department of Pharmacy, East West University, under the supervision and guidance of Nafisa Tanjia, Senior 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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Certificate by the Supervisor

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List of Abbreviations

Abbreviations	Full Name
RTE	Ready to Eat
EPEC	Enteropathogenic *E.coli*
EIEC	Enteroinvasive *E.coli*
ETEC	Enterotoxigenic *E.coli*
APW	Alkaline Peptone Water
BPW	Buffered Peptone Water
TSB	Trypticase Soy Broth
YE	Yeast Extract
TBX	Tryptone Bile X-glucoronide
TCBS	Thiosulfate Citrate Bile Salt-sucrose
BGA	Brilliant Green Agar
XLD	Xylose-Lysine Desoxycholate Agar
MIO	Motility Indole Ornithine
KIA	Kliglar's Iron Agar

Abstract

Street-vended foods are readily available sources of meals for many people around the world. However, the microbial safety of such food is always doubtful. In developing countries the major sources of food-borne illnesses are from street-vended foods. Again packaged foods are also detrimental to health if it is expired. The present research work was therefore untaken to find out the presence of enteric bacteria specially *E.coli, , Salmonella, Shigella* and *Vibrio* species from different types of expired and street-vended food items collected from different areas of Dhaka city, Bangladesh. Five agar media MacConkey, Tryptone Bile X-glucoronide (TBX) agar, Thiosulfate Citrate Bile Salt-sucrose (TCBS) agar, Brilliant Green Agar (BGA) and Xylose-Lysine Desoxycholate agar (XLD) were used to observe the presence of our targeted microorganisms in food items. Seven biochemical tests were performed to indentify the targeted organisms. The tests are KIA, citrate, motility, indole, ornithine, urease, and oxidase test. Out of total 35 food samples (expired and street) we have found the presence of enteric bacteria in 17 (48.6%) food samples containing *E.coli*, *Vibrio*, *shigella* and *Salmonella* species. All these enteric pathogens could be the potential cause for foodborne illnesses. So, government should take necessary steps to prevent the sale of expired food and unhygienic street food; as well as consumers also should be careful and cautious in purchasing foods to ensure their own health safety.

Keywords: Street-vended foods, Expired foods, Public health risk, Enteric bacteria, Biochemical test, *E.coli, Vibrio, shigella, Salmonella.*

Chapter 1

Introduction and Literature Review

1.1 Street-vended Foods

Street-vended foods are ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers especially in streets and other similar public places such as school, college, universities, market or fair, often from a portable food booth or food cart and are consumed on the streets without further preparation (Tambekar et al., 2011).

Street foods are popular due to industrialization which is forcing many city dwellers to eat their major daily meals out of home. Street food vending is a common feature of most cities and towns in developing countries (Rane, 2011). The types of street-vended food differ significantly on countries and cultures. In Bangladesh, the most popular and traditional street-vended foods includes jhal-muri, fuchka, vhel-puri, panipuri, bun, cake, danish, betel-leaf, chhola, peaju, sweet, sheekkabab, laddu, singara, somucha etc. In Dhaka city there are huge number of street food vendors, gather mainly at key points of transport such as train and bus stations, as well as in front of educational institutes (Rahman et al., 2014).

Figure 1.1: Street Food in Bangladesh

In developing countries food sold by street vendors is the major source of food-borne illness. Although food items from these outlets are appreciated mostly for their unique flavor and for their convenience, their microbiological safety is not always certain (Islam S, 2015). Street food vending has become an important public health issue and a great concern to everybody. This is due to widespread food borne diseases, due to the mushrooming of wayside food vendors who lack an adequate understanding of the basic food safety issues. Major sources contributing to microbial contamination are the place of preparation, utensils for cooking and serving, raw materials, time and temperature abuse

of cooked foods and the personal hygiene of vendors. Various studies have identified the sources of street food safety issues belonging to the microorganism genus *Bacillus, Staphylococcus, Clostridium, Vibrio, Campylobacter, Listeria,* and *Salmonella* (Rane, 2017).

However, questions have been raised about the safety and microbiological quality of these food products. Developing countries bear the brunt of the problem due to the presence of a wide range of food-borne diseases. Vendors generally use carts and stands, where they do not have easy access to running water, furthermore, dish and hand washing are done using the same bucket, sometimes even without soap (Okojie & Isah, 2014). Garbage and waste water are usually discarded in the streets nearby and therefore, rodents and insects come in contact with food (Kibret & Tadesse, 2013). 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 (Tambekar et al., 2011). Practices used during food preparation such as handling, cleaning, sorting and grading, packaging, storing and wrapping in low grade plastics are some of the critical factors that increase the risk of inadequate food safety. Rapid proliferation of the street food business has led to the growing concern for food safety. The consumption of these street foods potentially increases the risk of food borne diseases caused by a wide variety of pathogens which include *E.coli, Salmonella typhi, Pseudomonas* spp., *S.aureus* (Kwiri et al., 2014).

WHO reports that 20% of deaths among children under five are caused by diarrheal disease and UNICEF estimates that about 1,000 children below the age of five die every day in India, due to diarrhea. Food borne bacterial pathogens commonly detected in street vended foods are *Bacillus cereus* causes vomiting and diarrhea, *Clostridium perfringens* causes abdominal cramps and diarrhea, *Staphylococcus aureus* causes vomiting, diarrhea, loss of appetite, severe abdominal cramps and mild fever and *Salmonella* species causes typhoid, food poisoning and irritation and inflammation in the gastrointestinal tract (Sharma et al., 2015). Although there is a growing demand for RTE food products, no recent information is available regarding the microbiological quality of these products in Dhaka city, Bangladesh. The present study was hence undertaken to determine the microbiological quality and safety of a variety of street-vended RTE food products collected from several typical vendors surrounding different top private universities of Dhaka city.

1.2 Expired Foods

The expiration date is the date up to which the food maintains its microbiological and physical stability, and the nutrient content declared on the label. That means it's important to use that food before the expiration date to get the most nutritional value from it.

Expiration dates cover several different stages. For example,

- **The sell-by date** refers to the date when the store will discard the item.
- **The best before date** refers to the quality and taste of a product, not necessarily the health effects if consume it past that date.
- **The use-by date** refers to the last safe date before it starts to turn bad. However, this doesn't necessarily mean that the very next day the product is poisonous. It's a marker to show when the food degrades to a lower quality and a potentially dangerous state.

Most packages of food include a sell-by or use-by date that helps stores and consumers know how long each item is safe for consumption. Eating foods that have expired might increase the risk for certain illnesses or conditions. Foods that have an offending odor, flavor or appearance should not be eaten, the USDA (United States Department of Agriculture) Food Safety and Inspection Service says. Some important parameters related to expiration date of any food are as follows:

Nutritional Value

Foods eaten as soon as possible after purchase often taste better than older foods, and they might also be more nutritious as well. Sari Edelstein notes in her book, "Nutrition in Public Health: A Handbook for Developing Programs and Services," that eating food past its prime is often less nutritious than eating food when it is fresh. Even if the nutrition label indicates that a food is nutritious, it might not contain as much of each nutrient as the label suggests.

Food Poisoning

Uncooked meats and eggs are the usual suspects when it comes to food poisoning, but any food that has expired might lead to a growth of the bacteria that can lead to food-borne illness. Food poisoning occurs when dangerous bacteria ingested with food that are able to grow more easily on expired foods. This is most likely to occur with perishable foods such as beef, chicken, eggs and prepared foods such as macaroni salad or potato salad.

Physical Discomfort

Eating food that has expired might not cause a serious illness such as food poisoning, but it might cause physical discomfort, such as an upset stomach that might or might not be accompanied by vomiting. Gas and bloating are additional symptoms that also can occur. The USDA (United States Department of Agriculture) Food Safety and Inspection Service reports that moldy foods can cause allergies or respiratory distress, but some moldy foods can lead to illness.

Taste Change

Even if the expired food eating is safe for consumption, it might not taste as good as that of before the expiration date. As time passes, the quality of the food declines, which decreases the taste. The USDA (United States Department of Agriculture) Food Safety and Inspection Service notes that many expired foods can be safe to eat, but they might not be as flavorful as that of before the expiration date (Rahman et al., 2014).

1.3 Food-borne Illness

Food-borne illness, also called "food-borne disease," "food-borne infection," or "food poisoning, is a common, costly but preventable public health problem. Each year, 1 in 6 Americans gets sick by consuming contaminated foods or beverages. Many different disease-causing microbes, or pathogens, can contaminate foods, so there are many different food-borne infections. In addition, poisonous chemicals, or other harmful substances can cause food-borne diseases if they are present in food.

The Centers for Disease Control and Prevention (CDC) estimates that 48 million food-borne illness cases occur in the United States every year. At least 128,000 Americans are hospitalized, and 3,000 die after eating contaminated food each year. Food-borne illness costs Americans billions of dollars each year, and serves as a constant challenge for consumers, researchers, government and industry (Centers for Disease Control and Prevention [CDC] 2015).

1.4 Types of Microbial Food-borne Diseases

Food-borne diseases in humans result from the consumption of either food and water contaminated with viable pathogenic bacterial cells (or spores in the case of infant botulism) or food containing toxins produced by toxigenic bacteria and molds. On the basis of mode of illnesses, these can be arbitrarily divided into three groups: intoxication or poisoning, infection, and toxicoinfection.

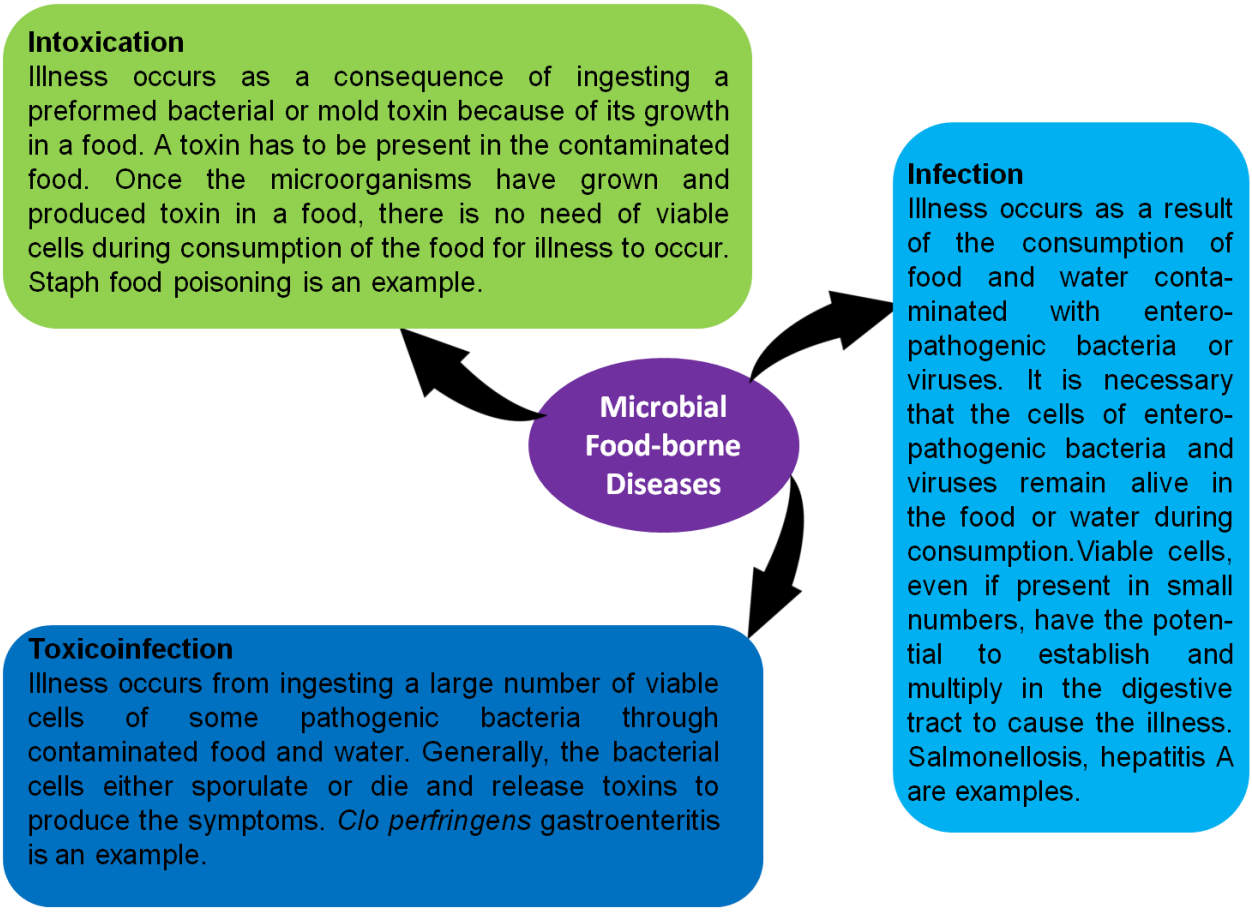


Figure 1.2: Microbial Food-borne Diseases

In addition to the pathogenic microorganisms associated with food-borne illnesses, some bacterial species and strains normally considered nonpathogenic can cause gastroenteritis, especially in susceptible individuals. They are designated as opportunistic pathogens. They are normally required to be alive and present in large numbers when consumed through a contaminated food (Ray, 2004).

1.5 Common Pathogens Responsible for Food-borne Diseases

Table 1.1: Common microorganisms responsible for Food-borne Diseases

Group of Pathogen		Example of Pathogen
Bacteria		*Salmonella* spp. *Campylobacter jejuni* *Shigella* spp. STEC O157:H7 *Listeria monocytognes* *Vibrio* spp. *Yersinia* spp.
Parasites		*Cryptosporidium* spp. *Cyclospora* spp. *Trichinella spiralis* *Giardia lamblia* *Toxoplasma caris* *Entamoeba histolytica*
Toxins	**Enterotoxins**	*Staphylococcus aureus* *Clostridium perfringens* *Bacillus cereus*
	Botulinum Toxin	*Clostridium botulinum*
	Fish Toxins	Scombrotoxin Ciguatera toxin Paralytic shellfish toxin
	Mushrooms	Amatoxin Phallotoxin
Miscellaneous		Niacin Monosodium glutamate

1.6 Factors Affecting Microbial Growth in Food

There are many important factors which directly or indirectly affect the growth of microorganisms in food products. When microorganisms grow in food they cause varying degrees of change in the food's characteristics as a result of metabolic activity. Some of

these changes, like those taking place during fermentation, are desirable, while others, like those resulting in food spoilage and food poisoning are undesirable (Jay, et al., 2005). The most important factors that affect microbial growth in foods can be summarized in the following categories:

1.6.1 Intrinsic factors

Factors related to the food itself, the "intrinsic factors," which include:

- Nutrient content
- Water activity
- pH value
- Redox potential
- The presence of antimicrobial substances and mechanical barriers to microbial invasion (Jay, et al., 2005).

1.6.2 Extrinsic Factors

Factors related to the environment in which the food is stored, the "extrinsic factors," include:

- The temperature of storage
- Relative humidity of environment
- The composition of gases in the atmosphere surrounding the food (Jay, et al., 2005).

1.6.3 Implicit Factors

Factors related to the microorganisms themselves. Implicit factors include interactions between the microorganisms contaminating the food and between these microorganisms and the food, e.g., their abilities to utilize different nutrient sources, tolerate stresses, and produce promoters or inhibitors of growth of other microorganisms, etc (Wiley Online Library, 2012).

1.6.4 Processing Factors

Processing factors include treatments such as heating, cooling, and drying that affect the composition of the food and also affect the types and numbers of microorganisms that remain in the food after treatment (Wiley Online Library, 2012).

1.6.5 Interactions

Interaction between the above-described factors can also affect the growth of microorganisms in foods in a complicated way; the combined effects may be additive or synergistic (Wiley Online Library, 2012).

1.7 Bacterial Agents of Food-borne Illness and Their Significant Features

1.7.1 *Escherichia coli*

E.coli is an almost universal inhabitant of the gut of humans and other warm-blooded animals where it is the predominant facultative anaerobe though only a minor component of the total microflora. Strains of *E.coli* were first recognized as a cause of gastroenteritis by workers in England investigating summer diarrhoea in infants in the early 1940s. Until 1982, strains producing diarrhoea were classified into three types based on their virulence properties: enteropathogenic *E.coli* (EPEC), enteroinvasive *E.coli* (EIEC), and enterotoxigenic *E.coli* (ETEC). They are not very common causes of food-borne illness in developed countries, but an important cause of childhood diarrhoea in less developed countries (Adams & Moss, 2008).

1.7.1.1 Characteristics

Escherichia is the type genus of the Enterobacteriaceae family and *E.coli* is the type species of the genus. It is a catalase-positive, oxidase-negative, fermentative, short, Gramnegative, non sporing rod. Genetically, *E.coli* is very closely related to the genus *Shigella*, although characteristically it ferments the sugar lactose and is otherwise far more active biochemically than *Shigella* spp. Late lactose fermenting, non-motile, biochemically inert strains of *E.coli* can however be difficult to distinguish from *Shigella.* *E.coli* can be differentiated from other members of the Enterobacteriaceae on the basis of a number of sugar-fermentation and other biochemical tests (Adams & Moss, 2008).

1.7.1.2 Pathogenesis and Clinical Features

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

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.

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. The infective dose of EIEC appears to be substantially higher than for *Shigella* and this is thought to be a reflection of the organism's greater sensitivity to gastric acidity.

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 afebrile 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 diarrhoea where it can cause serious dehydration.

Enterohaemorrhagic *E.coli* (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 thrombocytopaenic purpura (TTP) (Adams & Moss, 2008).

1.7.1.3 Isolation and Identification

Selective techniques for *E.coli* mostly exploit the organism's tolerance of bile and other surfactive compounds, a consequence of its natural habitat, the gut. Aniline dyes and the ability of many strains to grow at temperatures around 44°C are also used as selective agents. The first selective and differential medium was that originally devised by MacConkey in 1905. It has been variously modified since but its essential characteristics have remained unchanged. Bile salts (and sometimes the aniline dye, crystal violet) act as inhibitors of Gram-positive and some fastidious Gram-negative bacteria. Lactose is included as a fermentable carbohydrate with a pH indicator, usually neutral red. Strong acid producers like *Escherichia* and *Enterobacter* produce pink colonies; nonlactose fermenters such as *Salmonella, Proteus,* and *Edwardsiella,* with rare exceptions produce colorless colonies (Adams & Moss, 2008).

1.7.1.4 Association with Foods

Faecal contamination of water supplies and contaminated food handlers have been most frequently implicated in outbreaks caused by EPEC, EIEC and ETEC. A number of foods have been involved, including a coffee substitute in Romania in 1961, vegetables, potato salad, and sushi. In the United States, mould-ripened soft cheeses have been responsible for outbreaks in 1971, associated with EIEC in which more than 387 people were affected, and in 1983, caused by ETEC (ST). *E.coli* would not be expected to survive well in a fermented dairy product with a pH below 5 but, where contamination is associated with mould-ripening, the local increase in pH as a result of lactate utilization and amine production by the mould would allow the organism to grow. Outbreaks caused by EHEC serotype O157:H7 have mostly involved undercooked ground meat products and occasionally raw milk. Cattle seem to be an important reservoir of infection and O157:H7 has been isolated from 0.9–8.2% of healthy cattle in the UK (Adams & Moss, 2008).

1.7.2 *Salmonella* species

Salmonella is an important bacterial genus which causes one of the most common forms of food poisoning worldwide. It is one of the most extensively studied bacterial species in terms of its physiology, genetics, cell structure, and development. It is also one of the most extensively characterized bacterial pathogens and is a leading cause of bacterial gastroenteritis. *Salmonella* is capable of causing a variety of disease syndromes: enteric fever, bacteremia, enterocolitis, and focal infections (Darwin, 1999).

1.7.2.1 Characteristics

Salmonella is a rod-shaped, motile, aerobic and facultative anaerobe, non-spore forming and gram-negative organism. It can grow from 5°C up to 47°C, with an optimum temperature of 37°C. *Salmonella* is heat sensitive and can be readily destroyed at pasteurization temperature. *Salmonella* is a general name used for a group of more than 2,000 closely related bacteria that cause illness by reproducing in the digestive tract. Each *Salmonella* serotype shares common antigens and has its own name; *Salmonella enteritidis* was the commonest serotype isolated from human clinical specimens (Bayu et al., 2013).

1.7.2.2 Pathogenesis and Clinical Features

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 may result; case fatality of 16% reduced to 1% with antibiotic therapy (Adams & Moss, 2008).

1.7.2.3 Isolation and Identification

Salmonella is generally identified as being a non-lactose fermenting, (NLFs) Gram negative rod shaped organism, ranging 0.7 to 1.5 x 2 to 5 μm in size. With the exception of S. Pullorum and S. Gallinarum, they are motile with peritrichous flagellae. D-glucose is fermented with the production of acid and usually gas. Other carbohydrates usually fermented are L-arabinose, maltose, D-mannitol, D-mannose, L-rhamnose, D-sorbitol (except ssp VI), trehalose, D-xylose and dulcitol.

Salmonella is oxidase negative, catalase positive, indole and Voges Proskauer (VP) negative, methyl red and Simmons citrate positive, hydrogen sulphide producing and urea negative. Some of these characteristics are used for biochemical confirmation of *Salmonella*.

1.7.2.4 Association with Foods

Salmonellosis is described as a zoonotic infection since the major source of human illness is infected animals. Transmission is by the faecal–oral route whereby intestinal contents from an infected animal are ingested with food or water. Meat, milk, poultry, and eggs are primary vehicles; they may be undercooked, allowing the *salmonella*s to survive, or they may cross contaminate other foods that are consumed without further cooking. Cross-contamination can occur through direct contact or indirectly via contaminated kitchen equipment and utensils. 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 (Adams & Moss, 2008).

1.7.3 *Shigella* species

The genus *Shigella* was discovered as the cause of bacillary dysentery by the Japanese microbiologist Kiyoshi Shiga in 1898. It consists of four species *Sh. dysenteriae, Sh. flexneri, Sh. boydii* and *Sh. sonnei,* all of which are regarded as human pathogens though

they differ in the severity of the illness they cause. *Sh. dysenteriae* has been responsible for epidemics of severe bacillary dysentery in tropical countries but is now rarely encountered in Europe and North America where *Sh. sonnei* is more common. *Sh. Sonnei* causes the mildest illness, while that caused by *Sh. boydii* and *Sh. flexneri* is of intermediate severity (Adams & Moss, 2008).

1.7.3.1 Characteristics

Shigellas are members of the family Enterobacteriaceae. They are nonmotile, nonspore-forming, Gram-negative rods which are catalase positive (with the exception of *Shiga's bacillus, S. dysenteriae* serotype 1), oxidase-negative, and facultative anaerobes. They produce acid but usually no gas from glucose and, with the exception of some strains of *S. sonnei,* are unable to ferment lactose; a feature they share with most *salmonellas*. *Shigellas* are generally regarded as rather fragile organisms which do not survive well outside their natural habitat which is the gut of humans and other primates. They are typical mesophiles with a growth temperature range between 10–45 °C and heat sensitivity comparable to other members of the family. They grow best in the pH range 6–8 and do not survive well below pH 4.5 (Adams & Moss, 2008).

1.7.3.2 Pathogenesis and Clinical Features

Shigellas cause bacillary dysentery in humans and other higher primates. Studies with human volunteers have indicated that the infectious dose is low; of the order of 10–100 organisms. 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 are of abdominal pain, vomiting and fever accompanying a diarrhoea which can range from a classic dysenteric syndrome of bloody stools containing mucus and pus, in the cases of *Sh. dysenteriae, Sh. flexneri* and *Sh. boydii*, to a watery diarrhoea with *Sh. sonnei.* Illness lasts from 3 days up to 14 days in some cases and a carrier state may develop which can persist for several months. 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).

1.7.3.3 Isolation and Identification

Lack of interest in *Shigella* as a food-borne pathogen has meant that laboratory protocols for its isolation and identification from foods are relatively underdeveloped. A pre-enrichment procedure has been described based on resuscitation on a non-selective agar

before overlaying with selective media. Selective enrichment in both Gram-negative broth and selenite broth has been recommended. Selective plating media used is generally those employed for enumerating the Enterobacteriaceae or *Salmonella* although neither is entirely satisfactory. Rapid techniques for identification based on immunoassays which detect the virulence marker antigen, and on the polymerase chain reaction to detect the virulence plasmid by DNA/DNA hybridization has also been applied (Adams & Moss, 2008).

1.7.3.4 Association with Foods

In food-borne cases, the source of the organism is normally a human carrier involved in preparation of the food. In areas where sewage disposal is inadequate the organism could be transferred from human faeces by flies (Adams & Moss, 2008).

1.7.4 *Vibrio* Species

Historically, cholera has been one of the diseases most feared by mankind. It is endemic to the Indian subcontinent where it is estimated to have killed more than 20 million people in 19th century. It was Robert Koch who firmly established the causal link between *Vibrio cholerae* and cholera when working in Egypt in 1886.

1.7.4.1 Characteristics

Vibrios are Gram-negative pleomorphic (curved or straight), short rods which are motile with (normally) sheathed, polar flagella. Catalase and Oxidase-positive cells are facultatively anaerobic and capable of both fermentative and respiratory metabolism. Sodium chloride stimulates the growth of all species and is an obligate requirement for some. The optimum level for the growth of clinically important species is 1–3%.

1.7.4.2 Pathogenesis and Clinical Features

Cholera usually has an incubation period of between one and three days and can vary from mild, self-limiting diarrhoea to a severe, life threatening disorder. The infectious dose in normal healthy individuals is large when the organism is ingested without food or buffer, of the order of 1010 cells, but is considerably reduced if consumed with food which protects the bacteria from stomach acidity. Studies conducted in Bangladesh indicate that 103–104 cells may be a more typical infectious dose. Individuals with low stomach acidity (hypochlorohydric) are more liable to catch cholera. In severe cases, the hyper-secretion of sodium, potassium, chloride, and bicarbonate induced by the enterotoxin results in a profuse, pale, watery diarrhoea containing flakes of mucus,

described as rice water stools. Unless the massive losses of fluid and electrolyte are replaced, there is a fall in blood volume and pressure, an increase in blood viscosity, renal failure, and circulatory collapse. In fatal cases death occurs within a few days. In untreated outbreaks the death rate is about 30–50% but can be reduced to less than 1% with prompt treatment by intravenous or oral rehydration using an electrolyte/glucose solution (Adams & Moss, 2008).

1.7.4.3 Isolation and Identification

The enrichment media used for *vibrios* exploit their greater tolerance for alkaline conditions. In alkaline peptone water (pH 8.6 –9.0) the incubation period must be limited to 8 h to prevent overgrowth of the *vibrios* by other organisms. Tellurite/bile salt broth (pH 9.0 –9.2) is a more selective enrichment medium and can be incubated overnight. The most commonly used selective and differential agar used for *vibrios* is thiosulfate/citrate/bile salt/sucrose agar (TCBS). The medium was originally designed for the isolation of *V. parahaemolyticus* but other enteropathogenic *vibrios* grow well on it, with the exception of *V. hollisae. V. parahaemolyticus, V. mimicus, and V. vulnificus* can be distinguished from *V. cholerae* on TCBS by their inability to ferment sucrose which results in the production of green colonies. *V. cholerae* produces yellow colonies. Individual species can then be differentiated on the basis of further biochemical tests (Adams & Moss, 2008).

1.7.4.4 Association with Foods

Cholera is regarded primarily as a waterborne infection, though food which has been in contact with contaminated water can often serve as the vehicle. Consequently a large number of different foods have been implicated in outbreaks, particularly products such as washed fruits and vegetables which are consumed without cooking. Foods coming from a contaminated environment may also carry the organism, for example sea foods and frog's legs. In the current pandemic in South and Central America, an uncooked fish marinade, in lime or lemon juice, ceviche has been associated with some cases (Adams & Moss, 2008).

1.8 Prevalence of Food-borne Illness Around the world

According to WHO, A disease outbreak is the occurrence of cases of disease in excess of what would normally be expected in a defined community, geographical area or season. Expression of the similar symptoms or sickness by two or more of the individuals after

consumption of the same contaminated food is labeled as an outbreak of food-borne illness. The description of outbreak includes time, place, and person distribution (Jahan, 2012).

It is important that food-borne illness outbreaks are investigated timely and proper environmental assessments are done so that appropriate prevention strategies can be identified. According to CDC, the etiology of majority (68%) of reported food-borne illness outbreaks is unknown due to lack of timely reporting and lack of resources for investigations. In addition, persons who do not seek health care and limited testing of specimens are also the contributory factors in failure to determine the cause of food-borne illness outbreak (Lynch et al., 2009).

A number of food-borne illness outbreaks are reported from various parts of the world. Worldwide, a total of 4093 food-borne outbreaks occurred between 1988 and 2007. It was found that *Salmonella Enteritidis* outbreaks were more common in the EU states and eggs were the most frequent vehicle of infection. Poultry products in the EU and dairy products in the United States were related to *Campylobacter* associated outbreaks. In Canada, *Escherichia coli* outbreaks were associated with beef. In Australia and New Zealand, *Salmonella typhiumurium* outbreaks were more common (Greig & Ravel, 2009).

Daniels and colleague (2002) conducted a study in the United States, to describe the epidemiology of food-borne illness outbreaks in schools, colleges and universities. The data from January 1, 1973, to December 31, 1997 was reviewed. In majority (60%) of the outbreaks the etiology was unknown. Among the outbreaks with a known etiology, in 36% of outbreak reports *Salmonella* was the most commonly identified pathogen. However, the highest mortality was caused by *Listeria monocytogenes*. Viral pathogens were responsible for 33% of the outbreaks. Among the viral pathogens, norovirus was the most common causative agent (Lynch et al., 2006).

In 2002, a salmonellosis outbreak occurred in five states of U.S. It occurred after consuming ground beef. During this outbreak, forty seven cases were reported; out of which 17 people were hospitalized and one death was reported (Lynch et al., 2006).

In England and Wales, 2429 food-borne outbreaks were reported from 1992 to 2008. Approximately half of the outbreaks were caused by *Salmonella* spp. Poultry and red meat was the most commonly implicated foods in the causation of outbreaks. The associated factors in most outbreaks were cross-contamination, lack of adequate heat treatment and improper food storage (Gormley et al., 2011).

In central Taiwan, 274 outbreaks of food-borne illness including 12,845 cases and 3 deaths were reported during 1991 to 2000. Majority (62.4%) of the outbreaks were caused by bacterial pathogens. The main etiologic agents were *Bacillus cereus*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus*. The important contributing factor was improper handling of food. The implicated foods included seafood, meat products and cereal products (Chang & Chen, 2003).

In a study carried out from October 2004 to October 2005 in Catalonia, Spain, 181 outbreaks were reported; 72 were caused by *Salmonella* and 30 by norovirus (NoV) (Crespo et al., 2005).

In 2002, in the Netherlands a national study of food-borne illness outbreaks was performed. A total of 281 food-borne illness outbreaks were included. Most of these outbreaks were reported from nursing homes, restaurants, hospitals and day-care centers. The causative agents included norovirus (54%), *Salmonella* spp. (4%), rotavirus (2%), and Campylobacter spp. (1%) (Duynhoven et al., 2005).

A study conducted in Qassim province, Saudi Arabia, analyzed the food-borne illness surveillance data for the year 2006. During the study period, 31 food-borne illness outbreaks comprising of 251 cases, were reported. The most common etiologic agent was *Salmonella* spp, followed by *Staphylococcus* aureus (Jahan, 2012).

A study was conducted in 2015 to assess the microbiological quality of street vended food samples from Dhaka, Bangladesh. The study objective was to identify the presence of common pathogens (*Escherichia coli*, *Shigella* spp, *Salmonella* and *Vibrio* spp). Out of 50 food samples, six (12%) were confirmed to contain different species of *E.coli* and *Shigella* (Islam, et al., 2015).

Chapter 2

Objective of the Study

2.1 Research objective

The objective of this research work was to isolate and identify the presence of enteric bacteria especially *E.coli, Salmonella, Shigella* and *Vibrio* species

- from different expired food items; and
- from different types of street-vended food items collected from different university premises of Dhaka city, Bangladesh.

Chapter 3

Methodology

3.1 Bacteriological Subculture

3.1.1 Sample Collection

About 35 food samples were randomly chosen and collected from different private university premises of Dhaka city and from different shop (expired food). These samples were collected in different sealed poly bags to prevent their contact with any other source that can contaminate the samples.

3.1.2 Sample Processing

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

3.1.3 Enrichment of the Organisms

3.1.3.1 Enrichment of *E.coli* 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 37ºC for 18-24 h.

3.1.3.2 Enrichment of *Salmonella* and *Shigella* spp

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

Figure 3.1: Enrichment of the Organisms

3.1.4 Selective Growth of the Organisms

3.1.4.1 Selective Growth *E.coli* 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 ºC for 18-24 h for selective growth of the organism.

3.1.4.2 Selective Growth of *Salmonella* 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 ºC 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 ºC 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.

Figure 3.2: Autoclave and Hot air Oven

Figure 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.

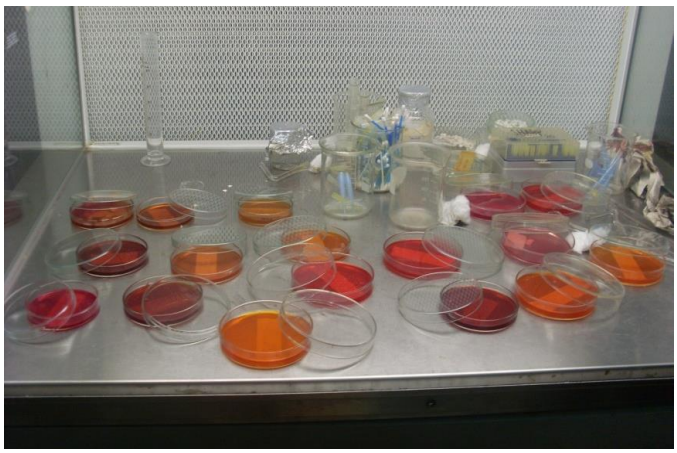


Figure 3.4: Petri dishes preparation

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.

Figure 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:

Organism	Media	Appearance
E.coli	MacConkey	Lactose fermenting pink colonies Non-lactose fermenting colorless colonies
	TBX	Blue colonies
Salmonella	BGA	Typical red colonies
	XLD	Red or clear colonies with black centers
Vibrio	TCBS	Large yellow colonies
Shigella	XLD	Typical red 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 stubbed into the butt to inoculate and the slant was streaked and incubated at 37°C for up to 24 hours.

Figure 3.6: Preparation of test tubes for KIA test

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 µl 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.

Figure 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.

Figure 3.8: Preparation of test tubes for Citrate test

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.

Figure 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
Catalase		Rapid bubble formation	No bubble formation
KIA	H_2S	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.

3.3 Colony Counting Methodology

3.3.1 Cell counting and serial dilutions

3.3.1.2 Theory

In quantitative microbiology, we are concerned with determining the concentration of colony forming units (CFUs) in our sample – i.e., the number of CFUs per ml or per gram

of the sample. More realistically, the concentration of CFUs in the sample could have been considerably greater. Counting the colonies on a plate inoculated with one ml of sample may be impossible. We would like to have "countable" plates – containing between 30 and 300 colonies. If fewer than 30, we run into greater statistical inaccuracy. If greater than 300, the colonies would be tedious to count and also would tend to run together.

So we now get into "dilution theory" to accomplish the equivalent of plating out succeedingly smaller amounts of sample. Making serial decimal dilutions (i.e., successive 1/10 dilutions, each made by adding one part of inoculum to 9 parts of diluent) and inoculating one ml into each of the plates, we can construct a plating procedure that is equivalent to the above.

3.3.1.3 Materials Required

- Tubes
- Micropipette with tips
- Distilled water
- Bacteria sample
- Nutrient agar
- Petri dishes
- Water bath
- Alchohol
- Colony counter
- Conical Flask
- Labeling Tape

3.3.1.4 Procedure

There are four major steps in the procedure:

1. Preparation of serial dilutions
2. Mixing the serial dilutions into agar
3. Counting the resulting bacterial colonies
4. Calculation of total numbers of viable bacteria from these counts.

3.3.1.5 Preparation of Serial Dilutions

- A sample was taken containing the bacteria to be counted.
- Four test tubes were taken and labeled them 10^{-1} to $10^{-4.}$
- Nine mL of distilled water was pipette into each of the tubes.

- One gm of the undiluted sample was given into the tube marked 10^{-1}. The contents were mixed and using a new pipette 1 mL from the 10^{-1} tube was pipette into the 10^{-2} tube.
- This was continued until transfers had been completed to the 10^{-4} tube.
- Therefore the following dilutions of the original sample were obtained.

Tubes	Dilution	Dilution	Dilution Factor
1	10^{-1}	1/10	10^{1}
2	10^{-2}	1/100	10^{2}
3	10^{-3}	1/1,000	10^{3}
4	10^{-4}	1/10,000	10^{4}

3.3.1.6 Mixing the dilutions into agar plates

1. Nutrient agar was prepared by autoclaving.
2. The bottle of molten agar was placed in a 50°C water bath and the agar was allowed to cool to 50°C.
3. Four empty sterile agar plates (Petri dishes) were marked 10^{-1} to 10^{-4} on the base of the plate NOT the lid. Other required details such as initials, sample type, date and culture conditions to the base of the plates were added.
4. Agar bottle from the 50°C water bath was removed and the outside of the bottle was wiped with paper toweling to remove water. Working quickly to avoid cooling of the agar to 42°C (this is the temperature at which it sets). About 15 mL of molten agar was poured into agar plates. The agar should be approximately 7 mm thick.
5. One mL of each of the dilutions was pipette into the base of correctly labeled plates using a separate pipette to avoid carryover errors.
6. Each plate was gently swirled to mix the 1 mL of diluted sample into the 15 mL of agar.
7. The plate was left without moving for at least 13 minutes to allow the agar to set.
8. When the agar was set, the plate was incubated as appropriate.

3.3.1.7 Counting bacterial colonies

1. After an appropriate incubation period the plates were examined for colonial growth.
2. Colonies will form on the top of the agar as well as in the agar. Those on top of the agar will be larger but all colonies must be counted.
3. Plates were selected that appear to have between 30 - 300 colonies in and on the agar as this gives the best statistical representation of the number of bacteria in the undiluted sample.
4. Using a light box or colony counter (if one is available) and marker pen (put a dot above each colony as you count it), the number of colonies were counted in each of the dilutions having between 30-300 colonies.

Chapter 4

Result

4.1 Bacterial colony morphology

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

Name of University	Sample (Street food)	Plates				
		MacConkey	**TBX**	**BGA**	**XLD**	**TCBS**
University of Information Technology and Sciences (UITS)	Cucumber Salad 1	Pink, Mucoid Pink	Blue	No growth	No growth	Yellow
	Chola	Pink, Colorless	No growth	No growth	No growth	Green, Yellow
	Misti Ruti	Pink, Mucoid Pink	No growth	No growth	No growth	No growth
	Tomato Sauce 1	No growth	No growth	No growth	No growth	No growth
East West University (EWU)	Cucumber Salad 2	No growth	No growth	No growth	No growth	Yellow
	Tomato Sauce 2	No growth	No growth	No growth	No growth	Yellow
	Chili Sauce	Mucoid Pink	No growth	No growth	No growth	Yellow
	Pudina Pata Sauce 1	Pink	No growth	No growth	No growth	No growth
	Jolpai Achar	No growth	No growth	No growth	No growth	No growth
	Boroi Achar	No growth	No growth	No growth	No growth	No growth
Bangladesh Institute of Science & Technology (BIST)	Cucumber Salad 3	Pink, Colorless	Blue	No growth	No growth	Yellow, Yellow with green dot
	Tetul Sauce	Colorless	No growth	No growth	No growth	No growth
	Pudina Pata Sauce 2	Pink, Colorless	Blue	No growth	No growth	No growth
	Misti Kumra Sauce	No growth	No growth	No growth	No growth	No growth
Southeast University (SEU)	Cucumber Salad 4	Pink	No growth	No growth	No growth	No growth
	Misti Petis	Pink	No growth	No growth	No growth	No growth
	Jhal Petis	Pink	No growth	No growth	No growth	Green
	Tetul Achar	No growth	No growth	No growth	No growth	No growth

Table 4.1 shows bacterial colony morphology isolated from different street vended food samples. Eighteen food samples were collected from four different private universities in Dhaka city. Of which, 13 samples show positive growth of our suspected organisms

(*E.coli, Vibio* spp., *Shigella* spp. *and Salmonella* spp.*)* and 5 samples shows no growth in these agar media.

Table 4.2: Bacterial colony morphology isolated from different expired food samples

Sample (Expired food)	**Plates**				
	MacConkey	**TBX**	**BGA**	**XLD**	**TCBS**
Butter bun 1	No growth	No growth	No growth	No growth	Yellow
Butter bun 2	No growth	No growth	No growth	No growth	Yellow, Black
Cake	Pink	No growth	Black centered red	No growth	No growth
Ovaltine cake	Mucoid Pink	No growth	No growth	No growth	Yellow
Chocolate cake	Mucoid Pink	No growth	No growth	No growth	Yellow
Fruit cake	Pink	No growth	No growth	No growth	No growth
Biscuit 1	Pink, Colorless	No growth	No growth	No growth	No growth
Biscuit 2	Pink	No growth	No growth	No growth	No growth
Salted biscuit	Pink	No growth	No growth	No growth	No growth
Chips 1	Mucoid Pink	No growth	No growth	No growth	No growth
Chips 2	Colorless	No growth	No growth	No growth	Yellow
Chips 3	No growth	No growth	No growth	No growth	No growth
Chips 4	No growth	No growth	No growth	No growth	No growth
Bread	Mucoid Pink	No growth	No growth	No growth	No growth
Milk bread	No growth	No growth	No growth	No growth	No growth
Toast 1	No growth	No growth	No growth	No growth	Yellow
Toast 2	No growth	Blue	No growth	No growth	No growth

Table 4.2 shows bacterial colony morphology isolated from different expired food samples. Seventeen food samples were collected from different areas in Dhaka city. Of which, 14 samples show positive growth of our suspected organisms (*E.coli, Vibio* spp., *Shigella* spp. *and Salmonella* spp.*)* and 3 samples shows no growth in these agar media.

Table 4.3: Number of food samples with growth of suspected organisms determined by colony morphology (n=35)

Types of food	**Name of University (for street food)**	**No. of samples with +ve growth by *E.coli***	**No. of samples with +ve growth by *Vibrio*s**	**No. of samples with +ve growth by *Shigella***	**No. of samples with +ve growth by *Salmonella***
Street food	UITS	2	0	1	0
	EWU	1	3	0	0
	BIST	0	0	3	0
	SEU	3	0	0	0
Expired food		8	5	0	1

Table 4.3 shows number of food samples with growth of suspected organisms determined by colony morphology. From total 35 food samples (street food and expired food), 27 (77.1%) samples were suspected to be contaminated with our targeted organisms (*E coli, Shigella, Salmonella* and *Vibrio* species). In total 27 samples, 14 (40%) samples were suspected to be contaminated with *E coli*, 8 (22.9%) with *Vibrio*, 4 (11.4%) with *Shigella* and 1(2.9%) with *Salmonella* species.

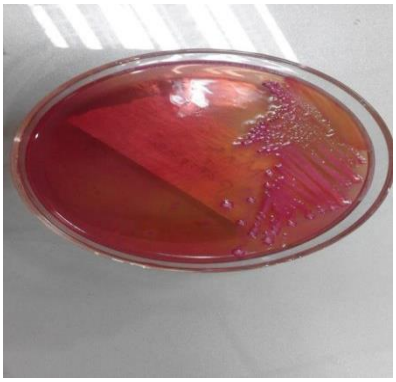


Figure 4.1: Bacterial colony (pink) on MacConkey agar plate

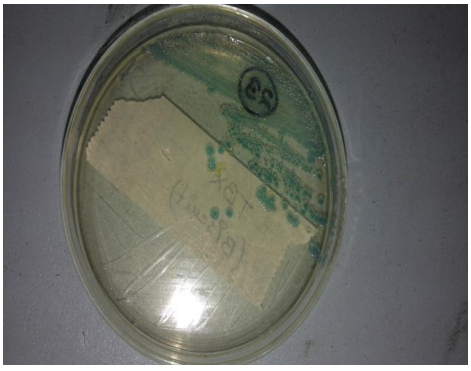


Figure 4.2: Bacterial colony (blue) on TBX agar plate

4.2 Suspected organism from different biochemical test

Table 4.4: Identification of the suspected organism (*E.coli* species) from different biochemical test

samples	**plates**	**colony morphology**	**M**	**I**	**O**	**Citrate**	**Urease**	**Oxidase**	**KIA**			**Organism**
									Slunt /butt	**H_2S**	**gas**	
Cucumber salad 4	MacConkey	Pink	+	+	-	+	-	-	A/A	-	+	*E.coli*
Pudina pata sauce 1	MacConkey	Pink	+	+	-	-	-	-	A/A	-	+	
Pudina pata sauce 2	TBX	Blue	+	+	-	+	-	-	A/A	-	+	
Misti ruti	MacConkey	Pink	-	+	-	+	-	-	A/A	-	+	
Ovaltine cake	MacConkey	Mucoid Pink	+	-	+	+	-	-	A/A	-	+	
Biscuit 1	MacConkey	Pink	+	-	+	+	-	-	A/A	-	+	
Toast biscuit 2	TBX	Isolated blue	+	+	+	-	-	-	A/A	-	+	

Table 4.4 shows identification of *E.coli* species from different biochemical test. Biochemical test results for the samples Cucumber salad 4, Pudina pata sauce 1, Pudina pata sauce 2, Misti ruti, Ovaltine cake, Biscuit 1 and Toast biscuit 2 matched with the standard results for *E.coli* species. So, we can say that the samples may contain the *E.coli* species.

Table 4.5: Identification of the suspected organism (*Vibrio* species) from different biochemical test

samples	plates	colony morphology	M	I	O	Citrate	Urease	Oxidase	KIA			Organism
									Slunt /butt	H_2S	gas	
Cucumber salad 2	TCBS	Yellow	+	+	-	+	-	-	K/A	-	-	*Vibrio* spp.
Cucumber salad 3	TCBS	Yellow	+	+	-	+	-	-	K/A	-	+	
Tomato sauce	TCBS	Yellow	+	+	-	+	-	-	K/A	-	-	
Chili sauce	TCBS	Yellow	-	+	-	+	-	-	K/A	-	-	
Chola	TCBS	Yellow	-	+	-	+	-	-	K/A	-	-	
Butter bun 1	TCBS	Yellow	+	+	+	+	-	-	K/A	-	-	
Chocolate cake	TCBS	Yellow	+	+	+	+	-	-	K/A	-	+	

Table 4.5 shows identification of *Vibrio* species from different biochemical test. Biochemical test results for the samples Cucumber salad 2, Cucumber salad 3, Tomato sauce, Chili sauce, Chola, Butter bun 1 and Chocolate cake matched with the standard results for *Vibrio* species. So, we can say that the samples may contain the *Vibrio* species.

Table 4.6: Identification of the suspected organism (*Shigella,* and *Salmonella* species) from different biochemical test

samples	plates	colony morphology	M	I	O	Citrate	Urease	Oxidase	KIA			Organism
									Slunt /butt	H_2S	gas	
Cucumber salad 3	MacConkey	Colorless	+	+	-	+	-	-	K/A	-	+	*Shigella* spp.
Tetul sauce	MacConkey	Colorless	-	+	-	+	-	-	K/A	-	+	
Pudina pata sauce 2	MacConkey	Colorless	-	+	-	+	-	-	K/A	-	+	
Chola	MacConkey	Colorless	-	+	-	+	-	-	K/A	-	+	
Cake	BGA	Black centered red	+	+	-	+	-	-	K/A	-	+	*Salmonella* spp.

Table 4.6 shows identification of *Shigella,* and *Salmonella* species from different biochemical test. Biochemical test results for the samples Cucumber salad 3, Tetul sauce, Pudina pata sauce 2 and Chola matched with the standard results for *Shigella.* So, we can say that the samples may contain the *Shigella* species. Biochemical test results for the sample Cake matched with the standard results for *Salmonella.* So, we can say that the sample may contain the *Salmonella* species.

Table 4.7: Presence of suspected organisms in selected food samples after biochemical test (n=19)

Types of food	Name of University (for street food)	*E.coli*	*Vibrios* spp.	*Shigella* spp.	*Salmonella* spp.
Street food	UITS	1	1	1	0
	EWU	1	3	0	0
	BIST	1	1	3	0
	SEU	1	0	0	0
Expired food		3	2	0	1

Table 4.7 shows presence of suspected organisms in selected food samples after biochemical test. From the results of biochemical test we found 19 of our suspected bacteria. Among them, from UITS we got 1 *E.coli*, 1 *Vibrio*, 1 *Shigella*; from EWU we got 1 *E.coli*, 3 *Vibrio*; from BIST we got 1 *E.coli*, 1 *Vibrio*, 3 *Shigella*; from SEU we got 1 *E.coli*; from different expired food we got 3 *E.coli*, 2 *Vibrio*, 1 *Salmonella*. In total we got 7 (36.8%) *E.coli*, 7 (36.8%) *Vibrio*, 4 (21%) *Shigella* and 1 (5.3%) *Salmonella* species.

Table 4.8: Presence of food borne pathogens in various street-vended and expired food samples (n=35)

Pathogens	**Food Categories**						
	Salad items (n=4)	**Sauce items (n=7)**	**Achar items (n=3)**	**Baked items (n=3)**	**Spicy items (n=1)**	**Expired food items (n=17)**	**Total food items (n=35)**
E.coli	1 (25%)	2 (29%)	Nd	1 (33%)	Nd	3 (18%)	7 (20%)
***Vibrio* spp.**	2 (50%)	2 (29%)	Nd	Nd	1 (100%)	2 (12%)	7 (20%)
***Shigella* spp.**	1 (25%)	2 (29%)	Nd	Nd	1 (100%)	Nd	4 (11%)
***Salmonella* spp.**	Nd	Nd	Nd	Nd	Nd	1 (6%)	1 (3%)

Table 4.8 shows the incidence of food borne pathogens in various food samples. Among 4 salad items, 1 (25%) sample was suspected to contain *E.coli*, 1 (25%) sample was suspected to contain *Shigella* and 2 (50%) samples were suspected to contain *Vibrio.* Among 7 sauce items, 2 (29%) samples were suspected to contain *E.coli*, 2 (29%) samples were suspected to contain *Vibrio* and 2 (29%) samples were suspected to contain *Shigella.* Among 3 baked items, 1 (33%) sample was suspected to contain *E.coli.* One spicy item was suspected to contain both *Vibrio* and *Shigella.* Among 17 expired food items, 3 (18%) samples were suspected to contain *E.coli*, 2 (12%) samples were suspected to contain *Vibrio* and 1 (6%) sample was suspected to contain *Salmonella* species.

4.3 Bacterial colony counting

After an appropriate incubation period the plates were examined for colonial growth. Colonies will form on the top of the agar as well as in the agar. Those on top of the agar will be larger but all colonies must be counted. Plates were selected that appear to have between 30 - 300 colonies in and on the agar as this gives the best statistical representation of the number of bacteria in the undiluted sample. Using a light box or colony counter and marker pen, the numbers of colonies were counted.

Table 4.9: Colony counting of various street food samples

Name of University	Sample (Street food)	Dilution 1	Dilution 2	Dilution 3	Dilution 4
University of Information Technology and Sciences (UITS)	Cucumber Salad 1	Uncountable	Uncountable	38	18
	Chola	Uncountable	65	28	13
	Misti Ruti	Uncountable	Uncountable	Uncountable	82
	Tomato Sauce 1	42	25	23	9
East West University (EWU)	Cucumber Salad 2	Uncountable	76	34	17
	Tomato Sauce 2	47	19	18	6
	Chili Sauce	Uncountable	32	11	10
	Pudina Pata Sauce 1	56	39	21	12
	Jolpai Achar	25	12	9	4
	Boroi Achar	8	5	2	1
Bangladesh Institute of Science & Technology (BIST)	Cucumber Salad 3	Uncountable	Uncountable	51	13
	Tetul Sauce	Uncountable	23	20	14
	Pudina Pata Sauce 2	41	22	10	10
	Misti Kumra Sauce	Uncountable	Uncountable	Uncountable	30
Southeast University (SEU)	Cucumber Salad 4	Uncountable	60	25	13
	Misti Petis	Uncountable	Uncountable	60	22
	Jhal Petis	Uncountable	Uncountable	Uncountable	48
	Tetul Achar	5	6	0	0

Table 4.9 shows Colony counting of various street food samples. For Cucumber Salad 1, plate 3 was selected as it has between 30-300 colonies. The other plates cannot be used because they are either too thick with colonies or too thin. The calculation of cell concentration in the original culture is:

38 colonies on plate 3 x dilution factor of 1000 = 38,000 cells/ml.

Dilution factor for plate 1: 10

Dilution factor for plate 2: 100

Dilution factor for plate 3: 1000

Dilution factor for plate 4: 10000

Using above mentioned formula and dilution factors we got the number of microorganism per ml of sample of street food items.

Table 4.10: Number of microorganism per ml of street food sample

Sample	Cucumber Salad 1	Chola	Misti Ruti	Tomato Sauce 1	Cucumber Salad 2	Tomato Sauce 2	Chili Sauce
Number of microorganism (cells/ml)	38,000	6,500	820,000	420	7,600	470	3,200
Sample	Pudina Pata Sauce 1	Cucumber Salad 3	Pudina Pata Sauce 2	Misti Kumra Sauce	Cucumber Salad 4	Misti Petis	Jhal Petis
Number of microorganism (cells/ml)	560	51,000	410	300,000	6,000	60,000	480,000

Table 4.11: Colony counting of various expired food samples

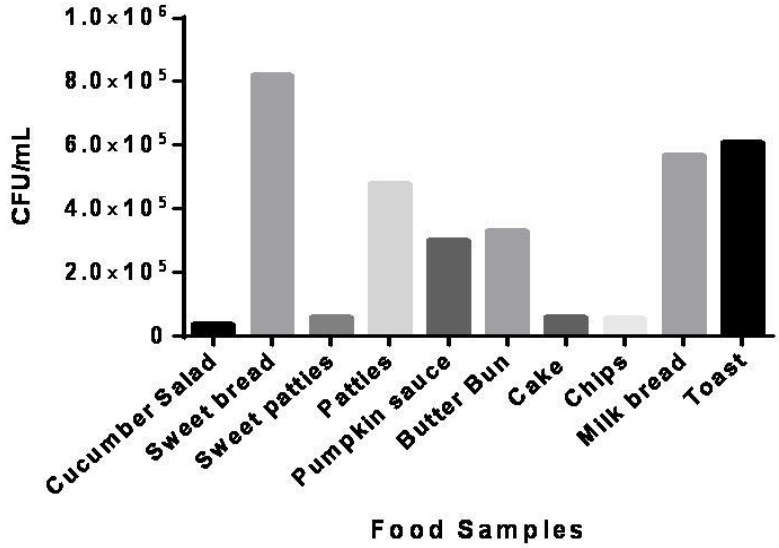
Sample (Expired food)	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Butter bun 1	Uncountable	Uncountable	50	42
Butter bun 2	Uncountable	Uncountable	Uncountable	33
Cake	Uncountable	42	29	23
Ovaltine cake	Uncountable	Uncountable	35	18
Chocolate cake	Uncountable	61	23	17
Fruit cake	Uncountable	Uncountable	80	54
Biscuit 1	46	29	22	16
Biscuit 2	Uncountable	66	42	31
Salted biscuit	28	19	13	5
Chips 1	Uncountable	Uncountable	55	44
Chips 2	Uncountable	78	47	30
Chips 3	Uncountable	Uncountable	Uncountable	34
Chips 4	Uncountable	Uncountable	36	17
Bread	Uncountable	56	29	11
Milk bread	Uncountable	Uncountable	Uncountable	57
Toast 1	Uncountable	Uncountable	90	88
Toast 2	Uncountable	Uncountable	Uncountable	61

Table 4.11 shows colony counting of various expired food samples. Using the same process followed for the street food items, we got the number of microorganism per ml of sample of expired food items.

Table 4.12: Number of microorganism per ml of expired food sample

Sample	Butter bun 1	Butter bun 2	Cake	Ovaltine cake	Chocolate cake	Fruit cake	Biscuit 1	Biscuit 2
Number of microorganism (cells/ml)	50,000	330,000	4,200	35,000	6,100	80,000	460	6,600
Sample	Chips 1	Chips 2	Chips 3	Chips 4	Bread	Milk bread	Toast 1	Toast 2
Number of microorganism (cells/ml)	55,000	7,800	340,000	36,000	5,600	570,000	90,000	610,000

Figure: 4.3 : **Bacterial count in different food samples**



Chapter 5

Discussion and Conclusion

5.1 Discussion and Conclusion

At present time, street food vending has become a major community health issue and matter of concern for all of us. A lot of food-borne disease outbreaks are occurring every year worldwide. The reasons behind this includes lack of appropriate knowledge and supervision on street food vending, preparation of food under insanitary conditions and displaying food openly which also lead to further contamination by dust, insects, rodents and hands of intending consumers. Similarly expired food also causes several health hazards. After the expiry date the food don't have the nutritional value as that of before the expiry date; moreover there might be growth of the bacteria that can lead to food-borne illness.

The present research work was therefore carried out to find out the presence of enteric bacteria specially *E.coli, Salmonella, Shigella* and *Vibrio* species from different types of expired foods and street-vended food items collected from different private universities of Dhaka city, Bangladesh.

Five agar media MacConkey, Tryptone Bile X-glucoronide (TBX) agar, Thiosulfate Citrate Bile Salt-sucrose (TCBS) agar, Brilliant Green Agar (BGA) and Xylose-Lysine Desoxycholate agar (XLD) were used to observe the presence of our targeted microorganisms in food items. MacConkey and TBX agar were used for the identification and isolation of *E.coli.* TCBS Agar is highly selective for *Vibrio* species isolation. XLD and BGA were used for isolation of *Salmonella* and *Shigella* species from food samples. Sometimes we didn't find any growth in agar media. The reason of no growth may include the following: a) sometimes fresh foods were collected early in the morning so no contamination occurred yet, b) sometimes food were hot which prevented growth of bacteria.

In this study, 35 different food samples were tested. Among them, 17 samples were expired food samples and remaining 18 samples were collected from 4 private university premises of Dhaka city. Among all 35 samples, we found contamination in 27 (77.1%) samples. Of which, 17 (48.6%) samples were suspected to be contaminated with our targeted organisms (*E coli, Shigella, Salmonella* and *Vibrio* species). In total 17 samples, 19 targeted organisms were suspected because 2 samples were suspected for containing two microorganisms. So, if we consider the different samples for each of the suspection then there were total 19 samples. Among them, 7 (20%) samples were suspected to be contaminated with *E coli*, 7 (20%) with *Vibrio*, 4 (11.4%) with *Shigella* and 1(2.9%) with

Salmonella species. From the results of biochemical test we got 19 of our suspected bacteria from 17 different samples. In total we got 7 (36.8%) *E.coli*, 7 (36.8%) *Vibrio*, 4 (21%) *Shigella* and 1 (5.2%) *Salmonella* species.

A study was conducted to assess microbiological safety of street vended foods from May to November, 2014 in Jigjiga City. One hundred thirty-two samples of street foods were aseptically collected from four 'kebeles' of Jigjiga City. The study revealed that 95(72%) of the food samples had pathogenic bacterial contaminations. Three different bacterial species were isolated: *E.coli* 68(51.5%), *S. aureus* 85(64.4%) and 26(19.7%) *Salmonella* species. The highest incidence of *S. aureus* 23/33(69%) was seen in 'Sambusa'; the highest incidence of *E.coli* 24/33(73.5%) was observed in 'Pasta', while the highest *Salmonella* incidence was observed in 'Ades' (Bereda et al., 2016). In our study we found *E.coli* in salad, sauce and expired food; we also found *salmonella* in expired food.

Study conducted in Amravati, India where forty water sample of panipuri were aseptically collected from eleven locations of Amravati City. 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%), *Klebsiella* spp. (20%), Pseudomonas spp. (5%) and yeast (3%). It is suggested that regular monitoring of the quality of street foods must be practiced to avoid any food-borne infection in future (Tambekar et al., 2011). In our study we have not seen any *Klebsiella* spp, or any *Pseudomonas* spp; however, we only observed the enteric pathogens like *E.coli, Vibrio, Shigella, Salmonella.*

A study has been done to analyze the microbiological quality of salads served along with street foods of Hyderabad. A total of 163 salad samples, 53 of carrot and 110 of onion samples, were collected from four different zones of Hyderabad. About 74% and 56% had *Staphylococcus aureus* in carrots and onions, respectively. Fifty-eight percent of carrots and forty-five percent of onions samples contained *Salmonella*, 68% of carrots and 24% of onions had Yersinia (Sabbithi et al., 2014). In our study we have found *Salmonella* in expired food item.

This present study indicated that the street vended foods of Dhaka city are highly contaminated with microorganism which can contribute to potential health risks for consumers. The risk factors to the contamination include the low educational background of the vendors, poor personal hygiene, improper handling and storage practice of foods. Most of the vendors handled food with bare hand and didn’t wear any gloves or hand

cover while handling money that can cause cross-contamination by introducing microbes on safe food. The study also indicated that the expired foods are highly contaminated with pathogenic bacteria. Ingestion of food after the expiration date indirectly causes the ingestion of hazardous pathogen which causes potential health risks. It is important to use that food before the expiration date to get the most nutritional value from it and to get rid of the affect of pathogenic organism.

Street food has become an important part of diet for many people as such food is easily accessible and affordable. It also plays an important role in providing employment for millions of men and women with limited education or skills, especially as the initial investment is low. In contrast to its benefits, it is also recognized that street-food vendors are often poor and uneducated and lack appreciation for safe food handling. In this study, among 35 food samples we have found suspected enteric pathogens in 19 foods. Of which, 7 (20%) samples were suspected to be contaminated with E coli, 7 (20%) with *Vibrio*, 4 (11.4%) with *Shigella* and 1(2.9%) with *Salmonella* species. So, it can be concluded that street foods are a major public health risk. So, the maintenance of these street vended/expired foods should be monitored cautiously. The government should take necessary steps to provide regular training and to create consciousness on food management and individual hygiene among street food vendors as well as consumers. Moreover, government should take necessary steps to prevent the sale of expired food products; as well as consumers also should be careful about the expiry date of their purchased foods to ensure their own health.

Chapter 6

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