Microbiological Assessment and Identification of enteric bacteria found in common street foods collected from different university premises in Dhaka city

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

Submitted by

Abuzar Ibn Faruk

ID: 2012-3-70-022

Department of Pharmacy

East West University

Declaration by the Candidate

I, Abuzar Ibn Faruk, hereby declare that the dissertation entitled "Microbiological Assessment and Identification of enteric bacteria found in common street foods collected from different university premises in Dhaka city" submitted by me to the Department of Pharmacy, East West University and in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, work carried out by me during the period 2016 of my research in the Department of Pharmacy, East West University, under the supervision and guidance of Ms. Nafisa Tanjia, 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.

_____

Abuzar Ibn Faruk
ID: 2012-3-70-022
Department of Pharmacy
East West University, Dhaka.

Certificate by the Supervisor

This is to certify that the thesis entitled "Microbiological Assessment and Identification of enteric bacteria found in common street foods collected from different university premises in Dhaka city" submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by Abuzar Ibn Faruk, ID: 2012-3-70-022, during the period 2016 of his 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.

_____

Nafisa Tanjia

Senior Lecturer

Department of Pharmacy

East West University, Dhaka.

Certificate by the Co-Supervisor

This is to certify that the thesis entitled "Microbiological Assessment and Identification of enteric bacteria found in common street foods collected from different university premises in Dhaka city" submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by Abuzar Ibn Faruk, ID: 2012-3-70-022, during the period 2016 of his 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.

_____

Dr. Sufia Islam

Professor

Department of Pharmacy

East West University, Dhaka.

Certificate by the Chairperson

This is to certify that the thesis entitled "Microbiological Assessment and Identification of enteric bacteria found in common street foods collected from different university premises in Dhaka city" submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by Abuzar Ibn Faruk, ID: 2012-3-70-022, during the period 2016 of his research in the Department of Pharmacy, East West University.

_____

Dr. Shamsun Nahar Khan

Associate Professor & Chairperson

Department of Pharmacy

East West University, Dhaka

ACKNOWLEDGEMENT

At first, I am grateful to Allah for the good health and wellbeing that were necessary to complete this research. I would like to express my deepest gratitude to my research supervisor, Nafisa Tanjia, Lecture, Department of Pharmacy, East West University and co-supervisor, Dr. Sufia Islam, Associate Professor, Department of Pharmacy, East West University, who had been always optimistic and full of passion and ideas. Their generous advice, constant supervision, intense support, enthusiastic encouragements and reminders during the research work not only helped shape this study but also molded me into being a better researcher. Their in-depth thinking, motivation, timely advice and encouragement have made it possible for me to complete this research.

Secondly, I am also indebted to the Department of Pharmacy, East West University. I am very proud to the part of this institute. To me it seems like second home. This institute is giving me an opportunity to learn about my future goals, to learn how to show respect to the pharmacy profession. I would like to show my gratitude to the Chairperson of Pharmacy Department, to the faculties who are teaching over the last four years to make us ready for the noble profession by becoming a pharmacist.

Third, my special thanks to Arzu Arafin Lisa, Tasnim Binte Shahjahan, Shahnaz Siddiqua, Md.Abu Bakar Siddique Bhuiyan and all of my friends, who helped me to conduct the research by being very co-operative to be the part of my study. Because of their tremendous support I could finish the work on time. I also, would like to help my fellow classmates, friends for their continuous support in my stay in this institute.

Finally, I am immensely grateful to my beloved parents, Md.Omar Faruk and Anwara Begum for their love and faith in me, especially for their unconditional love in my life. It is my parents who made me, who I am now! I also would like to express my heartfelt love to my family for their continuous support and love. I am fortunate to have such a nice family!

Table of Content

List of Abbreviations

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-glucuronide
BGA	Brilliant Green Agar
XLD	Xylose lysine deoxycholate
TCBS	Thiosulfate citrate-bile salts sucrose
KIA	Kliglar Iron Agar

Abstract

Street foods mean ready to eat foods and beverages that are prepared and sold especially in streets or similar public places. The street foods are prepared under unhygienic conditions and displayed openly to a high degree of contamination. The objective of this study was to isolate and identify the presence of enteric bacteria (*Escherichia coli*, *Klebsiella spp*, *Shigella* spp, *Salmonella spp* and *Vibrio* spp) in different street vended foods collected from different university premises in Dhaka city. Thirty food samples were collected from fixed and mobile vendors from different areas of Dhaka city. The tested samples were jhal-muri, fuchka, vhel-puri, panipuri, bun, cake, danish, chola, peaju, sweet, sheek-kabab, laddu, singara, somucha etc. Sterile polythene bags were used to collect three different samples from each university. They were tested for the presence of microorganisms following conventional microbiological processes. Biochemical tests were performed for the confirmation of

Escherichia coli, *Klebsiella spp*, *Shigella* spp, *Salmonella* and *Vibrio* spp. Among 30 samples divided into categories, 4 (13%) samples contained *E. coli,* 4 (13%) samples contained *Klebsiella pneumonia* and 2 (7%) samples contained *Vibrio* spp. All these enteric pathogens could be the potential cause for food-borne illnesses and provision of education to the vendors would improve quality of street foods.

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

CHAPTER 01

INTRODUCTION AND LITERATURE REVIEW

1.1 Street Vended Foods

Street foods feed millions of people daily with a wide selection of foods that are relatively cheap and easily accessible in Dhaka city. There are more than 100 variations of street foods available in Dhaka city for the young adults to choose. Each day 2.5 billion people prefer to consume street food worldwide due to its cost and convenience (FAO, 2010). Bangladesh is no exception in this situation. According to the FAO 2007 study, about 2.5 million people eat street food every day in Bangladesh. It signals that the proportion of business, opportunity exists in the street food sectors in Bangladesh. Since Bangladesh is among the low income countries, street food is still a lucrative and better option of food among the young generations. The cost of street food is cheaper than that of larger food establishments like restaurants, fast food outlets, which make it more popular to the consumers. In Bangladesh the demand of street food is increasing day by day among the young adults.

Figure 1.1: Different types of street food in Dhaka city

Street vended foods mean ready to eat foods and beverages that are prepared and sold especially in streets or similar public places by the street vendors or merchants for consumption at the location or later without any further preparation. The street vended foods are prepared under unhygienic conditions and displayed openly to a high degree of contamination. Street foods are sometimes stored at improper temperatures and sold from vending sites which includes kiosks, make-shift accommodation, and push carts as well as other temporary structures. In most cases running water is not available at vending sites, washing of hands and crockery are done in bowls or buckets and sometimes without soap. Thus from the health point of view, selling foods in the street is very controversial (Bereda et al., 2016).

The types of street-vended food differ significantly on countries and cultures. Foods from the street offer a source of readily available, reasonably priced meals with good nutritional values for the consumer (Rane, 2011).

In Bangladesh, the most popular and traditional street-vended foods includes jhal-muri, fuchka, vhel-puri, panipuri, bun, cake, danish, betel-leaf, chola, peaju, sweet, sheek-kabab, laddu, singara, somucha etc. Dhaka city has a huge number of street food vendors. These vendors gather mainly in the central business areas and at key points of transport such as train and bus stations, as well as in front of school where many people have these traditional foods (Rahman et al., 2014).

Street foods are mainly prepared from flour, meat, fish, vegetables, egg etc. These ingredients often contain microorganisms. So 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. Due to the handle of foods with unclean hands microbes get contaminated with these foods. These microbes are Staphyllococcus Aureus, S. Apedermides, species of Salmonella. Most of the time vendering machine and utensils are not cleared properly so the undesirable microbes take place in the street foods. These microbes are E. Coli, Enterobacter Aerogenus, Psycotrophic bacteria, species of Pseudomonous, Alcaligenus, Flavobacterium. A study of BCSIR shows that freshly squeezed or freshly prepared fruit juices sold by local market vendors in Dhaka city contain a lot of microbes. Total viable bacterial counts, fungal counts, total coliform, faecal coliform and the presence of pathogenic microorganisms such as E. coli, Bacillus cereus, Staphylococcus aureus, Salmonella, Streptococcuswere analyzed by standard methods. The total viable count of samples ranged from 3.00×102 to 9.60×108 and fungal counts ranged from 1.00×102 to 8.05×104. Out of 114 freshly prepared fruit juices samples collected 113 samples (99%) showed the presence of coliform and E. coli. The other bacteria like B. cereus, Staphylococcus aureus, Salmonella, Streptococcus were found in 64.91%, 6.14%, 7.89% and (5.26%) of the tested samples. The number and type of microorganisms recovered from the freshly squeezed fruit juices made them unsafe for drinking. Fast foods are preserved for a long time so some microbes can grow on the food items.

However, questions have been raised about the safety and microbiological quality of these food products. Food borne illnesses are a widespread problem globally. Developing countries bear the brunt of the problem due to the presence of a wide range of food-borne diseases. In Dhaka, street-food vending is everywhere; however there is a lacking of

information regarding food borne diseases related to street-vended foods. The vendors in Bangladesh lack education regarding the basic food safety issues. Vendors generally use carts and stands, where they do not have easy access to running water, furthermore dish and hand washing is done using the same bucket, sometimes even without soap (Okojie & Isah, 2014). Garbage and waste water is usually discarded in the streets nearby and therefore attracting and providing food for rodents and insects (Kibret & Tadesse, 2013). Toilets are not available nearby in some cases that force the vendors to eliminate their body wastes in nearby areas and come back to the vending sites without washing their hands. Environmental condition and practices like this often lead to contamination of cooked food. Vendors may purchase raw materials from doubtful sources which may either be contaminated with food borne pathogens or be unfit for consumption due to other reasons (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).

An estimated 2.5 billion people patronize food-vendors worldwide. Food-borne illness is a major international health problem with consequent economic reduction. According to Doyele and Evans (1999), food borne diseases are diseases resulting from ingestion of bacteria, toxins and cells produced by microorganism present in food (Monday et al., 2014). Street foods were responsible for 691 food poisoning outbreaks and 49 deaths from 1983 to 1992 in Shangdong Province of China (Rane, 2011).

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. In a national study, 37% of adults and 42% of children reported consuming fast food on one or both days of the survey. 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).

1.2 Types of Street Vended Foods

There are different types of street vended foods are available everywhere. The types of street vended foods vary from country to country according to their culture and geographical environment. Some street foods are common in every country such as salads, beef, chicken and gravy etc. Bangladesh has a huge number of street food vendors especially in Dhaka city. These vendors prefer to take their products to their customers in the central business areas and at key points of transport such as train and bus terminals, market places, as well as in front of schools. Such locations usually do not meet food and safety requirements. The most popular and traditional street-vended foods in Bangladesh include jhal-muri, fuchka, vhel-puri, panipuri, bun, cake, danish, betel-leaf, chola, peaju, sweet, sheek-kabab, laddu, singara, somucha etc (Rahman, Rahman &Ansary, 2014).

1.3 Food Borne Illnesses

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

1.4 Who Gets Food Borne Illnesses

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

1. ***Older adults:*** As anyone gets older, immune system may not respond as quickly and as effectively to infectious organisms as when younger.

2. ***Pregnant women:*** During pregnancy, changes in metabolism and circulation may increase the risk of food poisoning. The reaction may be more severe during pregnancy. Rarely, the baby may get sick, too.
3. ***Infants and young children:*** Their immune systems haven't fully developed.
4. ***People with chronic disease:*** Having a chronic condition such as diabetes, liver disease or AIDS or receiving chemotherapy or radiation therapy for cancer reduces immune response (Niddk.nih.gov, 2016).

1.5 Causes of Food Borne Illnesses

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

Table 1.1 Groups of Food borne Pathogens

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

1.6 Major sources contributing to microbial contamination

1.6.1 Vending Location: Food Handling and Waste Disposal

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

1.6.1.1 Food Handling

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

1.6.1.2 Waste Disposal

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

1.6.2 Quality of Raw Materials: Water and Other Material

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

1.6.2.1 Water

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

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

1.6.2.2 Other Raw Materials

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

1.6.3 Utensils and Equipments: Chemical and Microbial Contaminants

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

1.6.3.1 Chemical Contaminants

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

1.6.3.2 Microbial Contaminants

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

1.6.4 Food Preparation: Storage and Reheating

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

1.6.4.1 Storage

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

1.6.4.2 Reheating

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

1.6.5 Personal Hygiene of the Vendors or Food Handlers

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

1.7 Parameters of Foods That Affect Microbial Growth

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

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

1.7.1 Intrinsic Parameters

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

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

1.7.2 Extrinsic Parameter

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

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

1.8 Microorganisms Causing Food Borne Illnesses

1.8.1 *E. coli*

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

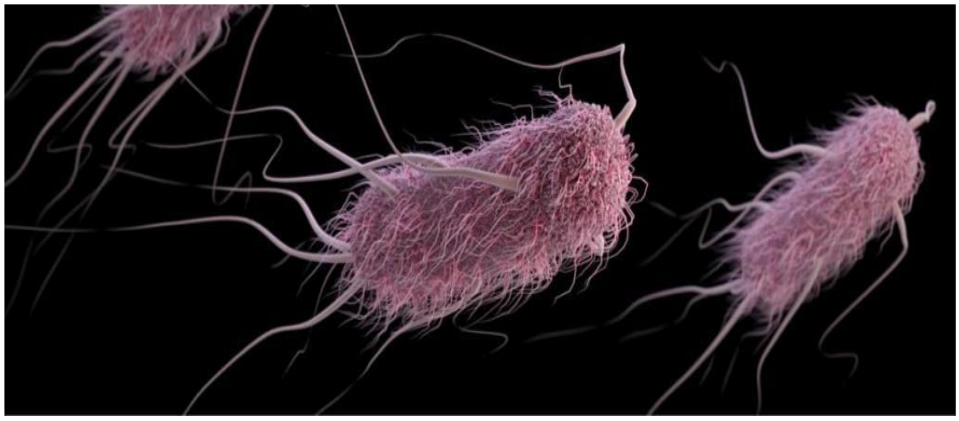

Fig. 1.2: *Escherichia coli* (*E. coli*)

1.8.1.1 Pathogenesis

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

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

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

1.8.1.2 Source of contamination

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

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

1.8.2 *Salmonella spp.*

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

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

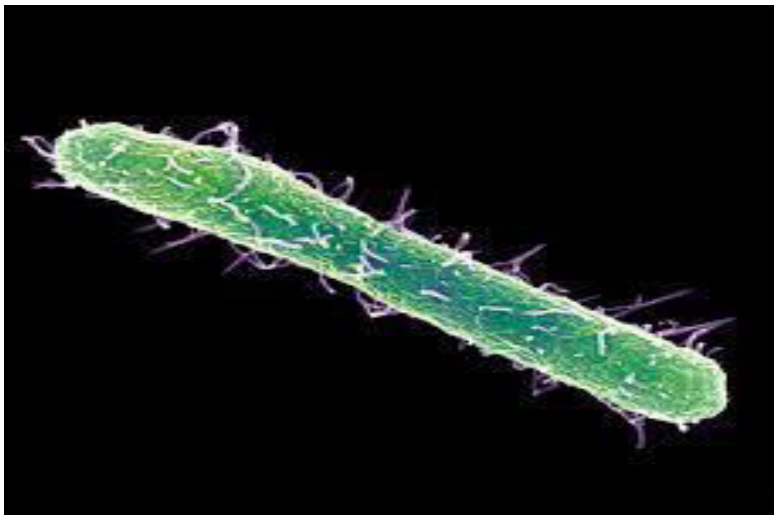

Fig. 1.3: ***Salmonella spp.***

1.8.2.1 Pathogenesis

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

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

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

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

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

1.8.2.2 Source of contamination

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

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

1.8.3 *Shigella spp.*

Shigella is a species of enteric bacteria that causes disease in humans and other primates. The disease caused by the ingestion of *Shigella* bacteria is referred to as shigellosis, which is most typically associated with diarrhea and other gastrointestinal symptoms.

Shigella spp. of the Enterobacteriaceae family, are gram-negative rod-shaped pathogenic bacteria. They are non-motile, non-encapsulated, and facultative anaerobes that do not ferment lactose, or do so slowly. Different serogroups, considered as species, can be

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

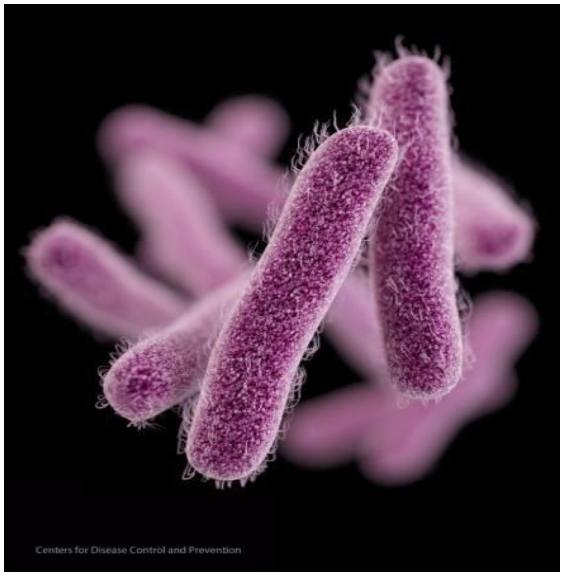

Fig. 1.4: *Shigella spp.*

1.8.3.1 Pathogenesis

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

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

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

- Fever
- Stomach cramps
- Rectal spasms

Milder forms of the illness are self-limiting and require no treatment but *Sh. dysenteriae* infections often require fluid and electrolyte replacement and antibiotic therapy.

Shigellosis is an invasive infection where the organism's invasive property is encoded on a large plasmid (Adams & Moss, 2008).

1.8.3.2 Source of contaminetion

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

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

1.8.4 *Vibrio spp.*

Vibrio spp. of Vibronaceae family are gram negative, non-spore forming, curved rod which are oxidase positive. They are very motile and have a single polar flagellum. The bacterium are 1- 3 µm by 0.5-0.8 µm, are a facultative anaerobe. The genus *Vibrio* consists of at least 28 species, and 3 that are often associated *with V. parahaemolyticus* in aquatic environments and seafood are *V. vulnificus, V. alginolyticus*, and *V. cholerae.*

Although most other known food-poisoning syndromes may be contracted from a variety of foods, *V. parahaemolyticus* gastroenteritis is contracted almost solely from seafood. When other foods are involved, they represent cross-contamination from seafood products. Another unique feature of this syndrome is the natural habitat of the etiological agent—the sea. In addition to its role in gastroenteritis, *V. parahaemolyticus* is known to cause extraintestinal infections in humans (Jay, Loessner & Golden, 2005).

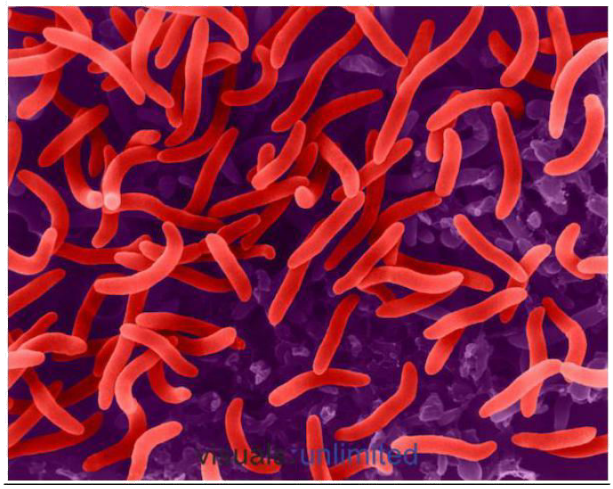

Fig. 1.5: ***Vibrio spp.***

1.8.4.1 Pathogenesis

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

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

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

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

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

1.8.4.1 Source of contamination

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

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

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

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

1.8.5 *Klebsiella spp.*

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

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

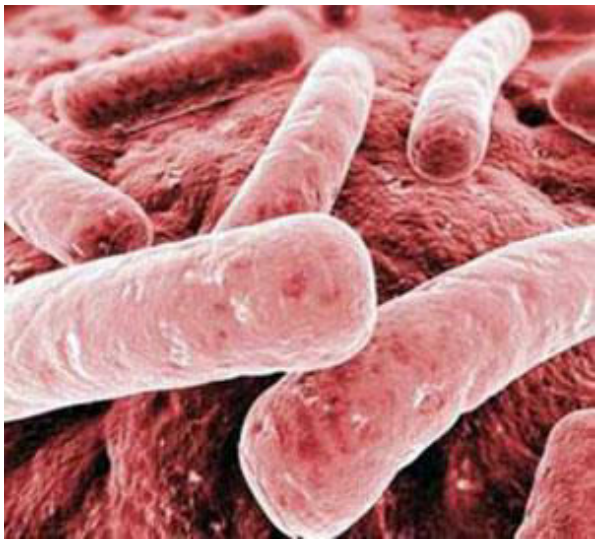

Fig. 1.6: *Klebsiella spp*

1.8.5.1 Pathogenesis

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

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

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

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

- Fever
- Chills

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

Pneumonia from K. pneumoniae can result in:

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

1.8.5.2 Source of contamination

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

1.9 Frequency of Food Borne Illness in Different Countries

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

1.9.1 Food borne Illness Outbreaks in the United States

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

1.9.2 Food Borne Illness Outbreaks in Australia

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

1.9.3 Food Borne Illness Outbreaks in Korea and Japan

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

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

1.10 Street Foods Condition in Dhaka city

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

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

CHAPTER 02

RESEARCH OBJECTIVE

Objective of this study

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

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

CHAPTER 03

METHODOLGY

3.1 Bacteriological Subculture

3.1.1 Sample Collection

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

3.1.1.1 Sample Category

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

3.1.2 Sample Processing

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

3.1.3 Enrichment of the Organisms

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

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

Fig. 3.1: Enrichment of the Organisms

3.1.4 Selective Growth of the Organisms

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

Cotton buds were dipped into the enrichment broths and swabbed onto MacConkey and TBX(Tryptone Bile X-glucuronide) agar plates, then streaked with sterile loops and incubated in aerobic condition at 37 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.

Fig. 3.2: Autoclave and Hot air Oven

Fig. 3.3: Laminar Air Flow Cabinet

3.1.6 Preparation of Petri dishes

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

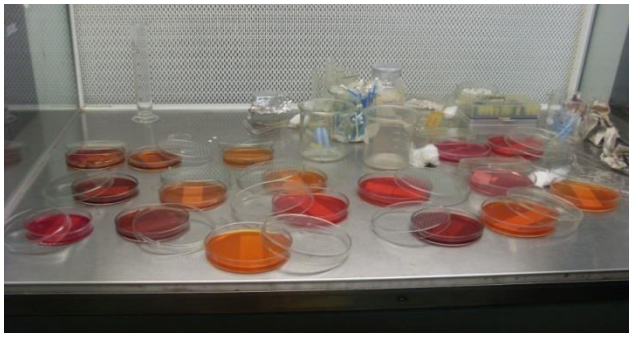

Fig. 3.4: Petri dishes preparation

3.1.7 Incubation

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

Fig. 3.5: Incubator

Table 3.1: Standard Colony Morphology of Suspected Organisms

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

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
Klebsiella	MacConkey	Pink colonies

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

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

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

3.2 Biochemical Tests

3.2.1 Kliglar Iron Agar Test (KIA Test)

3.2.1.1 Test Tube Preparation for KIA Test

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

3.2.1.2 Inoculation for KIA Test

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

Fig. 3.6: Preparation of test tubes for KIA test

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.

Fig. 3.7: Preparation of test tubes for MIO test

3.2.3 Citrate Test

3.2.3.1 Test Tube Preparation for Citrate Test

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

3.2.3.2 Inoculation for Citrate Test

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

Fig. 3.8: Preparation of test tubes for Citrate test

3.2.4 Urease Test

3.2.4.1 Test Tube Preparation for Urease Test

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

3.2.4.2 Inoculation for Urease Test

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

Fig. 3.9: Preparation of test tubes for Urease test

3.2.5 Oxidase test

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

3.2.6 Apparatus & reagent used for Biochemical Tests

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

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

Table 3.2: Standard Biochemical Test Results of Suspected Organisms

Biochemical Test		**Observation After Incubation**	
		Positive	**Negative**
MIO	Motility	Turbidity or haziness	No turbidity or haziness
	Indole	Red colored ring in surface	Yellow colored ring in surface
	Ornithine	Retention of purple color	Change in color
SCA (Simmon’s Citrate agar) test		Blue color	No change in color of media (green color)
Urease Test		Pink or purple color	No change in color (light orange)
Oxidase Test		Blue color of colony (avoid blue color after 10 seconds)	No color change of colony
KIA	H_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 alkali and A indicates acid. It can be K/A, A/K, K/K or even A/A for slant/butt.

4.1. Cell counting and serial dilutions.

4.2. 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 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.

4.3. Materials Required:

1. Tubes
2. Micropipette with tips
3. Distilled water
4. Bacteria sample
5. Nutrient agar
6. Petri dishes
7. Water bath
8. Alcohol
9. Colony counter
10. Conical Flask
11. Labeling Tape

4.4. 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.

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

Table 3.3 Dilution Factor

Plate	**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}

4.6. 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.

4.5. 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 04

RESULT

Result

Table 4.1. Bacteriological Colony Morphology

Name of University	Sample	Plates				
		MacConkey	TBX	BGA	XLD	TCBS
East West University (EWU)	Cake-1	Mucoid Pink	No growth	No growth	No growth	No growth
	Cake-2	No growth	No growth	No growth	No growth	Yellow
	Jilapi	Flat Pink	No growth	No growth	No growth	No growth
Govt. Titumir College	Danish	Pink	Blue	No growth	No growth	No growth
	Pakora-2	Pink	No growth	No growth	No growth	Yellow
	Patishapta Pitha	No growth	No growth	No growth	No growth	No growth
Popular Medical College	Biscuit-1	Pink	No growth	No growth	No growth	No growth
	Tehari-1	Pink	No growth	No growth	No growth	No growth
	Alur Chop	Colorless	No growth	No growth	No growth	No growth
Green University	Double Layer cake	Pink	No growth	No growth	No growth	No growth
	Biscuit-2	No growth	No growth	No growth	No growth	Yellow
	Samucha-4	Colorless	No growth	No growth	No growth	No growth
Bangladesh University Of professionals (BUP)	Amra Makha	Mucoid Pink	No growth	No growth	No growth	No growth
	Amra Makha	No growth	Blue	No growth	No growth	No growth
	Badam Makha	Flat pink	No growth	No growth	No growth	No growth

Table 4.1 shows bacterial colony morphology isolated from different street vended food samples. An estimate of 15 food samples was collected from the area around five different private universities in Dhaka city. In total 14 samples show growth of different pathogenic or non pathogenic microorganisms. Of which 10 samples show positive growth of our suspected organisms (*E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp.)* and 1 samples show 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 4.2: Bacterial colony morphology isolated from different street vended food

Name of University	Sample	Plates				
		MacConkey	TBX	BGA	XLD	TCBS
University of Asia Pacific (UAP)	Samucha-6	Pink	No growth	No growth	No growth	No growth
	Kalojam-2	No growth	No growth	No growth	No growth	No growth
	Vegetable roll	No growth	No growth	No growth	No growth	Yellow
Bangladesh Islamic University(BIU)	Chom chom	Mucoid pink	No growth	No growth	No growth	No growth
	Samucha-3	Colorless	No growth	No growth	No growth	No growth
	Chicken Ball	Flat pink	No growth	No growth	No growth	No growth
United International University(UIU)	Roll	No growth	No growth	No growth	No growth	Yellow
	Chola-2	Pink	No growth	No growth	No growth	Yellow
	Laddu -5	Mucoid pink	No growth	No growth	No growth	No growth
Ahsanullah Univerxity of Science and Technology(AUST	Dim Chop-6	Colorless	Blue	No growth	No growth	No growth
	Samucha-5	Pink	No growth	No growth	No growth	No growth
	Tikia	Pink	No growth	No growth	No growth	No growth
Primeasia University (PAU)	Chola-3	Colorless	No growth	No growth	No growth	Yellow
	Cup cake	No growth	No growth	No growth	No growth	No growth
	Misty Bar	Mucoid pink	No growth	No growth	No growth	Yellow

Table 4.2 shows bacterial colony morphology isolated from different street vended food samples.15 food samples were collected from the area around five different private universities in Dhaka city. In total 13 samples show growth of different pathogenic or non pathogenic microorganisms. Of which none of the samples show positive growth of our suspected organisms (*E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp.*) and 2 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 4.3: Number of food samples with growth of suspected organisms determined by colony morphology (n=30)

Name of University	No. of samples with +ve growth by E.coli	No. of samples with +ve growth by Klebsiella	No. of samples with +ve growth by Vibrio	No. of samples with +ve growth by Shigella	No. of samples with +ve growth by Salmonella
EWU	1	0	2	1	0
SDC	2	1	1	0	0
SU	2	0	1	0	0
GU	1	0	1	0	0
AUST	1	2	2	0	0
UAP	1	0	0	0	0
BIU	0	0	0	0	0
UIU	1	1	2	0	0
BU	2	0	0	0	0
PAU	3	0	1	0	0

Among 30 samples were collected from street vendors in the area around 10 private universities of Dhaka city. About 18 (60%) food samples were contaminated with pathogenic or non pathogenic microorganisms (Table 5.1 and Table 5.2). Of which 9 (30%) samples were suspected to be contaminated with our targeted organisms (*E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp.).*

Table 4.3 shows the number of food samples contaminated with the targeted organisms. In total 16 samples were suspected to be contaminated with either *E.coli* or *Klebsiella spp.*, 7 samples were suspected to be contaminated with *Vibio spp.* And 1 sample was suspected to be contaminated with *Shigella spp.*

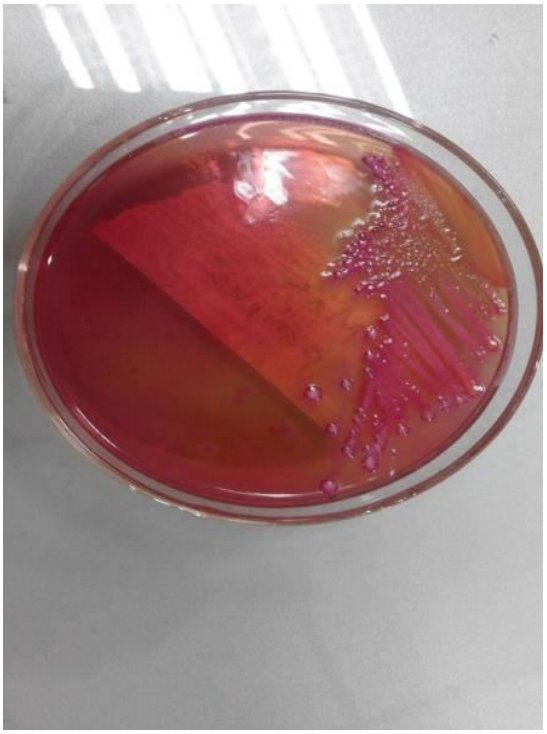

Figure 4.1: Bacterial colony (pink) on MacConkey agar plate

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

4.2 Suspected Organisms from Biochemical Tests

Table 4.4: Identification of the suspected organism from different biochemical test

Sample	Plates	Colony Morphology	M	I	O	Citrate	Urease	Oxidase	KIA			Suspected organism
									Slunt/ Butt	H_2S	Gas	
Cake-1	MacConkey	Mucoid Pink	-	+	-	+	-	-	K/A	-	+	*Klebsiella pneumoniae*
Cake-2	TCBS	Yellow	+	+	-	+	-	-	K/A	-	+	*Vibrio spp*
Pakora-2	MacConkey	Pink	+	+	-	-	-	-	A/A	-	+	*E.coli*
Biscuit-1	MacConkey	Pink	+	+	-	+	-	-	A/A	-	+	*E.coli*
Tehari-1	MacConkey	Pink	-	+	-	+	-	-	K/A	-	+	*Klebsiella pneumoniae*
Double Layer Cake	MacConkey	Pink	+	+	-	-	-	-	A/A	-	+	*E. coli*
Buscuit-2	TCBS	Yellow	+	+	-	+	-	-	K/A	-	+	*Vibrio spp*
Amra Makha	TBX	Blue	+	+	-	+	-	-	A/A	-	+	*E.coli*
	MacConkey	Mucoid Pink	-	+	-	+	-	-	A/A	-	+	*Klebsilla pneumoniae*
Badam Makha	MacConkey	Flat Pink	-	+	-	+	-	-	K/A	-	+	*Klebsiella pneumoniae*

Among 30 samples were collected from street vendors in the area around 10 private universities of Dhaka city. About 28 (93%) food samples were contaminated with pathogenic or non pathogenic microorganisms. Of which 9 (30%) samples were suspected to be contaminated with our targeted organisms (*E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp.).*

Table 4.4 shows identification of the suspected organism (*Klebsiella spp., Vibrio, E. coli*) from different biochemical test. In total 9 (30%) food samples were identified to be

contaminated with our suspected organism (*Klebsiella spp., Vibrio, E. coli*) from these biochemical tests.

Table 4.5: Presence of suspected organisms in no of food samples from different University (n=30)

Name of University	*E.coli*	*Klebsiella spp.*	*Vibrio spp.*	*Shigella spp.*	*Salmonella spp.*
EWU	0	1	1	0	0
GTC	1	0	0	0	0
PMC	1	1	0	0	0
GU	1	0	1	0	0
BUP	1	2	0	0	0

Table 4.5 shows presence of suspected organisms in number of food samples from different university. In total 9(30%) food samples from different university were suspected to be contaminated with our targeted organisms *E. coli, Klebsiella* spp, *Vibrio* spp and except *Shigella* spp, and *Salmonella* spp.

In EWU, 2 food samples were suspected to be contaminated with *Vibrio* spp and *Klebsiella* spp. In GTC, 1 food sample was suspected to be contaminated with *E. coli*. In PMC, 2 food samples were suspected to be contaminated with *Klebsiella* spp and *E. coli*. In GU, 2 food samples were suspected to be contaminated with *E, coli* and *Vibrio* spp. In BUP, 2 food samples were suspected to be contaminated with *E. coli* and *Klebsiella* spp.

Table 4.6: Incidence of food borne pathogens in various street vended food samples

Pathogen	Food Categories					
	Deep fried and fried items (n=16)	Rice items (n=1)	Noodles (n=0)	Baked items (n=9)	Others (n=4)	Total (n=30)
E.coli	1 (6%)	Nd	Nd	2 (22%)	1 (25%)	4 (13%)
Klebsiella spp.	Nd	1 (100%)	Nd	1 (11%)	2 (50%)	4 (13%)
Vibrio spp.	Nd	Nd	Nd	2 (22%)	Nd	2 (7%)
Shigella spp.	Nd	Nd	Nd	Nd	Nd	Nd
Salmonella spp.	Nd	Nd	Nd	Nd	Nd	Nd

Table 4.6 shows the incidence of food borne pathogens in various street vended food samples. Among 30 samples divided into catefories, 4 (13%) samples were suspected to contain *E. coli,* 4 (13%) samples were suspected to contain *Klebsiella pneumonia* and 2 (7%) samples were suspected to contain *Vibrio* spp.

4.7. Table: Colony counting of various samples

Sample Name	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Laddu	Uncountable	Uncountable	34	10
Tika	Uncountable	Uncountable	28	6
Plain Cake	39	24	8	6
Misty Bar	48	20	6	0
Alur Chop	Uncountable	Uncountable	Uncountable	62
Dim chop	Uncountable	Uncountable	43	24

For Laddu, 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:

34 colonies on plate 3 x dilution factor of 1000 = 34000 cells/ml.

For Tika, 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:

28 colonies on plate 3 x dilution factor of 1000 = 28000 cells/ml.

For Plain cake, plate 1 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:

39 colonies on plate 1 x dilution factor of 10 = 390 cells/ml.

For Misty Bar, plate 1 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:

48 colonies on plate 1 x dilution factor of 10 = 480 cells/ml.

For Alur chop, 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:

62 colonies on plate 4 x dilution factor of 10,000 = 620,000 cells/ml.

For Dim Chop, 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:

43 colonies on plate 3 x dilution factor of 1000 = 43000 cells/ml.

4.7.1. Table: Number of colonies per ml of sample

Sample Name	Number of microorganism (cells/ml)
Laddu	34,000
Tika	28000
Plain Cake	390
Misty Bar	480
Alur Chop	620,000
Dim chop	43,000

CHAPTER 05

DICUSSION

5. Discussion

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

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

A study was conducted 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 India, a study was conducted on some raw street vended Indian foods. *Staphylococcus aureus* were detected in 91 (60%) samples of coriander sauce, 87 (58%) samples of coconut slices and 129 (86%) samples of ready-to-eat salads. Twenty-three (15%) samples of coconut slices contained *Shigella* (18 *Sh. dysenteraie* type 1 and 5 *Sh. Flexneri* 2a), 13 (8%) samples of ready-to-eat salads and 10 (6%) samples of coriander sauce contained *Sh. flexneri* 2a. Spread of bacteria may have been facilitated by lack of access to potable water, toilet facilities and operated under poor hygiene conditions (Ghosh et al., 2007).

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, Klebsiella spp., Vibio spp.* and *Shigella spp* were found from 9 (30%) samples. From the biochemical test results of the colonies of MacConkey, XLD and TCBS agar media, 4 (10%) food samples were suspected to be contaminated with *E.coli*, 4 (27%) food samples were suspected to be contaminated with *Klebsiella spp.,* 1 (3%) food sample was suspected to be contaminated with *Vibrio spp.* from different university. No *Salmonella spp*. and *Shigella spp* were found from any food sample. Seven biochemical tests were performed for characterizing the organisms but PCR test was not done. Therefore it cannot be said confidently that colonies of the media plates are the claimed ones. In this research, due to limitations of facilities other tests were not performed.

Our study also shows the incidence of food borne pathogens in various street vended food samples divided into their category. Among 30 samples divided into categories, 4 (13%) samples were suspected to contain *E. coli,* 4 (13%) samples were suspected to contain *Klebsiella pneumonia* and 2 (7%) samples were suspected to contain *Vibrio* spp.

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

CHAPTER 05

CONCLUSION

5.2 Conclusion

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

CHAPTER 06

REFERENCE

References

Adams, M. R. & Moss, M. O. (2008) *Food Microbiology: Bacterial Agents of Food Borne Illness*. 3rd ed. Cambridge, UK: The Royal Society of Chemistry. p. 182-268.

Bennington-Castro. (2016) *What Is Klebsiella Pneumoniae?*. [Online] Available at: http://www.everydayhealth.com/klebsiella-pneumoniae/guide [Accessed 20th June 2016]

Bereda, T., Emerie, Y., Reta, M. & Asfaw, H. (2016) Microbiological Safety of Street Vended Foods in Jigjiga City, Eastern Ethiopia. *Ethiopa Journal of Health Science*. 26 (2). p. 161.

Cardinale, E., Gros-Claude, J. P., Tall, F., Guèye, E. & Salvat, G. (2005) Risk factors for contamination of ready-to-eat street-vended poultry dishes in Dakar, Senegal. *International Journal of Food Microbiology*. 103 (2). p. 157-165.

Centre for Disease Control. (2016) *CDC - Klebsiella pneumoniae in Healthcare Settings - HAI*. [Online] Available at: http://www.cdc.gov/HAI/organisms/klebsiella/klebsiella.html. [Accessed 19th June 2016]

Christison, C., Lindsay, D. & Holy, A. (2008) Microbiological survey of ready-to-eat foods and associated preparation surfaces in retail delicatessens, Johannesburg, South Africa. *Food Control*. 19 (7). p. 727-733.

Food and Drug Administration. (2016) *Vibrio vulnificus Health Education Kit Fact Sheet*. [Online] Available at: http://www.fda.gov/Food/ResourcesForYou/HealthEducators/ucm085365.htm. [Accessed 20th June 2016]

Foodsafety.gov. (2016) *Vibrio Infections | FoodSafety.gov*. [Online] Available at: http://www.foodsafety.gov/poisoning/causes/bacteriaviruses/vibrio_infections/. [Accessed 19th June 2016]

Ghosh, M., Wahi, S., Kumar, M. & Ganguli, A. (2007) Prevalence of enterotoxigenic

Staphylococcus aureus and *Shigella spp*. in some raw street vended Indian foods.

International Journal of Environmental Health Research. 17 (2). p. 151.

Gould, D., Kraa, E., Dalton, C., Givney, R., Gregory, J., Stafford, R. & Kirk, M. (2016) Food borne disease outbreaks in Australia, 1995 to 2000. *Communicable Diseases Intelligence Quarterly Report*. 28 (2). p. 211.

Islam, S., Nasrin, N., Rizwan, F., Nahar, L., Bhowmik, A., Esha, S. A., Talukder, K. A., Akter, M., Roy, A. & Ahmed, M. (2015) Microbial contamination of street vended foods from a university campus in Bangladesh. *Southeast Asian Journal Tropical Medicine and Public health*. 46(3). p. 1-6.

Jay, J., Loessner, M. & Golden, D. (2005) *Modern food microbiology*. 7th ed. New York: Springer. Käferstein, F. (2003) Food borne diseases in developing countries: etiology, epidemiology and strategies for prevention. *International Journal of Environmental Health Research.* 13 (1). p. 161-168.

Kirk, M., Pires, S., Black, R., Caipo, M., Crump, J., Devleesschauwer, B., Döpfer, D., Fazil, A., Fischer-Walker, C., Hald, T., Hall, A., Keddy, K., Lake, R., Lanata, C., Torgerson, P., Havelaar, A. & Angulo, F. (2015) World Health Organization Estimates of the Global and Regional Disease Burden of 22 Food borne Bacterial, Protozoal, and Viral Diseases, 2010: A Data Synthesis. *PLoS Med.* 12 (12). p. 21.

Mayoclinic.org. (2016) *Cholera Symptoms - Mayo Clinic*. [Online] Available at: http://www.mayoclinic.org/diseases-conditions/cholera/basics/symptoms/con-20031469. [Accessed 19th June 2016]

Mayoclinic.org. (2016) *Cholera Causes - Mayo Clinic*. [Online] Available at: http://www.mayoclinic.org/diseases-conditions/cholera/basics/causes/con-20031469. [Accessed 19th June 2016]

Mayoclinic.org. (2016) *E. coli Causes - Mayo Clinic*. [Online] Available at: http://www.mayoclinic.org/diseases-conditions/e-coli/basics/causes/con-20032105. [Accessed 19th June 2016]

Niddk.nih.gov. (2016) *Foodborne Illnesses*. [Online] Available at: http://www.niddk.nih.gov/health-information/health-topics/digestive-diseases/foodborne-illnesses/Pages/facts.aspx. [Accessed 19th June 2016]

Ohiokpehai, O. (2003) Nutritional Aspects of Street Foods in Botswana. *Pakistan Journal of Nutrition*. 2 (2). p. 76-81.

Omemu, A. & Aderoju, S. (2008) Food safety knowledge and practices of street food vendors in the city of Abeokuta, Nigeria. *Food Control.* 19 (4). p. 396-402.

Oranusi, S. U. & Braide, W. (2012) A study of microbial safety of ready-to-eat foods vended on highways: Onitsha-Owerri, south east Nigeria. *International Research Journal of Microbiology*. 3 (2). p. 66.

Puspanadan, S., Afsah-Hejri, L., Loo, Y. Y., Nillian, E., Kuan, C. H., Goh, S. G., Chang, W. S., Lye, Y. L., John, Y. H. T., Rukayadi, Y., Yoshitsugu, N., Nishibuchi, M. & Son, R. (2012) Detection of Klebsiella pneumoniae in raw vegetables using Most Probable Number-Polymerase Chain Reaction (MPN-PCR). *International Food Research Journal.*19 (4). p. 1757-1762.

Rahman, M. M., Rahman, M. H. & Ansary, N. P. (2014) Safety Issues of Street Foods in Bangladesh. *Time Journals of Biological Sciences and Technology*. 2. p. 21-32.

Rane, S. (2011) Street Vended Food in Developing World: Hazard Analyses. *Indian Journal of Microbiology*. 51 (1). p. 100-106.

Scallan, E., Griffin, P., Angulo, F., Tauxe, R. & Hoekstra, R. (2011) Foodborne Illness Acquired in the United States—Unspecified Agents. *Emerg. Infect. Dis.* 17 (1). p. 16-22.

Sharma, I. & Mazumdar, J. (2014) Assessment of bacteriological quality of ready to eat food vended in streets of Silchar city, Assam, India. *Indian Journal of Medical Microbiology*. 32 (2). p. 169.

Tambekar, D., Kulkarni, R., Shirsat, S. & Bhadange, D. (2011) Bacteriological Qual Ity Of Street Vended Food Panipuri: A Case Study Of Amravati City (Ms) India. *Bioscience Discovery*. 2 (3). p. 350.

WHO. (2016) *Salmonella (non-typhoidal)*. [Online] Available at: http://www.who.int/mediacentre/factsheets/fs139/en/. [Accessed 19th June 2016]

Won-Chang, L., Myeong-Jin, L., Jin-Suk, K. & Soon-Young, P. (2001) Foodborne Illness Outbreaks in Korea and Japan Studied Retrospectively. *Journal of Food Protection*. 6 (4). p. 899-902.