Study on Bacteriological Quality of Street-vended Foods Collected from Different Private Universities in Dhaka City, Bangladesh

A Dissertation Submitted to

East West University, Dhaka, Bangladesh

In the partial fulfillment of the requirements for the Degree of

Bachelor of Pharmacy

Submitted by

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Under the Guidance of

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June, 2016

Declaration by the Research Candidate

I, Nahida Akter, ID: 2012-1-70-023, hereby declare that the dissertation entitled— "Study on bacteriological quality of street-vended foods collected from different private universities in Dhaka City, Bangladesh" submitted by me, has been carried out under the joint supervision and guidance of Dr. Sufia Islam, Associate Professor and Nafisa Tanjia, lecturer, to the Department of Pharmacy, East West University in partial fulfillment of the requirement for the award of the degree of Bachelor of Pharmacy. It is further declared that the research work presented here is original, has not been submitted anywhere else for any degree or diploma.

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Certificate by the Supervisor

This is to certify that the thesis entitled "Study on bacteriological quality of street-vended foods collected from different private universities 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 is a bona fide record of original and genuine research work carried out by Nahida Akter, ID: 2012-1-70-023 in 2016 of her research in the Department of Pharmacy, East West University, under the supervision and guidance of me.

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Acknowledgement

At first, I am grateful to ALLAH for the good health and well-being that were necessary to complete this research. I would like to express my deepest gratitude to my research supervisor, Dr. Sufia Islam, Associate Professor, and co-supervisor Nafisa Tanjia, Lecturer Department of Pharmacy, East West University, who had been always optimistic and full of passion and ideas. Her 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. Her 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 be a part of this institution. To me it seems like second home. This institution gave 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. I am thankful to Ajoy Roy, Lab instructor, for his co-operation.

Thirdly, I want to give special thanks to Mir Shafaly, Shampa Mutsuddy, Zahid Hasan and all of my friends, who gave me support for my research work and for being very co-operative in my work. Because of their tremendous support I could finish the work on time.

Finally, I am immensely grateful to my beloved parents, Md. Kamrul Huda and Nasima 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 genuine love to my other family members for their continuous support and love. I am fortunate to have such a nice family!

Dedication

To
My Beloved
Parents & Research
Supervisors

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List of Abbreviations

RTE	Ready to Eat
APW	Alkaline Peptone Water
BPW	Buffered Peptone Water
TBX	Tryptone Bile X-glucoronide
TCBS	Thiosulfate Citrate Bile Salt-sucrose
BGA	Brilliant Green Agar
XLD	Xylose-Lysine Desoxycholate agar
YE	Yeast Extract
ETEC	*Enterotoxigenic E. coli*.
EIEC	*Enteroinvasive E. coli*
EPEC	*Enteropathogenic E. coli*
EHEC	*Enterohaemorrhagic E. coli*
VTEC	Verotoxin producing *E. coli*
CDC	Centers for Disease Control and Prevention
MIO	Motility Indole Ornithine
KIA	Kliglar's Iron Agar

Abstract

Street-vended foods are readily available sources of meals for many people around the world, but the microbial safety of such food is always doubtful. In developing countries the major sources of food-borne illnesses are street-vended foods. The present research work was therefore untaken to find out the presence of enteric bacteria specially *E. coli, Klebsiella, Salmonella, Shigella* and *Vibrio* species from different types of street-vended food items collected from different private universities of Dhaka city, Bangladesh. Five agar media MacConkey, Tryptone Bile X-glucoronide (TBX) agar, Thiosulfate Citrate Bile Salt-sucrose (TCBS) agar, Brilliant Green Agar (BGA) and Xylose-Lysine Desoxycholate agar (XLD) were used to observe the presence of our targeted microorganisms in food items. Seven biochemical tests were performed to indentify the targeted organisms. The tests are motility, indole, ornithine, citrate, urease, oxidase and KIA test. In this study, 30 different food samples were collected from 10 private universities. Among them, we found contamination in 28 (93.3%) samples. Of which, 22 (73.3%) samples were suspected to be contaminated with our targeted organisms (*E coli, Klebsiella, Shigella, Salmonella* and *Vibrio* species). In total 22 samples, 14 (46.7%) samples were suspected to be contaminated with *E coli*, 14 (46.7%) with *Klebsiella*, 14 (46.7%) with *Vibrio*, 1 (3.3%) with *Shigella* and 1(3.3%) with *Salmonella* species. From the results of biochemical test we got 13 of our suspected bacteria from 13 different samples. In total, we got 5 (38%) *Klebsiella*, 4 (31%) *Vibrio*, 3 (23%) *E. coli* and 1 (8%) *Shigella* species. This study indicated that the street vended foods of Dhaka city are highly contaminated with pathogenic bacteria which can contribute to potential health risks for consumers. Regular monitoring of the quality of street foods must be practiced to avoid any food-borne illness in future. In addition, health education to improve the awareness of food vendors on food safety and hygiene practices is essential.

Keywords: Street-vended foods, Microbial safety, Agar media, Biochemical test, Contamination, Dhaka city.

Chapter 1

Introduction and Literature Review

1.1 Street-vended Foods

Street-vended foods are foods from street vendors which are ready-to-eat food (RTE) or drink prepared on the streets or at home and also sold in street or other public place, such as school, college, universities, market or fair, often from a portable food booth or food cart and are consumed on the streets without further preparation (Tambekar et al., 2011).

Figure 1.1 Street-vended Food

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, chhola, 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).

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).

Although there is a growing demand for RTE food products, no recent information is available regarding the microbiological quality of these products in Dhaka city, Bangladesh. The present study was hence undertaken to determine the microbiological quality and safety of a variety of street-vended RTE food products collected from several typical vendors surrounding different top private universities of Dhaka city.

1.2 Microbiology of Food

In the early 20th century, studies continued to understand the association and importance of microorganisms, especially pathogenic bacteria in food. Specific methods were developed for their isolation and identification. The importance of sanitation in the handling of food to reduce contamination by microorganisms was recognized. Specific methods were studied to prevent growth as well as to destroy the spoilage and pathogenic bacteria. There was also some interest to isolate beneficial bacteria associated with food fermentation, especially dairy fermentation, and study their characteristics. However, after the 1950s, food microbiology entered a new era.

Availability of basic information on the physiological, biochemical, and biological characteristics of diverse types of food, microbial interactions in food environments and microbial physiology, biochemistry, genetics, and immunology has helped open new frontiers in food microbiology (Ray, 2004).

1.2.1 Factors Affecting Growth of Microorganisms

Intrinsic Parameters

These parameters are as follows:

- ✓ pH
- ✓ Moisture content
- ✓ Oxidation-reduction potential (Eh)
- ✓ Nutrient content (water, source of energy, source of nitrogen, vitamins and related growth factors, minerals)
- ✓ Antimicrobial constituents
- ✓ Biological structures

Extrinsic Parameters

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

The food processor reduces potential problems from microorganisms in several ways:

- ✓ Removing or destroying them by trimming, washing, heating, pickling, by adding chemicals, or by encouraging competition by acid- or alcohol-forming organisms.
- ✓ Minimizing contamination from equipment, people, the environment, and from unprocessed food.
- ✓ Minimizing microbial growth on equipment, by cleaning and sanitizing, and in the product itself by adjusting storage temperature, pH, and other environmental factors (Ray, 2004).

1.2.2 Personal Hygiene of the Vendors or Food Handlers

According to WHO, 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. A study in Santa Fe de Bogota, Colombia revealed that over 30% of a group of food handlers examined were carriers of pathogenic microorganisms including Salmonella typhi, *Staphylococcus aureus*, *Salmonella enteritidis* and *Shigella* (Rane, 2011).

1.3 Food-borne Illness

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

The US food supply is among the safest in the world, but organisms that we can't see, smell, or taste – bacteria, viruses, and tiny parasites – are everywhere in the environment. The Centers for Disease Control and Prevention (CDC) estimates that 48 million food-borne illness cases occur in the United States every year. At least 128,000 Americans are hospitalized, and 3,000 die after eating contaminated food each year. Food-borne illness costs Americans billions of dollars each year, and serves as a constant challenge for consumers, researchers, government and industry (CDC, 2015).

1.3.1 Types of Microbial Food-borne Diseases

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

- **Intoxication**

Illness occurs as a consequence of ingesting a preformed bacterial or mold toxin because of its growth in a food. A toxin has to be present in the contaminated food. Once the microorganisms have grown and produced toxin in a food, there is no need of viable cells during consumption of the food for illness to occur. Staph food poisoning is an example.

- **Infection**

Illness occurs as a result of the consumption of food and water contaminated with enteropathogenic bacteria or viruses. It is necessary that the cells of enteropathogenic bacteria and viruses remain alive in the food or water during consumption. Viable cells, even if present in small numbers, have the potential to establish and multiply in the digestive tract to cause the illness. Salmonellosis and hepatitis A are examples.

- **Toxicoinfection**

Illness occurs from ingesting a large number of viable cells of some pathogenic bacteria through contaminated food and water. Generally, the bacterial cells either sporulate or die and release toxins to produce the symptoms. *Clo perfringens* gastroenteritis is an example.

In addition to the pathogenic microorganisms associated with foodborne illnesses, some bacterial species and strains normally considered nonpathogenic can cause gastroenteritis, especially in susceptible individuals. They are designated as opportunistic pathogens.

They are normally required to be alive and present in large numbers when consumed through a contaminated food (Ray, 2004).

1.4 Bacterial Agents of Food-borne Illness

1.4.1 *Salmonella* species

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

1.4.1.1 Microbiological Characteristics

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

1.4.1.2 Pathogenesis and Clinical Features

Generalized systemic enteric fever, headache, malaise, anorexia, enlarged spleen, and constipation followed by more severe abdominal symptoms; rose spots on trunk in 25% of Caucasian patients; complications include ulceration of Peyer's patches in ileum, can produce hemorrhage or perforation; Common enterocolitis may result without enteric fever; characterized by headache, abdominal pain, nausea, vomiting, diarrhea, dehydration may result; case fatality of 16% reduced to 1% with antibiotic therapy (Adams & Moss, 2008).

1.4.1.3 Association with Foods

Salmonellosis is described as a zoonotic infection since the major source of human illness is infected animals. Transmission is by the faecal–oral route whereby intestinal contents from an infected animal are ingested with food or water. Meat, milk, poultry, and eggs are primary vehicles; they may be undercooked, allowing the salmonellas to survive, or they may cross-contaminate other foods that are consumed without further cooking. Cross-

contamination can occur through direct contact or indirectly via contaminated kitchen equipment and utensils. Human carriers are generally less important than animals in the transmission of salmonellosis. Human transmission can occur if the faecally contaminated hands of an infected food handler touch a food which is then consumed without adequate cooking, often after an intervening period in which microbial growth occurs (Adams & Moss, 2008).

1.4.2 *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).

1.4.2.1 Characteristics

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

1.4.2.2 Pathogenesis and Clinical Features

Shigellas cause bacillary dysentery in humans and other higher primates. Studies with human volunteers have indicated that the infectious dose is low; of the order of 10–100 organisms. The incubation period can vary between 7 h and 7 days although food-borne outbreaks are commonly characterized by shorter incubation periods of up to 36 h. Symptoms are of abdominal pain, vomiting and fever accompanying a diarrhoea which can range from a classic dysenteric syndrome of bloody stools containing mucus and pus, in the cases of *Sh. dysenteriae, Sh. flexneri* and *Sh. boydii,* to a watery diarrhoea with *Sh.*

sonnei. Illness lasts from 3 days up to 14 days in some cases and a carrier state may develop which can persist for several months. Milder forms of the illness are self-limiting and require no treatment but *Sh. dysenteriae* infections often require fluid and electrolyte replacement and antibiotic therapy. Shigellosis is an invasive infection where the organism's invasive property is encoded on a large plasmid (Adams & Moss, 2008).

1.4.2.3 Isolation and Identification

Lack of interest in *Shigella* as a food-borne pathogen has meant that laboratory protocols for its isolation and identification from foods are relatively underdeveloped. A pre-enrichment procedure has been described based on resuscitation on a non-selective agar before overlaying with selective media. Selective enrichment in both Gram-negative broth and selenite broth has been recommended. Selective plating media used are generally those employed for enumerating the Enterobacteriaceae or Salmonella although neither are entirely satisfactory. Rapid techniques for identification based on immunoassays which detect the virulence marker antigen, and on the polymerase chain reaction to detect the virulence plasmid by DNA/DNA hybridization have also been applied (Adams & Moss, 2008).

1.4.2.4 Association with Foods

Food-borne cases of shigellosis are regarded as uncommon though some consider the problem to be greatly underestimated. The limited range of hosts for the organism certainly suggests that it is relatively insignificant as a food-borne problem when compared with say *Salmonella.* In food-borne cases, the source of the organism is normally a human carrier involved in preparation of the food. In areas where sewage disposal is inadequate the organism could be transferred from human faeces by flies (Adams & Moss, 2008).

1.4.3 *Vibrio* Species

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

1.4.3.1 Characteristics

Vibrios are Gram-negative pleomorphic (curved or straight), short rods which are motile with (normally) sheathed, polar flagella. Catalase and Oxidase-positive cells are facultatively anaerobic and capable of both fermentative and respiratory metabolism.

Sodium chloride stimulates the growth of all species and is an obligate requirement for some. The optimum level for the growth of clinically important species is 1–3%.

1.4.3.2 Pathogenesis and Clinical Features

Cholera usually has an incubation period of between one and three days and can vary from mild, self-limiting diarrhoea to a severe, life threatening disorder. The infectious dose in normal healthy individuals is large when the organism is ingested without food or buffer, of the order of 1010 cells, but is considerably reduced if consumed with food which protects the bacteria from stomach acidity. Studies conducted in Bangladesh indicate that 103–104 cells may be a more typical infectious dose. Individuals with low stomach acidity (hypochlorohydric) are more liable to catch cholera. In severe cases, the hyper-secretion of sodium, potassium, chloride, and bicarbonate induced by the enterotoxin results in a profuse, pale, watery diarrhoea containing flakes of mucus, described as rice water stools. Unless the massive losses of fluid and electrolyte are replaced, there is a fall in blood volume and pressure, an increase in blood viscosity, renal failure, and circulatory collapse. In fatal cases death occurs within a few days. In untreated outbreaks the death rate is about 30–50% but can be reduced to less than 1% with prompt treatment by intravenous or oral rehydration using an electrolyte/glucose solution (Adams & Moss, 2008).

1.4.3.3 Isolation and Identification

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

1.4.3.4 Association with Foods

Cholera is regarded primarily as a waterborne infection, though food which has been in contact with contaminated water can often serve as the vehicle. Consequently a large

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

1.4.4 *Escherichia coli*

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

1.4.4.1 Characteristics

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

1.4.4.2 Pathogenesis and Clinical Features

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 afebrile diarrhoea to a severe choleralike syndrome of watery stools without blood or mucus, stomach pains and vomiting. The illness is usually self-limiting, persisting for 2–3 days, although in

developing countries it is a common cause of infantile diarrhoea where it can cause serious dehydration.

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 precedes the passage of stools containing blood, mucus, and faecal leukocytes. The infective dose of EIEC appears to be substantially higher than for *Shigella* and this is thought to be a reflection of the organism's greater sensitivity to gastric acidity.

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.

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 foodborne 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).

1.4.4.3 Isolation and Identification

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

acid producers like *Escherichia*, *Klebsiella*, and *Enterobacter* produce pink colonies; non-lactose fermenters such as *Salmonella*, *Proteus*, and *Edwardsiella*, with rare exceptions produce colourless colonies (Adams & Moss, 2008).

1.4.4.4 Association with Foods

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

1.4.5 *Klebsiella pneumoniae*

Bacteria belonging to the genus *Klebsiella* frequently cause human nosocomial infections. In particular, the medically most important *Klebsiella* species, *Klebsiella pneumoniae*, accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections. The principal pathogenic reservoirs for transmission of *Klebsiella* are the gastrointestinal tract and the hands of hospital personnel. Because of their ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks (Podschun & Ullmann, 1998).

1.4.5.1 Characteristics

Klebsiella pneumoniae 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).

1.4.5.2 Pathogenesis and Clinical Features

Nosocomial *Klebsiella* infections most commonly involve the urinary and respiratory tracts. Since these two body sites differ considerably with respect to the host defense mechanisms, it should be expected that the pattern of virulence factors found in UTI-causing strains of *Klebsiella* will differ from that observed in strains isolated from pulmonary sources of patients with pneumonia.

Typical *Klebsiella pneumoniae* is an opportunistic pathogen, which mostly affects those with weakened immune systems and tends to cause nosocomial infections. A subset of hypervirulent *K. pneumoniae* serotypes with elevated production of capsule polysaccharide can affect previously healthy persons and cause life-threatening community-acquired infections, such as pyogenic liver abscess, meningitis, necrotizing fasciitis, endophthalmitis and severe pneumonia. *K. pneumoniae* utilizes a variety of virulence factors, especially capsule polysaccharide, lipopolysaccharide, fimbriae, outer membrane proteins and determinants for iron acquisition and nitrogen source utilization, for survival and immune evasion during infection (Li et al., 2014).

1.4.5.3 Isolation and Identification

Klebsiella species are usually identified and differentiated according to their biochemical reactions. The genus is defined as containing gram-negative, nonmotile, usually encapsulated rod-shaped bacteria of the family Enterobacteriaceae, which produce lysine decarboxylase but not ornithine decarboxylase and are generally positive in the Voges-Proskauer test (Podschun & Ullmann, 1998).

1.4.5.4 Association with Foods

Klebsiella pneumoniae (K. pneumoniae) is one of the most important members of *Klebsiella* genus in Enterobacteriacae family, which is responsible for pneumonia (the destructive lung inflammation disease). Vegetables are known as source of contamination with K. pneumonia. Raw vegetables are usually consumed in salads and other dishes (Puspanadan et al., 2012).

1.5 Occurrence of Food-borne Illness in Different Countries of 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).

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

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

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

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

handling of food. The implicated foods included seafood, meat products and cereal products (Chang & Chen, 2003).

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

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

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

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

Chapter 2

Objective of the Study

2.1 Research objective

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

- To find out the presence of enteric bacteria specially *E. coli*, *Klebsiella*, *Salmonella*, *Shigella* and *Vibrio* species from different types of street-vended food items collected from different private universities of Dhaka city, Bangladesh.

Chapter 3

Methods and Materials

3.1 Study Area

10 private universities of Dhaka city which are North South University (NSU), BRAC University, East West University (EWU), South East University (SEU), United International University (UIU), Independent University Bangladesh (IUB), University of Asia Pacific (UAP), University of Liberal Arts Bangladesh (ULAB), Stamford University (SU) and American International University-Bangladesh (AIUB).

3.2 Study Duration

This study was carried out over a period of 7 months from September 2015 to March 2016.

3.3 Bacteriological Subculture

3.3.1 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 aseptically in different sealed poly bags to prevent their contact with any other source that can contaminate the samples.

3.3.1.1 Category of Foods

Deep Fried and Fried Items (n=13)	**Spicy Preparations (n=4)**	**Baked Items (n=7)**	**Sweet Items (n=4)**	**Others (n=2)**
Singara, aluchop, egg chop pakora, nargis kabab, shik kabab, kathi kabab,	Pani fuchka, chhola	Cake, Danish, biscuit, nimkey	Laddu goja	Noodles & vegetable roll

3.3.2 Sample Processing

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

3.3.3 Enrichment of the Organisms

3.3.3.1 Enrichment of *Salmonella* and *Shigella* Species

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

3.3.3.2 Enrichment of *E. coli* and *Klebsiella* Species

5 gm solid sample 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.

Figure 3.1: Enrichment for targeted organisms

3.3.3.3 Enrichment of *Vibrio* Species

5 gm solid sample 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.

3.3.4 Selective Growth of the Organisms

3.3.4.1 Selective Growth of *Salmonella* and *Shigella* Species

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

3.3.4.2 Selective Growth *E.coli* and *Klebsiella* Species

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

3.3.4.3 Selective Growth of *Vibrio* Species

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

3.3.5 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

3.3.6 Preparation of Petri dishes

The different types of prepared Agar solution were poured into each of the three 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.

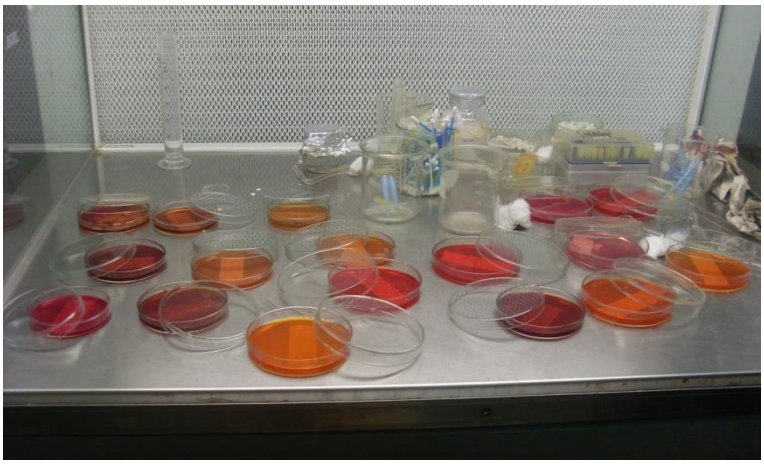

Figure 3.4: Petri dishes preparation

3.3.7 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

3.3.8 Standard Colony Morphology of Suspected Organism in Different Media

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

Table 3.1: Standard Colony Morphology of Suspected Organism

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
	MacConkey	Smooth non-lactose fermenting transparent colony
Klebsiella	MacConkey	Pink colonies

3.3.9 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

3.4 Biochemical Tests

3.4.1 Kliglar Iron Agar Test (KIA Test)

3.4.1.1 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.

3.4.1.2 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

3.4.2 MIO Test

3.4.2.1 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.

3.4.2.2 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

3.4.3 Citrate Test

3.4.3.1 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).

3.4.3.2 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

3.4.4 Urease Test

3.4.4.1 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.

3.4.4.2 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

3.4.5 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.

3.4.5 Standard Biochemical Test results of Suspected Organism

Table 3.2: Biochemical Test Observation

Biochemical Test		**Observation After Incubation**	
		Positive	**Negative**
MIO	Motility	Turbidity or haziness	No turbidity or haziness
	Indole	Red colored ring in surface	Yellow colored ring in surface
	Ornithine	Retention of purple color	Change in color
SCA (Simmon's Citrate agar) test		Blue color	No change in color of media (green color)
Urease Test		Pink or purple color	No change in color (light orange)
Oxidase Test		Blue color of colony (avoid blue color after 10 seconds)	No color change of colony
Catalase		Rapid bubble formation	No bubble formation
KIA	H_2S	Black color	No Black color
	Gas production	Bubble production	No bubble in test tube

For KIA test, slant and butt portion of test tube is also observed for acid and alkali production to identify whether the organisms are lactose fermenting or not. K indicates acid and A indicates alkali. It can be K/A, A/K, K/K or even A/A for slant/butt.

3.4.6 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

Chapter 4

Result

4.1 Bacterial colony morphology

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

Name of University	Sample	Plates				
		MacConkey	**TBX**	**BGA**	**XLD**	**TCBS**
North South University (NSU)	Nimkey	Pink	Blue	White	Yellowish	Yellow
	Nargis kabab 1	No growth	White	No growth	Whitish	No growth
	Aluchop 1	No growth	White	No growth	No growth	Yellow
BRAC University	Danish 1	Flat pink dot	White	White	No growth	No growth
	Singara 1	No growth	White	No growth	Whitish	No growth
	Nargis kabab 2	No growth	No growth	Whitish	Yellow	Yellow
East West University (EWU)	Pakora	No growth	White	No growth	No growth	Yellow
	Chhola 1	No growth	No growth	No growth	No growth	No growth
	Danish 2	Oval pink	No growth	No growth	Yellow	No growth
South East University (SEU)	Kabab 1	Mucoid pink	White	White	No growth	No growth
	Alu chop 2	Colorless	Colorless	No growth	Yellow dot	Yellow
	Chhola 2	No growth	White	Yellowish	Yellow	No growth
United International University (UIU)	Egg chop 1	Pink	White	Whitish	Yellow	No growth
	Kabab 2	No growth	No growth	Yellow	Yellow	Yellow
	Cake 1	Colorless	No growth	No growth	No growth	Yellow

Table 4.1 (Bacterial colony morphology isolated from different street-vended food samples) shows bacterial colony morphology isolated from different street vended food samples. 15 food samples were collected from five different private universities in Dhaka city. In total 14 samples show growth of different pathogenic or non pathogenic microorganisms. Of which, 11 samples show positive growth of our suspected organisms (*E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp.)* and 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.

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

Name of University	Sample	Plates				
		MacConkey	**TBX**	**BGA**	**XLD**	**TCBS**
Independent University Bangladesh (IUB)	Egg chop 2	Flat pink	White	No growth	No growth	No growth
	Laddu 1	No growth	White	No growth	Yellow	No growth
	Pani fuchka 1	Colorless	No growth	Yellowish	Yellow	Dark green
University of Asia Pacific (UAP)	Noodles	No growth	No growth	No growth	No growth	Yellow
	Biscuit 1	No growth	No growth	No growth	No growth	No growth
	Cake 2	Pink	Bluish	Yellowish	Yellow	Yellow
University of Liberal Arts Bangladesh (ULAB)	Vegetable roll	Colorless	No growth	No growth	Yellow	No growth
	Kathi kabab	No growth	No growth	Whitish	Yellow	Yellow
	Laddu 2	No growth	White	Yellowish	No growth	No growth
Stamford University (SU)	Goja	Mucoid pink	White	No growth	No growth	No growth
	Pani fuchka 2	No growth	No growth	Yellowish	Yellow	No growth
	Biscuit 2	No growth	White	White	Yellow	Yellow & black
American International University-Bangladesh (AIUB)	Shik kabab 1	Pink	No growth	No growth	No growth	Yellow
	Shik kabab 2	No growth	White	White	Red flat	Yellow
	Laddu 3	Colorless	White	Yellowish	No growth	No growth

Table 4.2 (Bacterial colony morphology isolated from different street-vended food samples) shows bacterial colony morphology isolated from different street vended food samples.15 food samples were collected from five different private universities in Dhaka city. In total, 14 samples show growth of different pathogenic or non pathogenic microorganisms. Of which, 11 samples show positive growth of our suspected organisms (*E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp.*) and 1 sample shows no growth in these agar media. 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 Vibrios	No. of samples with +ve growth by Shigella	No. of samples with +ve growth by Salmonella
NSU	1	1	2	0	0
BRAC	1	1	1	0	0
EWU	1	1	1	0	0
SEU	2	2	1	0	0
UIU	2	2	2	0	0
IUB	2	2	1	0	0
UAP	1	1	2	0	0
ULAB	1	1	1	0	0
SU	1	1	1	0	0
AIUB	2	2	2	1	1

From total 30 food samples collected from street vendors, we found contamination in 28 (93.3%) samples (Table 5.1 and Table 5.2). Of which, 22 (73.3%) samples were suspected to be contaminated with our targeted organisms (*E coli, Klebsiella, Shigella, Salmonella* and *Vibrio* species).

In total 22 samples, 14 (46.7%) samples were suspected to be contaminated with *E coli*, 14 (46.7%) with *Klebsiella*, 14 (46.7%) with *Vibrio*, 1 (3.3%) with *Shigella* and 1(3.3%) with *Salmonella* species.

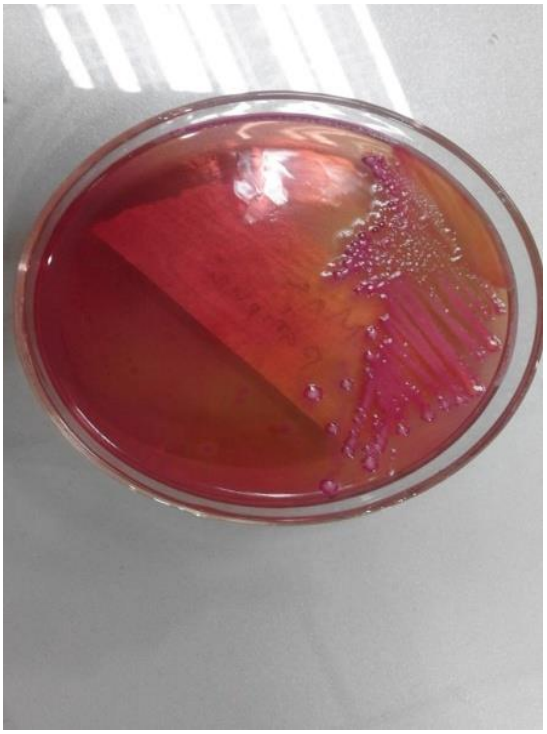

Figure 4.1: Bacterial colony (pink) on MacConkey agar plate

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

4.2 Suspected organism from different biochemical test

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

Samples	**Plates**	**Colony Morpho-logy**	**M**	**I**	**O**	**Ci-trate**	**Urease**	**Oxi-dase**	**KIA**		
									Slunt /butt	**gas**	**H_2s**
Nimkey	TBX	Blue	_	+	_	+	_	_	K/A	_	+
Egg chop 1	MacConky	Pink	_	+	_	+	_	_	K/A	_	+
Kabab 1	MacConky	Mucoid Pink	_	+	_	+	_	_	K/A	_	+
Goja	MacConky	Mucoid pink	_	+	_	+	_	_	K/A	_	+
Cake 2	MacConky	Pink	_	+	_	+	_	_	K/A	_	+

Biochemical test results for the samples nimkey, egg chop 1, kabab 1, goja and cake 2 matched with the standard results for *Klebsiella* species. So, we can say that the samples may contain the *Klebsiella* species.

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

Samples	**Plates**	**Colony Morphology**	**M**	**I**	**O**	**Citrate**	**Urease**	**Oxidase**	**KIA**		
									Slunt/ butt	**gas**	**H_2S**
Pakora	TCBS	Yellow	+	+	+	+	_	_	K/A	_	+
Nargis kabab 2	TCBS	Yellow	+	+	+	+	_	_	K/A	_	+
Shik kabab 1	TCBS	Yellow	+	+	+	+	_	_	K/A	_	+
Kathi kabab	TCBS	Yellow	+	+	+	+	_	_	K/A	_	+

Biochemical test results for the samples pakora, nargis kabab 2, shik kabab 1 and kathi kabab matched with the standard results for *Vibrio* species. So, we can say that the samples may contain the *Vibrio* species.

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

Samples	**Plates**	**Colony Morpho-logy**	**M**	**I**	**O**	**Citrate**	**Urease**	**Oxidase**	**KIA**			**Suspected Microorganisms**
									Slunt/ butt	gas	H_2S	
Danish 1	Mac Conky	Flat pink dot	+	+	_	_	_	_	A/A	_	+	E.Coli
Danish 2	Mac Conky	Oval pink	+	+	_	+	_	_	K/A	_	+	E.Coli
Egg chop 2	Mac Conky	Flat pink	+	+	_	+	_	_	K/A	_	+	E.Coli
Shik kabab 2	XLD	Red flat	_	+	_	+	_	_	K/A	_	+	Shigella

Biochemical test results for the samples Danish 1, Danish 2 and egg chop 2 matched with the standard results for *E. coli* species. So, we can say that the samples may contain the *E. coli* species. Test for shik kabab 2 matched with standard result for *Shigella* species. So, we can say that the samples may contain the *Shigella* species.

Table 4.7: Presence of suspected organisms in no of food samples (n=13)

Name of University	*E.coli*	*Klebsiella spp.*	*Vibrio spp.*	*Shigella spp.*	*Salmonella spp.*
NSU	0	1	0	0	0
BRAC	1	0	1	0	0
EWU	1	0	1	0	0
SEU	0	1	0	0	0
UIU	0	1	0	0	0
IUB	1	0	0	0	0
UAP	0	1	0	0	0
ULAB	0	0	1	0	0
SU	0	1	0	0	0
AIUB	0	0	1	1	0

From the results of biochemical test we got 13 of our suspected bacteria from 13 different samples. Among them, from NSU we got 1 *Klebsiella*, from BRAC 1 *E. coli* and 1 *Vibrio*, from SEU 1 *Klebsiella*, from UIU 1 *Klebsiella*, from IUB 1 *E. coli*, from UAP 1 *Klebsiella*, from ULAB 1 *Vibrio*, from SU 1 *Klebsiella* and from AIUB 1 *Vibrio* and 1 *Shigella* species. In total, we got 5 (38%) *Klebsiella*, 4 (31%) *Vibrio*, 3 (23%) *E. coli* and 1 (8%) *Shigella* species.

Table 4.8: Incidence of food borne pathogens in various street-vended food samples (n=30)

Pathogens	**Food Categories**					
	Deep Fried and Fried Items (n=13)	**Spicy Preparations (n=4)**	**Baked Items (n=7)**	**Sweet Items (n=4)**	**Others (n=2)**	**Total (n=30)**
Klebsiella	2 (15%)	Nd	2 (27%)	1 (25%)	Nd	5 (17%)
Vibrios	4 (31%)	Nd	Nd	Nd	Nd	4 (13%)
E. coli	1 (8%)	Nd	2 (27%)	Nd	Nd	3 (10%)
Shigella	1 (8%)	Nd	Nd	Nd	Nd	1 (3%)

Nd= Not detected

Table 4.8 (Incidence of food borne pathogens in various street vended food samples) shows the presence of pathogens in different categories of food samples. Here, 2 *Klebsiella* were found in deep fried and fried items, 2 from baked items and 1 from sweet items. 4 *Vibrios* were found in deep fried and fried items. 1 *E. coli* was found in deep fried and fried items, and 2 in baked items 1 *Shigella* was found in deep fried and fried items. From total 30 samples, 17 % *Klebsiella*, 13% *Vibrios*, 10% *E. coli* and 3% *Shigella* species were obtained.

Chapter 5

Discussion

5.1 Discussion

At present time, street food vending has become a major community health issue and matter of concern for all of us. A lot of food-borne disease outbreaks are occurring every year worldwide. The reasons behind this includes lack of appropriate knowledge and supervision on street food vending, preparation of food under insanitary conditions and displaying food openly which also lead to further contamination by dust, insects, rodents and hands of intending consumers.

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

Five agar media MacConkey, Tryptone Bile X-glucoronide (TBX) agar, Thiosulfate Citrate Bile Salt-sucrose (TCBS) agar, Brilliant Green Agar (BGA) and Xylose-Lysine Desoxycholate agar (XLD) were used to observe the presence of our targeted microorganisms in food items. MacConkey and TBX agar were used for the identification and isolation of *E. coli* and *Klebsiella.* TCBS Agar is highly selective for *Vibrio* species isolation. XLD and BGA were used for isolation of *Salmonella* and *Shigella* species from food samples.

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).

A study has been done to analyze the microbiological quality of salads served along with street foods of Hyderabad. A total of 163 salad samples, 53 of carrot and 110 of onion samples, were collected from four different zones of Hyderabad. About 74% and 56% had *Staphylococcus aureus* in carrots and onions, respectively. Fifty-eight percent of

carrots and forty-five percent of onions samples contained *Salmonella*, 68% of carrots and 24% of onions had *Yersinia* (Sabbithi et al., 2014).

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 this study, 30 different food samples were collected from 10 private universities. Among them, we found contamination in 28 (93.3%) samples. Of which, 22 (73.3%) samples were suspected to be contaminated with our targeted organisms (*E coli, Klebsiella, Shigella, Salmonella* and *Vibrio* species). In total 22 samples, 14 (46.7%) samples were suspected to be contaminated with *E coli*, 14 (46.7%) with *Klebsiella*, 14 (46.7%) with *Vibrio*, 1 (3.3%) with *Shigella* and 1(3.3%) with *Salmonella* species. From the results of biochemical test we got 13 of our suspected bacteria from 13 different samples. In total, we got 5 (38%) *Klebsiella*, 4 (31%) *Vibrio*, 3 (23%) *E. coli* and 1 (8%) *Shigella* species.

This study indicated that the street vended foods of Dhaka city are highly contaminated with pathogenic bacteria which can contribute to potential health risks for consumers. The risk factors to the contamination include the low educational background of the vendors, poor personal hygiene, improper handling and storage practice of foods. Most of the vendors handled food with bare hand and didn’t wear any gloves or hand cover while handling money that can cause cross-contamination by introducing microbes on safe food.

5.2 Conclusion

Hygiene in handling and cooking of street foods is very essential. Personal hygiene is also very much important for food safety because human are the largest source of contamination. So it is very important to maintain cleanliness. From this study, it clear that all the samples are microbiologically unacceptable to eat. Strict public health regulations should be established to control the situation. The maintenance of these street vended foods should be monitored cautiously. The government should take necessary steps to provide regular training and to create consciousness on food management and individual hygiene among street food vendors as well as consumers.

Chapter 6

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