Microbiological Quality of Street-Vended, Expired and Cafeteria Food Items Collected from Different Places 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

Tasmia Ferduse

ID: 2013-3-70-013

Under the Guidance of

Sufia Islam, Ph. D

Professor

Department of Pharmacy

East West University

Declaration by the Research Candidate

I, Tasmia Ferduse, hereby declare that the dissertation entitled "Microbiological Quality of Street-Vended, Expired and Cafeteria Food Items Collected from Different Places in 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 2017 of my research in the Department of Pharmacy, East West University, under the joint supervision and guidance of Sufia Islam Ph. D, Professor and 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.

_____

Tasmia Ferduse
ID: 2013-3-70-013
Department of Pharmacy
East West University
Dhaka, Bangladesh.

Certificate by the Supervisor

This is to certify that the thesis entitled "Microbiological Quality of Street-Vended, Expired and Cafeteria Food Items Collected from Different Places in Dhaka City, Bangladesh" 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 Tasmia Ferduse, ID: 2013-3-70-013, during the period 2017 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.

_____

Sufia Islam Ph. D
Professor
Department of Pharmacy
East West University
Dhaka, Bangladesh.

Certificate by the Co-Supervisor

This is to certify that the thesis entitled "Microbiological Quality of Street-Vended, Expired and Cafeteria Food Items Collected from Different Places in Dhaka City, Bangladesh" submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree of Bachelor of Pharmacy, was carried out by Tasmia Ferduse, ID: 2013-3-70-013, during the period 2017 of her research in the Department of Pharmacy, East West University, under the co-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.

_____

Nafisa Tanjia
Senior Lecturer
Department of Pharmacy
East West University
Dhaka, Bangladesh.

Certificate by the Chairperson

This is to certify that the thesis entitled "Microbiological quality of street-vended, expired and cafeteria food items collected from different places in Dhaka City, Bangladesh" 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 Tasmia Ferduse, ID: 2013-3-70-013, during the period 2017 of her research in the Department of Pharmacy, East West University.

_____

Dr. Chowdhury Faiz Hossain

Professor and Chairperson

Department of Pharmacy

East West University

Dhaka, Bangladesh.

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ETEC	Enterotoxigenic *E. coli*
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
EHEC	Enterohaemorrhagic *E. coli*
VTEC	Verotoxin-producing *E. coli*
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-glucoronide
BGA	Brilliant Green Agar
XLD	Xylose Lysine Deoxycholate
TCBS	Thiosulfate Citrate –bile Salts
KIA	Kliglar Iron Agar

Abstract

Food-borne illnesses generally cause disorders of the digestive tract; however, they can also lead to more serious consequences. Food-borne illness is the result of having contaminated, expired, or toxic food items. In developing countries food sold by street vendors is the major source of food-borne illness. Food in the student cafeteria can cause food-borne illness if they are contaminated. As a result safety and well-being of the students are affected. Harmful bacteria grow in the expired food items which lead to food-borne disease. The objective of this study was to isolate and identify the presence of enteric bacteria (*Escherichia coli*, *Shigella*, *Salmonella* and *Vibrio species)* in different expired foods, cafeteria foods and street foods. Among thirty five food items 8, 4 and 23 were street foods, expired foods and foods from cafeteria respectively. The street vended foods, expired foods and cafeteria foods were collected from different areas in Dhaka city. The tested food samples were Hotdog, Shingara, Mayonnaise, Bhel-puri, Beguni, Bun, Cake, Danish, Chola, Chips, Laddu, Pattice etc. Sterile polythene bags were used to collect 3 different samples. They were tested for the presence of microorganisms following conventional microbiological processes. Biochemical tests were done for the confirmation of *Escherichia coli*, *Shigella*, *Salmonella* and *Vibrio* species. Out of thirty five food samples 7 (20%) food samples were suspected to be contaminated with *E. coli*, 2 (5%) food samples were suspected to be contaminated with *Shigella spp.*, 10 (28%) food samples were suspected to be contaminated with *Vibrio spp*. All these enteric pathogens could be the potential cause for food-borne illnesses. Further study is needed to find out the contamination of different types of food items with large sample size.

Key Words: Street foods, *Escherichia coli*, *Shigella spp, Vibrio spp*, Expired foods, Cafeteria foods, Biochemical tests.

Chapter- 01: Introduction and Literature Review

1.1 Street Foods

Foods and beverages which are prepared and sold by the sellers on places like streets other similar places, festival areas and consumed by the consumers on the run are known as street food.

Some street foods are regional but many are not, having spread beyond their place of origin and enjoyed locally for their exotic or unusual ingredients and flavors. Street food satisfies the food consumption need of a significant section of the population. 2.5 billion People worldwide eat street food every day, according to a 2007 Food and Agriculture Organization study.

The food sold on the streets is relatively cheap and readily available for the consumer. It is sometimes brought to the door step of the customers. Street food, therefore, not only meets the food requirements particularly of those of the low income categories but also the busy customers who do not have much time either to prepare their own food or to go to other eating houses where probably the food is more expensive and servicing is time consuming.

Figure 1.1: Street-vended Foods

While there is a growing demand for street food products and there is no current information is available regarding the microbiological quality of these foodstuffs in Dhaka city, Bangladesh. The present study was hence undertaken to find out the

microbiological quality and safety of a variety of street-vended food products collected from several typical vendors surrounding different private universities of Dhaka city.

1.2 Expired Foods

Expiration Date refers to the last date a food should be eaten or used. When we buy food items at local grocery store, one may notice a printed sell by date**,** use by date or best before date on the wrapping or item itself. This sell by date or use by date or best before date indicate to the last date that a product, as food, should be used before it is considered spoiled or ineffective, usually specified on the label or package. The aware consumer should fully enlighten about the true "shelf life" of the most popular food items. Nearly all food is still edible after these printed expiration dates have passed. When reviewing the best-by date, or any printed date on a food item in question, the following facts associated with the shelf life of foods:

- **Food can be sold after a Date Expires**– Supplies are not legally required to eradicate food from the shelf once the expiration date has passed. The expiration dates are strictly "advisory" in nature and are left completely to the discretion of the manufacturer, thus not truly investigative of an items true Shelf Life.

- **Food Dates Are Not Required By Law** – With the exception of infant formula and baby food, the Food and Drug Administration (FDA) does not need food companies to place dates on their food products. The only obligation is that the food is wholesome and fit for consumption.

- **Laws Vary by State**– States have varying food dating laws. For example, many states require that milk and other perishables be sold before the expiration date, while others do not (Eatbydate.com. n. d.)

Injurious bacteria grow in the expired food items which lead to food-borne disease. The present study was conducted to determine the microbial condition of the expired foods cafeteria food collected from different universities of Dhaka city, Bangladesh.

1.3 Cafeteria Food

The Canteen and Cafeteria provide reasonably priced quality food to students and a cookery service to the School, university and colleges encouraging healthy eating, whilst

ensuring to minimize the economic cost of the food operation to the School. A cafeteria is a type of food service place in which there is little or no waiting staff table service, whether a restaurant or within an organization such as a large office building or school; a school dining location is also referred to as a dining hall or cafeteria.

An outbreak of cryptosporidiosis occurred on a Washington, DC university campus in September and October of 1998. Among 88 cases and 67 controls, eating in 1 of 2 cafeterias was associated with diarrheal illness (Quiroz et al. 2000).

In Bangladesh the demand of cafeteria food is increasing day by day among the young adults. There is no present information is available regarding the microbiological quality of the cafeteria foodstuffs in Dhaka city, Bangladesh. The present study was conducted to determine the microbiological quality of some cafeteria food collected from different universities of Dhaka city, Bangladesh.

1.3.1 Categories of Cafeteria Food

In cafeteria we can enjoy delicious food at a reasonable price. Usually in cafeteria we can get breakfast item foods, lunch item foods and snacks item foods. In breakfast Porota, Dal-vaji, ruti, Vegetable vaji, coffee, tea items are available. In lunch item rice, Fried rice, Pulao, Biryani, Chicken curry, Beef curry etc is easily available. In snacks item Burger, Pizza, Hot-dog, Pattice, Egg chop, Shingara, Beguni are available.

1.4 Street Vendors

Street foods mainly sold by the street vendors. Street vendors could be stationary and occupy space on the pavements or other public or private areas, or could be mobile, and move from place to place carrying their wares on push carts or in cycles or baskets on their heads, or could sell their wares in moving buses. They have low-cost seating facilities which are sometimes rudimentary.

1.4.1 Socio-Demographic Characteristics of Street Food Vendors

The street food vendors are identified as the informal sector where their businesses are conducted as a form of irregular, unstable. As such there is no systematic documentation of the numbers of street food vendors, their scale of businesses. After rickshaw-pulling, street vending is probably the second most important employment opportunities for the urban poor in Bangladesh, and particularly important for young and middle-aged men

who have migrated to Dhaka in the past five to ten years. About 300,000 street vendors live and work in Dhaka (Rahman, Rahman & Ansary 2014).

1.5 Significant of Street Foods

Street foods often reflect traditional local cultures and exist in an endless variety. Most street foods are considered both finger food and fast food and are more reasonably priced than restaurant meals. These food items are usually sold by vendors and hawkers in the streets or other similar public places. Street vended foods are appreciated for their unique flavors. In contrast to these potential benefits, it is also recognized that street food vendors are often poor, uneducated, and lack knowledge in safe food handling, environment, sanitation and hygiene. Mode of food display, food service and hand washing, sources of raw materials, and use of potable water are also contribute to food safety. Street foods are perceived to be a major public health risk. Dhaka city is the capital of Bangladesh and is one of the most densely populated cities of the world. At present Dhaka is the residence of approximately 14 million people and it has been estimated that there are around 2 million street food vendors currently engaged in food vending in the city. A few published reports on street food vendors in Dhaka suggests poor microbial quality of street food and bear the risk of transmitting enteric disease in the communities (Redzwan 2016, Islam et al. 2015). Most of the consumers of street foods are male (98%). A research shows that the generally food consumers are rickshaw puller, laborers and informal sectors (43%), workers (19%), students, children (12%) and others (26%) (Rahman, Rahman & Ansary 2014).

1.6 Street Food Sector in Bangladesh

As a developing country a lot of street foods are found in the street and open places in Bangladesh (especially in Dhaka). In developing countries like Bangladesh, urbanization and population growth are increasing in a continuous streak, which demands that street food sectors should be expanded into the next century (WHO, 1996).

1.6.1 Types of Street Food Available In Bangladesh

Street foods are very much popular to the Bangladeshi people living in the urban areas due to its cheap price and sharp taste. There are 128 varieties of street foods found in

Bangladesh. Among them Fuchka, Chotpothi, Velpuri, Samucha, DaalPuri, Lassi, Pakura, Halim, Beguni, Jhalmuri, different types of Achar are most popular. Street foods are an extremely heterogeneous food category encompassing meals, drinks, snacks (Rahman, Rahman & Ansary 2014).

Figure 1.2: Different types of Street Food Available in Bangladesh

1.7 Safety of Street Foods

Some studies on street foods chemical composition have shown that a several numbers of banned food additives were being used by the street vendors in different food preparations. Presence of banned coloring, non-food-grade additives such as textile coloring agents were used to make the appearance of the street foods better. 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). Street food is harmful for our health because when the sellers make some alur chop, samucha, beguni, singara, they use burnt oil. They also use harmful chemical in food to make them delicious. Street foods are mainly prepared from flour, meat, fish, vegetables, egg etc. These ingredients often contain microorganisms. That's why most of the time the microbial condition of the street foods are not satisfactory. These foods are prepared in open places by the street vendors. The water they use is not filtered rather it

contains bacteria and microorganisms like *E. coli* species of *Alcoligeus, Proteus, Bacillus, Salmonella.* Due to the handle of foods with unclean hands microbes get contaminated with these foods. Many street food sellers don't cut their nail regularly but we know that in nail 150000 lakh bacteria remain there. Most of the time vending machine and utensils are not clear properly so the undesirable microbes take place in the street foods. Freshly squeezed or freshly prepared fruit juices sold by street vendors contain a lot of microbes.

1.8 Food Borne Illness

Food borne illnesses of microbial origin are a major health problem associated with street foods. In addition, resistance of food-borne microorganisms in multi-drug made the food safety situation more vulnerable in public health. Approximately, 30 million people in Bangladesh are suffering from food-borne illnesses each year. Diarrheal diseases are the most common food poisoning cases in Bangladesh and in some cases, these can cause death. The diseases are caused by either toxin from the microbe or by the human body's reactions to the microbe. The traditional processing methods that are used in the preparation, inappropriate holding temperature, and poor personal hygiene of food handlers are some of the main causes of contamination of street foods. Also the foods are not effectively protected from flies and dust. In Bangladesh, street foods are mostly prepared and processed manually and sold to the public at various lorry terminals, by the roadside or by roaming vendors. A study result demonstrated that 25% street food vendors are illiterate and cannot write their names and have no formal education. As street food business requires low investment, most of the vendors (88%) were found to own the business. They reportedly work for 13–18 hours a day without having toilet facilities. Most of the vending shops (68%) were located on the foot path and 30% vending carts were placed near the municipal drain and 18% near the sewerage. Microbiological study of different foods items, drinking water, and hand swab samples showed the prevalence of overwhelmingly high numbers of aerobic bacteria, coli form bacteria, and pathogens. People, who patronize street food, have been reported to suffer from food borne diseases like diarrhea, cholera, typhoid fever, jaundice and food poisoning (CDC, 2016, Islam S, 2015)

Table 1.1 Causes of Food-borne Diseases

Group of Pathogens	Example Of Pathogens	Sign And Symptoms
Bacteria	*Salmonella spp.* *Camphylobacter jejuni* *Shigella spp* STEC O157:H7 *Listeria monocytognes* *Vibrio spp.* *Yersinia spp.*	Abdominal pain; diarrhea; chills; fever; vomiting; dehydration; nausea; jaundice; Anorexia.
Parasites	*Crysptospridium spp.* *Cyclospora spp.* *Giardia lamblia* *Toxoplasma caris* *Entamoeba histolica*	Severe diarrhea; lowgrade fever; and severe intestinal distress; abdominal pain; cramps.
Toxins **Enterotoxins**	*Staphylococcus aureus* *Clostridium perfringens* *Bacillu cereus*	Abdominal pain; diarrhea; vomiting; nausea;
Botulinumtoxins	*Clostridium botulinum*	Vertigo; double vision; difficulty in swallowing; weak muscles; respiratory paralysis; frequently fatal.
Fishtoxins	Scombrotxin Ciguatera toxin Paralytic sheefish toxin	Abdominal pain; dizziness; vertigo; numbness; tingling and muscle pain; diarrhea.
Mushrooms	Amatoxin Phallotoxin	Severe Seizures of abdominal pain; persistent vomiting; watery diarrhea; extreme thirst and lack of urine production.
Miscellaneous	Niacin Monosodium glutamate	Visual hallucination and ataxia in children.

(Jay & Golden 2005)

1.8.1 Populations at Risk for Food-borne Diseases

Food-borne diseases can occur in anyone. The occurrence of food-borne illnesses depends on the type of organisms that are present in the food, amount of exposure, age, health, co-morbidities etc. The following groups are more prone food borne diseases:

- Infants and children
- Pregnant women and their fetuses
- Older adults
- People with weak immune systems

These groups also have a greater risk of developing severe symptoms or complications of food-borne illnesses (NIDDK n. d.).

1.9 Factors Affecting Growth of Microorganisms in Food

There are 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.

The most important factors that affect microbial growth in foods can be summarized in the following categories:

1.9.1 Intrinsic Factors

Factors related to the food itself, the “intrinsic factors,” which include;

- Nutrient content,
- Water activity,
- pH value,

Redox potential and the presence of antimicrobial substances and mechanical barriers to microbial invasion.

1.9.2 Extrinsic Factors

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

- The temperature of storage, and
- The composition of gases and relative humidity in the atmosphere surrounding the food

1.9.3 Implicit Factors

Factors related to the microorganisms themselves, the "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.

1.9.4 Processing Factors

Processing factors, which 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.

1.9.5 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 (eds. Bhat 2012).

1.10 Description of Some Common Microorganism Responsible for Food borne Diseases

1.10.1 *Escherichia Coli*

Escherichia coli (commonly abbreviated *E. coli*) is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2, and by preventing the establishment of pathogenic bacteria within the intestine. *E. coli* and related bacteria constitute about 0.1% of gut flora, and fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause diseases (Adams & Moss, 2007)

Figure 1.3: *Escherichia Coli*

1.10.1.1 Characteristics

Enteropathogenic *Escherichia coli* (EPEC) are in the family Enterobacteriaceae. The bacteria are non-spore forming, motile with peritrichous flagella or non motile, and grow on MacConkey agar (colonies are 2 to 3 mm in diameter and red or colorless). They can grow under aerobic and anaerobic conditions and do not produce enterotoxins.

1.10.1.2 Pathogenesis

E. coli consists of a diverse group of bacteria. Pathogenic *E. coli* strains are categorized into pathotypes. Six pathotypes are associated with diarrhea and collectively are referred to as diarrheagenic *E. coli*. They are:

1.10.1.3 Shiga toxin-producing *E. coli* (STEC)

STEC may also be referred to as Verocytotoxin-producing *E. coli* (VTEC) or enterohemorrhagic *E. coli* (EHEC). This pathotype is the one most commonly known bacteria associated with food-borne outbreaks.

First recognized as a cause of human disease in 1982, EHEC causes bloody diarrhea (hemorrhagic colitis), non-bloody diarrhea and hemolytic uremic syndrome (HUS).

The key virulence factor for EHEC is Stx, which is also known as verocytotoxin (VT). Stx consists of five identical B subunits that are responsible for binding the holotoxin to the glycolipid globotriaosylceramide (Gb3) on the target cell surface, and a single A subunit that cleaves ribosomal RNA, causing protein synthesis to cease12. The Stx family contains two subgroups Stx1 and Stx2 that share approximately 55% amino acid homology. Stx is produced in the colon and travels by the bloodstream to the kidney, where it damages renal endothelial cells and occludes the microvasculature through a combination of direct toxicity and induction of local cytokine and chemokine production,

resulting in renal inflammation. This damage can lead to HUS, which is characterized by hemolytic-anaemia, thrombocytopoenia and potentially fatal acute renal failure. Stx also induces apoptosis in intestinal epithelial cells, a process that is regulated by the Bcl-2 family 44 (Adams & Moss, 2007).

1.10.1.4 Enterotoxigenic *E. coli* (ETEC)

ETEC causes watery diarrhea, which can range from mild, self-limiting disease to severe purging disease. The organism is an important cause of childhood diarrhea in the developing world and is the main cause of diarrhea in travelers to developing countries. ETEC colonizes the surface of the small bowel mucosa and elaborates enterotoxins, which give rise to intestinal secretion. Colonization is mediated by one or more proteinaceous fibrillar colonization factors (CFs), which are designated by CFA (colonization factor antigen), CS (coli surface antigen) or PCF (putative colonization factor) followed by a number. More than 20 antigenically diverse CFs have been characterized, yet epidemiological studies indicate that approximately 75% of human ETEC express either by CFA/I, CFA/II or CFA/IV (Adams & Moss, 2007).

1.10.1.5 Enteropathogenic *E. coli* (EPEC)

EPEC was the first pathotype of *E. coli* to be described. Large outbreaks of infant diarrhea in the United Kingdom led Bray, in 1945, to describe a group of serologically distinct *E. coli* strains that were isolated from children with diarrhea but not from healthy children.

A characteristic intestinal histopathology is associated with EPEC infections; known as ‘attaching and effacing’ (A/E), the bacteria intimately attach to intestinal epithelial cells and cause striking cytoskeletal changes, including the accumulation of polymerized actin directly beneath the adherent bacteria. The microvilli of the intestine are effaced and pedestal-like structures on which the bacteria perch frequently rise up from the epithelial cell. The ability to induce this A/E histopathology is encoded by genes on a 35-kb pathogenicity island (Adams & Moss, 2007).

1.10.1.6 Enteroinvasive *E. coli* (EIEC)

EIEC are biochemically, genetically and pathogenically closely related to *Shigella* spp. numerous studies have shown that *Shigella* and *E. coli* are taxonomically indistinguishable at the species level. EIEC might cause an invasive inflammatory colitis, and occasionally dysentery, but in most cases EIEC elicits watery diarrhea that is

indistinguishable from that due to infection by other *E. coli* pathogens (Adams & Moss, 2007).

1.10.1.7 Diffusely Adherent *E. coli* (DAEC)

DAEC are defined by the presence of a characteristic, diffuse pattern of adherence to HEp-2 cell monolayers. DAEC have been implicated as a cause of diarrhea in several studies, particularly in children >12 months of age (Kaper 2004).

1.10.2 *Salmonella* Species

Salmonella species are ubiquitous human and animal pathogens, and salmonellosis. It has a rod-shaped conformation and is aerobic. This bacterium is also Gram-negative, meaning that it has a three-layer cell membrane, essentially. The first layer is the outer membrane, in the center is a thin peptidoglycan layer followed be an inner plasma membrane. The thin peptidoglycan layer is characteristic of all Gram-negative bacteria. The bacterial cell *Salmonella typhi* is a motile bacterium and is able to move due to flagella. Flagella are a special organelle on the outside of the bacteria that looks like an eyelash or the tail of a sperm. 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 & Miller 1999).

Figure 1.4: *Salmonella* Species

1.10. 2.1 Characteristics

Salmonella enterica is one of two *Salmonella* species (*enterica and bongori*) and a member of the Enterobacteriaceae family. *Salmonella enterica* spp. is subdivided into 6 subspecies (*enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV) and *indica* (VI)). The usual habitat for subspecies enterica is warm-blooded animals. The

usual habitat for subspecies II, IIIa, IIIb, IV and VI is cold-blooded animals and the environment. All species of *Salmonella* can infect humans. *Salmonella enterica* subspecies *enterica* has 2610 different serotypes; the most well known being serotypes Typhi, Paratyphi, Enteriditis, Typhimurium and Choleraesuis. The serotypes are characterized by three surface antigens: the flagellar "H" antigen, the oligosaccharide "O" antigen and the polysaccharide "Vi" antigen (found in Typhi and Paratyphi serotypes). *Salmonella enterica* is a facultative anaerobe and is a gram negative, motile and non-sporing rod that is 0.7-1.5 by 2.0-5.0 µm in size.

1.10.2.2 Pathogenesis

Salmonellosis includes several syndromes (gastroenteritis, enteric fevers, septicemia, focal infections, and an asymptomatic carrier state). Particular serovars show a strong propensity to produce a particular syndrome (*S typhi*, *S paratyphi-A*, and *S schottmuelleri* produce enteric fever; *S choleraesuis* produces septicemia or focal infections; *S typhimurium* and *Senteritidis* produce gastroenteritis); however, on occasion, any serotype can produce any of the syndromes. In general, more serious infections occur in infants, in adults over the age of 50, and in subjects with debilitating illnesses.

Most non-typhoidal s*almonellae* enter the body when contaminated food is ingested. Person-to-person spread of *salmonellae* also occurs. To be fully pathogenic, *salmonellae* must possess a variety of attributes called virulence factors. These include (1) the ability to invade cells, (2) a complete lipopolysaccharide coat, (3) the ability to replicate intracellularly, and (4) possibly the elaboration of toxin(s). After ingestion, the organisms colonize the ileum and colon, invade the intestinal epithelium, and proliferate within the epithelium and lymphoid follicles. The mechanism by which *salmonellae* invade the epithelium is partially understood and involves an initial binding to specific receptors on the epithelial cell surface followed by invasion. Invasion occurs by the organism inducing the enterocyte membrane to undergo "ruffling" and thereby to stimulate pinocytosis of the organism. Invasion is dependent on rearrangement of the cell cytoskeleton and probably involves increases in cellular inositol phosphate and calcium. Attachment and invasion are under distinct genetic control and involve multiple genes in both chromosomes and plasmids.

After invading the epithelium, the organisms multiply intracellularly and then spread to mesenteric lymph nodes and throughout the body via the systemic circulation; they are

taken up by the reticuloendothelial cells. The reticuloendothelial system confines and controls spread of the organism. However, depending on the serotype and the effectiveness of the host defenses against that serotype, some organisms may infect the liver, spleen, gallbladder, bones, meninges, and other organs. Fortunately, most serovars are killed promptly in extraintestinal sites, and the most common human *Salmonella* infection, gastroenteritis remains confined to the intestine (NCBI, 1996).

1.10.3 *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).

1.10.3.1 Characteristics

Shigella spp., of the Enterobacteriaceae family, is 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 differentiated by their biochemical properties, phage or colicin susceptibility, and polyvanlent antisera can detect specific polysaccharide antigens. *S. dysenteriae* is considered the most virulent, and can produce a potent cytotoxin known as Shigatoxin.

Figure 1.5: *Shigella Species*

1.10.3.2 Pathogenesis

Shigella causes 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 diarrhea 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 diarrhea with *Sh. sonnei.* Illness lasts from 3 days up to 14 days in some cases and a carrier state may 250 Bacterial Agents of Food borne Illness develop which can persist for several months. Milder forms of the illness are self-limiting and require no treatment but Sh. dysenteries 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 and Moss, 2008).

1.10.4 *Vibrio* Species

Vibrio is a family of Gram-negative bacteria that can cause a variety of illnesses in humans. The most famous form of *Vibrio* is *Vibrio cholerae*, the bacterium that causes cholera. *Vibrio cholerae* has been the cause of seven worldwide pandemics and countless deaths over the last couple of centuries. Except for *Vibrio cholerae* and *Vibrio mimicus*, all require saltwater for growth. Therefore, seawater and raw or undercooked shellfish are common infection routes for *Vibrio*.

Figure 1.6: *Vibrio Species*

1.10.5.1 Characteristics

Vibrios are 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.

1.10.5.2 Pathogenesis

Cholera usually has an incubation period of between one and three days and can vary from mild, self-limiting diarrhea 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.

Cholera is a non-invasive infection where the organism colonizes the intestinal lumen and produces a potent enterotoxin. In severe cases, the hyper secretion of sodium, potassium, chloride, and bicarbonate induced by the enterotoxin results in a profuse, pale, watery diarrhea containing flakes of mucus, described as rice water stools. The diarrhea, which can be up to 20 l day1 and contains up to 108 *vibrios* ml1, is accompanied by vomiting, but without any nausea or fever. 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 and Moss, 2008).

1.11 Food-borne Disease Out-breaks around the World

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).

Fecal contamination of the salad was documented which caused outbreak of gastrointestinal illness among students and employees in college in Florida (Lieb S, 1985).

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 management 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 incorporated norovirus (54%), *Salmonella spp.* (4%), rotavirus (2%), and *Campylobacter spp.* (1%) (Duynhoven et al, 2005).

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 spp.* (Islam, et al., 2015).

Chapter- 02:

Research Objective

2.1 Research Objectives

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

To isolate and identify the presence of enteric bacteria especially *E. coli, Salmonella, Shigella* and *Vibrio* species from different expired foods, cafeteria foods and street foods. The street vended foods, expired foods and cafeteria foods were collected from different places in Dhaka city.

Chapter- 03: Methodology

3.1 Bacteriological Subculture

3.1.1 Sample Collection

About 35 solid food samples 4 street foods, 8 cafeteria foods and 23 expired food samples were randomly chosen and collected from different areas in Dhaka city 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 (Shingara, Hot-dog, Pattice, Beguni), spicy items (Fuchka, chola), baked items (Cake, Danish, Biscuit) 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*

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 spp* 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*

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 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 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

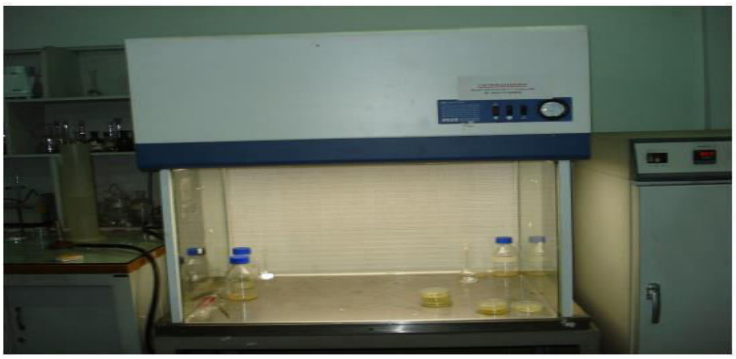

Figure 3.3: Laminar Air Flow Cabinet

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 spp.	BGA	Typical red colonies
	XLD	Red or clear colonies with black center
Vibrio spp.	TCBS	Large yellow colonies
Shigella spp.	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 and Kovac's reagent
- Agar:
  - ✓ Kliglar's Iron Agar,
  - ✓ MIO agar,
  - ✓ Christensen's Urea Agar
- Simmons citrate medium
- Analytical balance and 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 (Simmions 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 ten seconds	No color change in colony
KIA	H2S	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.

3.3.1 Cell Counting and Serial Dilutions

3.3.2 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. It is desirable 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 succeeding smaller amounts of sample. Making serial decimal dilutions (i.e., successive 1/10 dilutions, each made by adding one part of inoculums to 9 parts of diluents) and inoculating one ml into each of the plates, we can construct a plating procedure that is equivalent to the above.

3.3.3 Materials Required:

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

3.3.4 Procedure:

There are four major steps in the procedure:

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

3.3.5 Preparation of Serial Dilutions

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

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

Table 3.3: Calculation of Dilution Factors

Plate	Dilution	Dilution	Dilution Factor
1	10-1	1/10	101
2	10-2	1/100	102
3	10-3	1/1,000	103
4	10-4	1/10,000	104

3.3.6 Mixing the Dilutions into Agar Plates

1.1 Nutrient agar was prepared by autoclaving.

1.2 The bottle of molten agar was placed in a 50°C water bath and the agar was allowed to cool to 50°C.

1.3 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.

1.4 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.

1.5 One mL of each of the dilutions was pipette into the base of correctly labeled plates using a separate pipette to avoid carryover errors.

1.6 Each plate was gently swirled to mix the 1 mL of diluted sample into the 15 mL of agar.

1.7 The plate was left without moving for at least 13 minutes to allow the agar to set.

1.8 When the agar was set, the plate was incubated as appropriate.

3.3.7 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. 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- 04:

Result

4.1 Bacterial colony morphology

Table 4.1: Bacterial colony morphology isolated from different canteen foods and street foods

Name of University	Sample	Plates				
		Macconkey	**TBX**	**BGA**	**XLD**	**TCBS**
East West University	Noodles 1	Pink	Blue	No growth	No growth	No growth
	Myaonnaise	Colorless	No growth	No growth	No growth	Yellow
	Hot dog	No growth	No growth	No growth	No growth	Yellow and yellow with black dot
	Cucumber and Onion salad	Pink and Mucoid pink	Blue	No growth	No growth	Yellow
	Ghunni	Colorless	Blue	No growth	No growth	Yellow
	Vegetable vaji	Pink	No growth	No growth	No growth	Yellow
North South University	Noodles 2	Pink	White	No growth	Red	Yellow
	Beguni	Pink	Green	No growth	No growth	No growth
Bangladesh Institute of Science And Technology	Laddu	Colorless	No growth	No growth	No growth	Yellow and black
	Pudina pata sauce 2	No growth	No growth	No growth	No growth	No growth
University of Liberal Arts Bangladesh	Tomato sauce	No growth	No growth	No growth	No growth	No growth
	Jilapi	No growth	No growth	No growth	No growth	No growth

Table 4.1: (Bacterial colony morphology isolated from different canteen food samples) shows bacterial colony morphology isolated from different street vended food samples.

Around 12 food samples were collected from four different private universities in Dhaka city. In total 5 samples show growth of different pathogenic or non pathogenic microorganisms. Of which, 8 samples show positive growth of our suspected organisms of *E.coli and* 6 samples show positive growth of our suspected organisms of *Vibio spp* and 2 sample shows *Shigella flexneri and* one sample shows no growth in these agar media. The reason for observing no growth in sample 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.

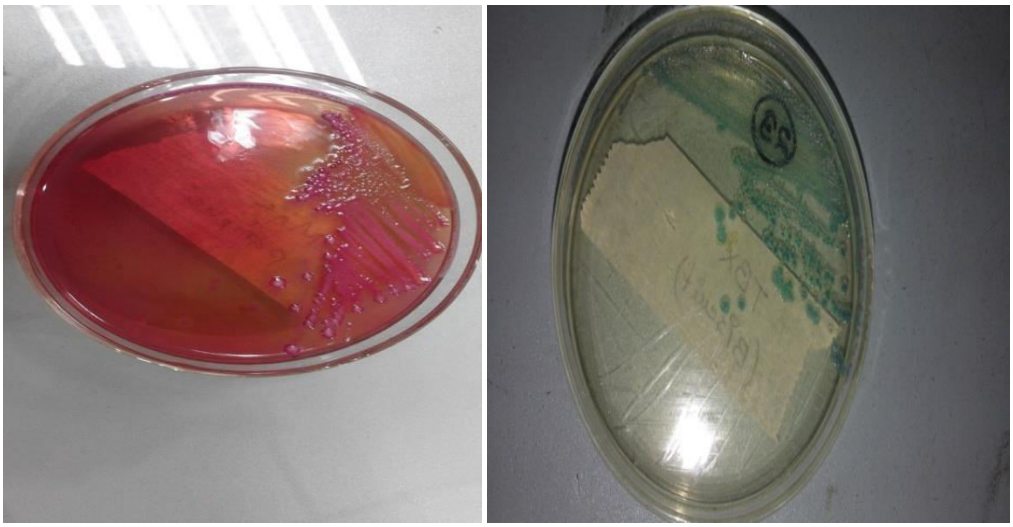

Figure 4.1: Bacterial colony on ager plate

Table 4.1.1: Number of food samples with growth of suspected organisms determined by colony morphology (n=12)

Name of Universities	**No. of of samples with +ve growth *E. coli***	**No. of of samples with +ve growth *Vibrio spp.***	**No. of of samples with +ve growth *Salmonella spp.***	**No. of of samples with +ve growth *Shigella Spp.***
East West University	4	5	Nd	1
North South University	2	2	Nd	Nd
Bangladesh Institute Of Science And Technology	Nd	Nd	Nd	Nd
University Information And Technology	Nd	Nd	Nd	1

Table 4.1.1: (Number of food samples with growth of suspected organisms determined by colony morphology**)** shows in total 12 samples, 6 (17%) samples were suspected to be contaminated with *E coli*, with *Vibrio* 7 (50%)*,* with *Shigella* and 2 (5%) with *Salmonella* 0 (0%) species.

Table 4.2: Bacterial colony morphology isolated from different expired foods

Samples	Agar Plates				
	MacConkey	**TBX**	**BGA**	**XLD**	**TCBS**
Bun	No Growth	No Growth	No Growth	No Growth	Green
Honey Comb Bread	No Growth	No Growth	No Growth	No Growth	No Growth
Spong Cake	No Growth	No Growth	No Growth	No Growth	Yellow
Plane Cake	No Growth	No Growth	No Growth	No Growth	Yellow
All time Bun	No Growth	No Growth	No Growth	No Growth	Yellow
Dora Cake	No Growth	No Growth	No Growth	No Growth	No Growth
Tiffin Cake	No Growth	No Growth	No Growth	No Growth	Green
Chocolate Biscut	No Growth	No Growth	No Growth	No Growth	No Growth

Table 4.3: Bacterial colony morphology isolated from different expired foods

Samples	Agar Plates				
	MacConkey	**TBX**	**BGA**	**XLD**	**TCBS**
Biscuit 1	Pink White dot Mucoid Pink	No Growth	No Growth	No Growth	Yellow
Salty toast biscuit	Pink	No Growth	No Growth	No Growth	No Growth
Sweet toast biscuit	Pink	No Growth	No Growth	No Growth	No Growth
Anarkoli Biscuit	No Growth	No Growth	No Growth	No Growth	No Growth
Dual Chocolate Cake	No Growth	No Growth	No Growth	No Growth	No Growth
All time cake	Pink	No Growth	No Growth	No Growth	No Growth
All-time milk bread	Pink	No Growth	No Growth	No Growth	No Growth
Maria toast	No Growth	No Growth	No Growth	No Growth	No Growth

Table 4.4: Bacterial colony morphology isolated from different expired foods

Samples	Agar Plates				
	MacConkey	**TBX**	**BGA**	**XLD**	**TCBS**
Danish Bread	No Growth	No Growth	No Growth	No Growth	Yellow
Motor Vaja	No Growth	No Growth	No Growth	No Growth	Yellow
Sweet Bread	No Growth	No Growth	No Growth	No Growth	Yellow
Bon Ruti	No Growth	No Growth	No Growth	No Growth	No Growth
Potato Chips	No Growth	No Growth	No Growth	No Growth	No Growth
Kaju Delight Biscuit	No Growth	No Growth	No Growth	No Growth	No Growth
Chalpata Chips	No Growth	No Growth	No Growth	No Growth	No Growth

Table 4.2, 4.3, and 4.4: (Bacterial colony morphology isolated from different expired foods) shows that, from 23 expired food samples were collected from different areas in Dhaka city in total, 12 samples show growth of different pathogenic or non pathogenic microorganisms. Of which, 2 samples show positive growth of our suspected organisms *Vibrio spp.* and 10 samples shows no growth in these agar media. The reason for observing no growth in sample 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.

Table 4.5: Number of food samples with growth of suspected organisms determined by colony morphology (n=23)

Samples	No. of of samples with +ve growth *E. coli*	No. of of samples with +ve growth *Vibrio spp.*	No. of of samples with +ve growth *Salmonella spp.*	No. of of samples with +ve growth *Shigella Spp.*
Expired Food	Nd	2	Nd	Nd

Table 4.5: (Number of food samples with growth of suspected organisms determined by colony morphology) shows that, in total 23 samples, 0 (0%) samples were suspected to be contaminated with *E coli*, with *Vibrio* 2 (8%) with *Shigella* 0 (0%) and with *Salmonella* 0 (0%) species.

4.2 Suspected Organisms From Different Biochemical Tests:

Table 4.6: Identification of the suspected organism (*E. coli and Shigella flexneri)* from different biochemical test

Samples	Plates	Colony Morphology	M	I	O	Citrae	Urease	Oxidase	KIA			Organism
									Slunt/ Butt	Gas	H2s	
Cucumber and Onion salad	Macconkey	Pink	+	+	-	+	-	-	A/A	-	+	*E. coli*
	TBX	Blue	+	+		+	-	-	A/A	-	+	
	TCBS	Yellow	+	+		+	-	-	K/A	-	+	
Vegetable Vaji	Macconkey	Pink	+	+	-	+	-	-	A/A	-	+	
Noodles 1	TBX	Blue	+	+	-	+	-	-	A/A	-	+	
	Macconkey	Pink	+	+		-	-	-	A/A	+	-	
Noodles 2	Macconkey	Pink	+	+		+	-	-	A/A	-	+	
Beguni	Macconkey	Pink	+	+	+	+	-	-	A/A	-	+	
Hot dog	TBX	Blue		+	-	+	-	-	A/A	-	+	
Mayonnaise	TCBS	Yellow		+	-	+	-	-	K/A	-	-	*Shigella flexneri*
Laddu	Macconkey	Colorless		+		+	-	-	K/A	-	-	

Table 4.7: Identification of the suspected organism (*Vibrio spp*.) from different biochemical tests

Samples	Plates	Colony Morphology	M	I	O	Citrae	Urease	Oxidase	KIA			Organism
									Slunt /Butt	Gas	H2s	
Cucumber and Onion salad	TCBS	Yellow	+	+	-	+	-	-	K/A	-	+	Vibrio spp.
Vegetable Vaji	TCBS	Yellow	+	+	-	+	-	-	K/A	+	-	
Mayonnaise	TCBS	Yellow	-	+	-	+	-	-	K/A	-	-	
Noodles 1	TCBS	Yellow	-	+	-	+	-	-	K/A	-	-	
Noodles 2	TCBS	Yellow	+	+	-	+	-	-	K/A	-	-	
Beguni	TCBS	Yellow	+	+	-	+	-	-	K/A	-	-	
Hot dog	TCBS	Yellow	+	+	-	+	-	-	K/A	-		
	TCBS	Yellow with black dot		+	-	-	-	-	K/A	-	-	
Danish bread	TCBS	Yellow	+	+	+	+	-	-	K/A	-	+	
Motor vaja	TCBS	Yellow	+	+	-	+	-	-	K/A	-	-	

Table 4.6 & 4.7 shows that, among 20 (70%) food samples were subjected for different biochemical test to identify our targeted organisms. Biochemical test results of about 10 (40%) food samples show similarities with the standard biochemical test results of our targeted organisms *(E. coli, Vibio spp*., *Shigella spp*. and *Salmonella spp.)* as compared.

Table 4.8: Incidence of food borne pathogens in various street-vended food samples

Pathogens	**Food categories**					
	Salad items (n = 1)	**Sauce items (n = 3)**	**Baked Items (n = 2)**	**Spicy items (n =6)**	**Expired items (n = 23)**	**Total food items (n = 35)**
E.Coli	1 (25%)	Nd	Nd	6 (100%)	Nd	7 (20%)
Shigella flexneri	Nd	1 (33%)	1 (50%)	Nd	Nd	2 (5%)
Vibrio spp	1 (25%)	1 (33%)	Nd	6 (100%)	2 (8%)	10 (28%)
Salmonella spp.	Nd	Nd	Nd	Nd	Nd	Nd

Table 4.9 shows the incidence of food borne pathogens in various food samples. Among one salad items 1 (25%) samples was suspected to contain *E. coli* and 1 (25%) sample was suspected to contain *Vibrio.* Among 3 sauce items, 1 (33%) samples were suspected to contain *Shigella flexneri,* 1 (33%) sample was suspected to contain *Vibrio.* Among 2 baked items, 1 (50%) samples were suspected to contain *Shigella flexneri.* Among 6 spicy items, 6 (100%) samples were suspected to contain *E. coli* and 6 (100%) samples were suspected to contain *Vibrio.* Among 23 expired food items, 2 (8%) samples were suspected to contain *Vibrio.*

4.3 Colony Counting of Various Samples

Table 4.10.1: Colony counting of various street foods, cafeteria foods and Expired foods

Sample Name	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Honey comb bread	20	36	53	70
Spong cake	10	28	45	75
Cucumber with onion salad	Uncountable	Uncountable	Uncountable	300
Bun	Uncountable	100	64	42
Tiffin cake	32	24	20	13
Dora cake	27	20	17	9
Chocolate biscuit	Uncountable	190	92	56
Anarkoli biscuit	Uncountable	Uncountable	Uncountable	400
Hot dog	Uncountable	Uncountable	Uncountable	Uncountable
Ghunni	Uncountable	Uncountable	Uncountable	Uncountable
Beguni	Uncountable	Uncountable	Uncountable	600
Vegetable vaji	Uncountable	Uncountable	Uncountable	Uncountable
Noodles 1	Uncountable	Uncountable	Uncountable	Uncountable
Noodles 2	Uncountable	Uncountable	205	350
Mayonnaise	Uncountable	Uncountable	Uncountable	560
Pudina pata sauce	Uncountable	Uncountable	Uncountable	Uncountable
Tomato sauce	12	32	48	57
Laddu	10	25	40	74
Jilapi	19	20	27	60
Motor vaja	Uncountable	Uncountable		
All-time milk bread	Uncountable	Uncountable	132	206
Chalpata chips	Uncountable	Uncountable	36	40

Sample Name	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Maria	200	142	120	62
Salty toast Biscuit	Uncountable	Uncountable	Uncountable	Uncountable
Sweet bread	Uncountable	Uncountable	Uncountable	Uncountable
Dual chocolate	Uncountable	100	80	70
Biscuit 1	Uncountable	Uncountable	64	150
Danish bread	Uncountable	Uncountable	Uncountable	Uncountable
Bon ruti	Uncountable	Uncountable	Uncountable	Uncountable
Poatato chips	120	95	55	44
Kaju delight biscuit	80	170	277	300
Plane cake	Uncountable	Uncountable	Uncountable	
All time Bun	280	190	180	160

Table 4.10: Colony counting of various samples:

For Honey Comb Bread 1plate 2 were 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:

36 colonies on plate 2X dilution factor of 1,000 = 3600 cells/ml.

For Tiffin cake 1 plate 1was 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:

32 colonies on plate 1X dilution factor of 10= 320cells/ml.

For Spong cake 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:

45 colonies on plate 3X dilution factor of 1,000= 45,000cells/ml.

For Cucumber with onion salad plate 4 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:

300 colonies on plate 4X dilution factor of 10,000=3,000, 000cells/ml.

For hot dog plate 4was 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:

540 colonies on plate 4X dilution factor of 10,000= 54, 00,000cells/ml.

For Mayonnaise plate 4was 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:

560 colonies on plate 4X dilution factor of 10,000= 56, 00,000cells/ml.

Table 4.11: Number of colonies per ml of sample

Sample Name	Number of Microorganism (cells/ml)
Honey comb bread	3600 cells/ml.
Tiffin cake	320cells/ml
Spong cake	45,000cells/ml
Cucumber with onion salad	3,000,000cells/ml
Hot dog	54, 00,000cells/ml.
Mayonnaise	56, 00,000cells/ml.
Maria	200 cells/ml

Sample Name	Number of Microorganism (cells/ml)
Chocolate biscuit	190,000cells/ml.
Anarkoli Biscuit	4,000,000cells/ml.
Noodles 2	205,000cells/ml.
Beguni	6,000,000cells/ml
Pudina pata sauce	3,780,00cells/ml.
Tomato sauce	32,00cells/ml.
Laddu	40,000cells/ml
Jilapi	60,0000cells/ml
Motor vaja	30000cells/ml
All time milk bread	132,000 cells/ml.
Chalpata chips	36,000cells/ml
Biscuit 1	64,000cells/ml.
Sweet toast biscuit	3,000cells/ml
Dual chocolate	10,000cells/ml.
Plane cake	4,200cells/ml.
All time bun	28,000cells/ml.

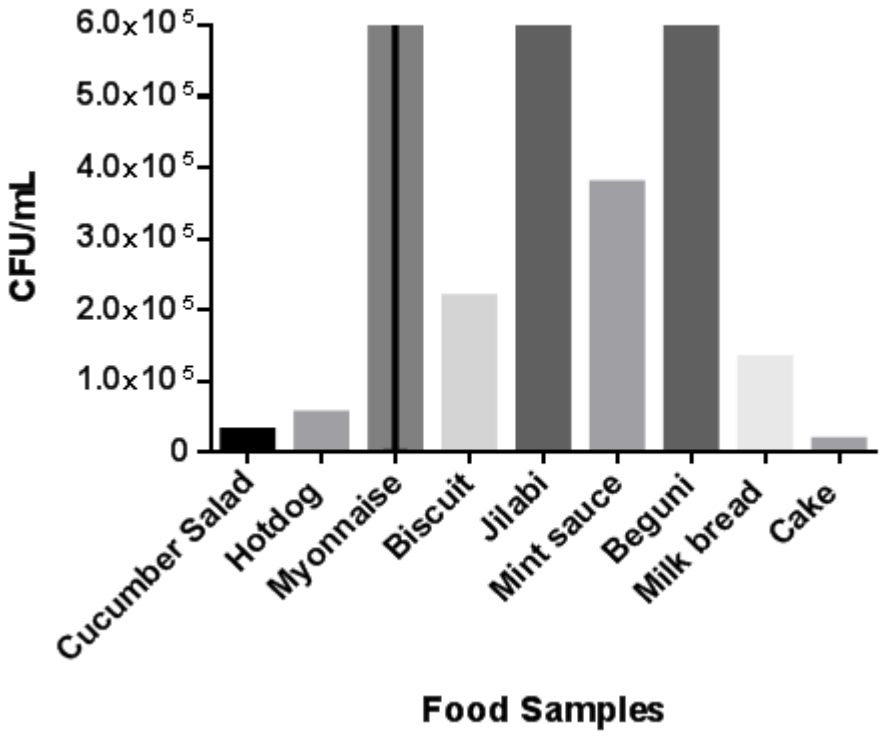

Figure 4.2: Bacterial count in different food samples

Chapter- 05: Discussion & Conclusion

5.1 Discussion and Conclusion

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 especially *E. coli, Vibio spp., Shigella spp. and Salmonella spp.* from different types of expired, cafeteria and street foods.

A total of 35 samples were collected from different areas 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.Out of thirty five foodsamples 7 (20%) food samples were suspected to be contaminated with *E. coli*, 2 (5%) food samples were suspected to be contaminated with *Shigella flexneri*, 10 (28%) food samples were suspected to be contaminated with *Vibrio spp*.

Fecal contamination of the salad was documented which caused outbreak of gastrointestinal illness among students and employees in college in Florida. Common symptoms were diarrhea, nausea, weakness, abdominal cramps, chills, vomiting, and low-grade fever. Cases of illness were identified in 40% of 628 students and 15% of 162 employees who responded to a survey. Among students, there was a sevenfold excess risk associated with eating one or more meals at the campus cafeteria (Lieb et al. 1985).

Nine spices with Acaroid mites in University canteen condiments were identified. Here thirteen kinds of condiments of the public cafeteria and 29 kinds of condiments of the canteen warehouse storage were collected (Song, Duan & Li 2015).

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 bacteria 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).

A study was conducted in Amravati, India. 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 present study, 3 suspected organisms *E. coli, Vibio spp.* and *Shigella spp.* were found from 10 (40%) samples. From the biochemical test results of the colonies of MacConkey, XLD and TCBS agar media, 7 (20%) food samples were suspected to be contaminated with *E. coli*, 2 (5%) food samples were suspected to be contaminated with *Shigella flexneri,* 10 (28%) food sample was suspected to be contaminated with *Vibrio* spp. No *Salmonella spp*. were found from any food sample.Seven biochemical tests were performed for characterizing the organisms but the serological test and PCR technology was not used to confirm the presence of pathogenic bacteria. Therefore it cannot be said confidently that colonies of the media plates are the claimed ones.

Our study also shows the incidence of food-borne pathogens in various street vended food samples. Among 35 samples divided into 6 categories, one salad items 1 (25%) sample was suspected to contain *E. coli* and 1 (25%) sample was suspected to contain *Vibrio* spp. Among 3 sauce items, 1 (33%) sample was suspected to contain *Shigella flexneri,* 1 (33%) sample was suspected to contain *Vibrio.* Among 2 baked items, 1 (50%) sample was suspected to contain *Shigella flexneri.* Among 6 spicy items, 6 (100%) samples were suspected to contain *E. coli* and 6 (100%) samples were suspected to contain *Vibrio.* Among 23 expired food items, 2 (8%) samples were suspected to contain *Vibrio.*

Street vended foods and cafeteria 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 in developing country like Bangladesh. It is also suggested that regular monitoring of the quality of street foods must be practiced to avoid food-borne infection in future.

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.

Chapter- 06:

Reference

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