MICROBIOLOGICAL ANALYSIS OF STREET VENDED FOODS FROM DIFFERENT PRIVATE UNIVERSITIES IN DHAKA CITY

A Dissertation submitted to the Department of Pharmacy, East West University, Bangladesh, in partial fulfillment of the requirements for the Degree of Bachelor of Pharmacy

Submitted by
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Declaration by the Research Candidate

I, Mir Shafaly Akter, ID: 2012-3-70-025, hereby declare that the dissertation entitled— Microbiological analysis of street vended foods from different private universities in Dhaka city. Submitted by me to the Department of Pharmacy, East West University in partial fulfillment of the requirement for the award of the degree of Bachelor of Pharmacy is a record of research work under the supervision and guidance of Dr. Sufia Islam,Associate Professor and Nafisa Tanjia, Lecturer, Department of Pharmacy, East West University, Dhaka.

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Certificate by the Supervisor

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Dr. Shamsun Nahar Khan
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List of abbreviation

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
KIA	Kliglar Iron Agar
MIO	Mortility Indole Ornithine

Abstract

This study was analyzed the microbial quality of street vended food samples. The main objective of this study was to find out the presence of enteric bacteria specially *Escherichia coli, Shigella, Vibrios, Klebsiella* and *Salmonella* in different food samples. Total 30 samples were collected from the street vended shops beside the 10 university in Dhaka city, Bangladesh. Five different agar media were used for isolation and identification of our suspected organisms from the samples. The agar media were MacConeyk, 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. In our present study, 3 suspected organisms *E.coli, Klebsiella spp.* and *Vibio spp.* were found from 13 (43%) samples. From the biochemical test results of the colonies of MacConkey, XLD and TCBS agar media, 6 (14%) food samples were suspected to be contaminated with *E.coli*, 6 (14%) food samples were suspected to be contaminated with *Klebsiella spp.* and 1 (3%) food sample was suspected to be contaminated with *Vibrio spp.* No *Salmonella spp*. was found from any food sample. Seven biochemical tests were performed for characterizing the organisms but PCR test was not done. Therefore it cannot be said confidently that colonies of the media plates are the claimed ones. In this research, due to limitations of facilities other tests were not performed. Therefore, to reduce the frequency of food-borne illness future studies will be needed to determine the presence of various microorganisms responsible for food-borne illnesses.

Key Words: Street vended food, Food-borne illness, *E.coli, Klebsiella spp.*, MacConkey Agar, TCBS (Thiosulfate citrate-bile salts sucrose), Urease Test, MIO (Mortility Indole Ornithine) Test.

CHAPTER 1
INTRODUCTION & LITERATURE REVIEW

1.1 Street vended foods

'Street food' refers to a wide variety of foods and beverages prepared and sold by vendors and hawkers especially in streets around trading centers and other public places for immediate consumption or consumption at a later time without further processing or preparation (Tesfaye W. et al., 2016).

In Bangladesh the most popular and traditional street-vended foods include jhal-muri, fuchka, vhel-puri, panipuri, cake, danish, chola, laddu, singara, samucha 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, college and universities where many people have these traditional foods (Rahman, M. M., Rahman, M. H. & Ansary, N. P., 2014).

Figure 1.1: street vended foods

1.1.1 Benefits of street vended foods

Street-vended foods provide:

•A source of inexpensive, convenient and often nutritious food for urban and rural people,

• A source of attractive and varied food for tourists and the economically advantaged,

• A chance for self-employment and the opportunity to develop business skills with low Capital investment.

While street-vended foods are appreciated for their unique flavours as well as their convenience, they are also often essential for maintaining the nutritional status of the population (Rane, 2011).

The Street foods provide a source of affordable nutrients to the majority of the people especially the low-income group in the developing countries (Kibret M. & Tadesse M., 2013).

1.1.2 Problems of street vended foods

Street-vended foods are predisposed to contaminations because they are sold in the open and often not covered. Additionally, since street vendors prefer to take their products to their customers, they often operate from places such as bus terminals, industrial areas, schools, universities, market places and streets. Such locations usually do not meet food and safety requirements. Sale of food in the streets is very controversial from a health point of view. The main health hazard associated with street foods is microbial contamination (Tesfaye W. et al., 2016).

Street foods displayed on open work area can easily be contaminated by dust, exhaust smoke, insects, and hands of the buyers. In most cases, tap water is not available for washing hands and utensils at vending sites; hand and utensil washing are usually done in one or more buckets, and sometimes without soap. Toilets, waste disposal and refrigeration facilities are rarely available. Wastewater and garbage are therefore discarded nearby, providing nutrients for insects and other house hold rodents, which may carry food borne pathogens (Kibret M. & Tadesse M., 2013).

Lack of awareness about food safety and hygiene among vendors also results in food contamination. The vendors can be carriers of pathogens like *E.coli, Salmonella, Shigella, Campylobacter* and *S.aureus*, who eventually transfer these food-borne pathogens to consumers. Street food vendors are often poor, less aware of food safety and untrained on food preparation, handling and storage. As a result, ready-to-eat street foods are exposed to contamination by a variety of microorganisms. Moreover, antibiotic resistant bacteria have been isolated from different street vended foods. Consequently, street foods are perceived to be a major public health risk (Rane, 2011).

1.2 Food-borne illness

Food-borne illness often shows itself as flu-like symptoms such as nausea, vomiting, diarrhea, or fever, so many people may not recognize the illness is caused by bacteria or other pathogens on food. Thousands of types of bacteria are naturally present in our environment. Not all bacteria cause disease in humans. For example, some bacteria are used

beneficially in making cheese and yogurt. Bacteria that cause disease are called "pathogens." When certain pathogens enter the food supply, they can cause food-borne illness. Only a few types cause millions of cases of food-borne illness each year. Ironically, most cases of food-borne illness can be prevented. Proper cooking or processing of food destroys bacteria. They can grow in just about any food, but are fond of protein foods, such as meat, poultry, seafood, eggs, and dairy products in particular, as well as high-protein vegetables such as beans and grains (Braide W. et al., 2012)

1.2.1 Classification of Food-borne Illnesses Caused by Pathogens

Food-borne Illness occurs when a pathogen is ingested with food and establishes itself (and usually multiplies) in the human host, or when a toxic pathogens establishes itself in a food product and produces a preformed toxic microbial product is then ingested by the human host. Thus, food-borne illness is generally classified into two main categories, food-borne infection and food-borne intoxication, as follows:

Food-borne Infections occur as a consequence of growth of the pathogen in the human body. Since an incubation period is usually involved, the time from ingestion until symptoms occur is much longer than that of food-borne intoxications. The two basic categories of food-borne infections are:

- ✓ Invasive Infections which are caused by pathogens that invade bodily tissues and organs. Included in this group are the viruses, parasitic protozoa, other parasites, and invasive bacteria (e.g., *Salmonella, Aeromonas, Campylobacter, Shigella, Vibrio parahaemolyticus, Yersinia and enteric-type Escherichia coli*).
- ✓ Toxicoinfections which are caused by infective bacteria that are not considered invasive in nature, but are capable of multiplication or colonization in human intestinal tract and produce toxins. Included in this group are: *Vibrio cholerae, Bacillus cereus* (diarrheal-type), *C. botulinum* (in infants), *C. perfringens* and *E. coli* (E. coli O157:H7 and others) (Altekruse et al., 2008).

1.2.2 Mode of Action of Food-borne Pathogens

Pathogens enter the body through the digestive tract. These organisms differ in how they establish themselves (and may or may not cause illness), and in the number of microorganisms (infective dose) or quantity of toxin (toxic dose) required to cause illness. A major factor in the ability of these organisms to cause illness is the characteristics of the host.

Humans can be generally characterized as low risk and high risk in terms of susceptibility. Low risk or healthy individuals may be resistant to many (but not all) of the food-borne illnesses. However, high-risk populations (e.g., immune compromised, immune suppressed, infirmed, elderly, small children, etc.) have much lowered (and highly varied) resistance.

A person infected with a food-borne pathogen can be a carrier and a source of infection to others. Even infected individuals that do not show signs of illness can be chronic carriers. While this is especially true for persons infected with viruses (e.g. *hepatitis A*), chronic carrier states occur for many infective food-borne bacteria as well which can be excreted during the illness period (e.g. *Salmonella* spp., *Shigella* spp.). While the carrier state usually ceases after several weeks, it can for longer time periods in certain individuals and for certain pathogens (e.g. *Salmonella typhi*) (Altekruse et al., 2008).

1.3 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 (Ray, 2004).

1.4 Food-borne Disease Outbreaks

Centers for Disease Control and Prevention (CDC) has a long history of summarizing outbreak reports from local and state health departments. Data reported for each outbreak include the number of illnesses, hospitalizations, and deaths; the pathogens, toxins, and chemical agents that caused illnesses; the implicated food; the settings of food preparation and consumption; and factors contributing to food contamination. CDC's Food-borne Disease Outbreak Surveillance System gathers data on food-borne disease outbreaks from state, local, territorial, and tribal health departments.

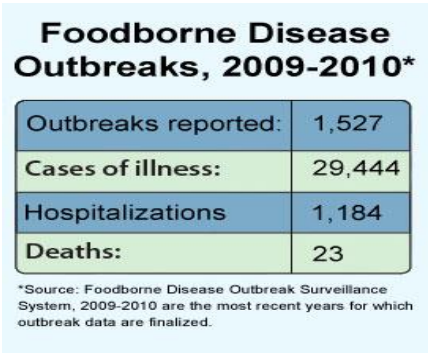

Figure 1.2: Data on Food-borne Disease Outbreaks during 2009-2010

During January 1, 2009 through December 31, 2010, public health departments reported 1,527 food-borne disease outbreaks, resulting in 29,444 cases of illness, 1,184 hospitalizations, and 23 deaths.

- Among the 790 outbreaks with a laboratory-confirmed illness, norovirus was the most commonly reported infection, accounting for 42% of outbreaks; followed by *Salmonella*, with 30% of outbreaks.
- Of the 29,444 outbreak-related illnesses, 1,184 (4%) resulted in hospitalization. *Salmonella* caused the most outbreak-related hospitalizations (49%), followed by Shiga toxin-producing *E. coli* (16%), and norovirus (9%).
- Outbreaks caused by some pathogens were particularly severe. For example, *Listeria* outbreaks resulted in the highest proportion of persons hospitalized (82%), followed by *Clostridium botulinum* (67%), and paralytic shellfish poisoning (67%).
- Among the 23 deaths, 22 were linked to bacteria (9 *Listeria,* 5 *Salmonella*, *E. coli* O157, 3 *Clostridium perfringens,* and 1 *Shigella*), and 1 was linked to norovirus (CDC, 2014).

1.5 Worldwide Food-borne Illness Data

A study was conducted in Zaria, Nigeria, where out of 160 food samples tested, 42 (26.3%) were contaminated with *B. cereus*, 24 (15.9%) with *S. aureus*. Six (18.3%) of the 32 coagulase positive *S. aureus* isolates tested produced enterotoxin A (SEA). More than 50% of the coagulase positive *S. aureus* were resistant to the common antimicrobial drugs used in the treatment of staphylococcal and wound infections (Umoh V.J. & Odoba M.B., 2016).

The present study was aimed to investigate the hygienic conditions of the vendors and microbial quality of street foods in West Delhi. It was found that their food handling practices were very poor. For microbial analysis, 5 street foods and 5 franchise's food products were taken from most popular shops. In local street foods, the bacterial load in veg-momos (130±2.1- 34.5±1.5 CFU/g) and non-veg momos (360±2.55-4±0.60 CFU/g) was high than the other samples. In branded food product samples, burger (362.5±2.55 CFU/g), non-veg momos (262.5±2.41-2.0±0.30 CFU/g), veg-momos (85.0±1.92-9.0±0.95 CFU/g), gol-gappa (237.5±2.37- 8±0.90 CFU/g) were assessed. The presence of coliform indicated faecal contamination of the processing water as well as the prevailing unhygienic conditions related to the lo-cation of food preparation (Sharma A., Bhardwaj H. & Ravi I., 2015).

A study was performed in Harare, Zimbabwe on 200 samples of mostly vended ready to eat foodstuffs (comprising chicken and beef stew, egg rolls, doughnuts and boiled mealie cobs) between the month of October and November 2012. Samples were analyzed against different types of indicator micro-organisms namely total aerobics, coliforms *Escherichia coli* and pathogens (*Salmonella spp.* and *Staphylococcus aureus*). Nearly 85.5% and 53% of the samples were highly contaminated with *S. aureus* and *E. coli*. (Kwiri R., 2014).

A study was performed 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 spp*, 68% of carrots and 24% of onions had *Yersinia spp* (Kumar N. et al., 2014).

A analysis of Street Vended Foods in Gondar, Ethiopia revealed that 64.3% of the food samples were contaminated with one or more bacteria. The isolates were *S. aureus* accounts 29 (53.7%) and *E. coli* 25 (46.3%). No *Salmonella species* was isolated from any food

sample. The study indicates that the probability of street foods contamination was high in Gondar town (Derbew G.et al., 2013).

A study was 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 microbiological analysis was conducted in Alice, Eastern Cape Province, South Africa on 252 samples which included vegetables, potatoes, rice, pies, beef and chicken stew. Bacterial growth was present in all the food types tested; high levels of total aerobic count were observed in vegetables, 6.8 ± 0.07 followed by rice, 6.7 ± 1.7 while pies had the lowest count (2.58 ± 0.24). Organisms isolated included: *Listeria spp.* (22%), *Enterobacter spp.* (18%), *Aeromonas hydrophila* (12%), *Klebsiella oxytoca* (8%), *Proteus mirabilis* (6.3%), *Staphylococcus aureus* (3.2%) and *Pseudomonas luteola* (2.4%). Interestingly, *Salmonella spp.* and *Escherichia coli* were not isolated in any of the samples. The results indicated that most of the ready-to-eat food samples examined in this study did not meet bacteriological quality standards, therefore posing potential risks to consumers (Nyenje M. et al., 2012).

From 1992 to 2008, 2429 food-borne outbreaks were reported in England and Wales. 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).

According to CDC, the etiology of majority (68%) of reported food-borne illness outbreaks is unknown. In addition, ill 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).

In Spain, 971 and 1227 outbreaks were reported in 2002 and 2003, respectively. A substantial proportion of outbreaks were associated with consumption of eggs and egg products. During the study period, 49 states reported 350 outbreaks. During these outbreaks 8,598 cases were reported; out of which 1,493 (17%) were hospitalized, and 40 (0.5%) died. Transmission route for 183 (52%) was food-borne and the food vehicle for 75 (41%) food-borne outbreaks was ground beef (Sparling et al., 2008).

1.6 Microorganisms responsible for food-borne diseases

Some bacteria cause more serious illness than others, but only a few are responsible for the majority of cases. Below is information regarding five prominent bacteria.

1.6.1 *Escherichia coli*

Since 1885, when it was first isolated from childrens' faeces and described by the German bacteriologist Theodor Escherich. *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 in England 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).

Figure 1.3 : *Escherichia coli*

1.6.1.1 Characteristics

Escherichia is the type genus of the *Enterobacteriaceae* family and *E. coli* is the type species of the genus. It is a catalase-positive, oxidase-negative, fermentative, short, Gram-negative, non-sporing rod. Genetically, *E. coli* is very closely related to the genus *Shigella*, although characteristically it ferments the sugar lactose.

1.6.1.2 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 diarrhoea to a severe cholera like syndrome of watery stools without blood or mucus, stomach pains and vomiting. The illness is usually self-limiting, persisting for 2–3 days, although in developing countries it is a common cause of infantile diarrhoea where it can cause serious dehydration.

Enteroinvasive E. coli (EIEC). Infection by EIEC results in the classical symptoms of an invasive bacillary dysentery. EIEC invades and multiplies within the epithelial cells of the colon causing ulceration and inflammation. Clinical features are fever, severe abdominal pains, malaise and often a watery diarrhoea which precedes the passage of stools containing blood or mucus.

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,vomiting and diarrhoea 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. *E. coli* O157:H7 is the most common EHEC serotype reported. The illness it causes can range from a non-bloody diarrhoea, through haemorrhagic colitis, to the life threatening conditions haemolytic uraemic syndrome.

1.6.1.3 Isolation and Identification

Selective techniques for *E. coli* mostly exploit the organism's tolerance of bile, a consequence of its natural habitat, the gut. 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.

1.6.1.4 Association with Foods

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. 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).

1.6.2 *Salmonella spp.*

Salmonella spp. 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.

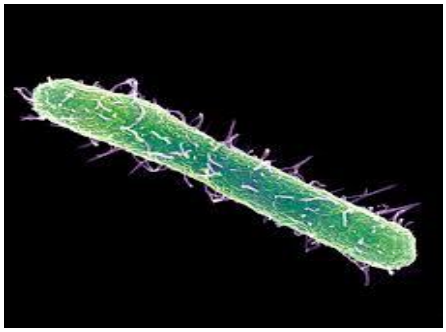

Figure 1.4: *Salmonella spp.*

1.6.2.1 History

Salmonella spp. was named after Daniel Elmer Salmon (1850-1914), an American veterinary pathologist, who described *Salmonella enteric* (formerly *S. choleraesuis*). However, it was his colleague and subordinate Theobald Smith, who first discovered the bacterium in 1885, from pigs, in an investigation for the cause of hog cholera (Bayu et al., 2013).

1.6.2.2 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 (Bayu et al., 2013).

1.6.2.3 Pathogenesis and Clinical Features

Generalized systemic enteric fever, headache, enlarged spleen, and constipation followed by more severe abdominal symptoms. 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).

1.6.2.4 Association with Foods

Salmonellosis is described as a zoonotic infection since the major source of human illness is infected animals. Transmission is by the faecal-oral route whereby intestinal contents from an infected animal are ingested with food or water. Meat, milk, poultry, and eggs are primary vehicles; they may be undercooked, allowing the salmonellas to survive, or they may cross-contaminate other foods that are consumed without further cooking. (Adams & Moss, 2008).

1.6.3 *Klebsiella spp.*

Bacteria belonging to the genus *Klebsiella spp.* frequently cause human nosocomial infections. In particular, the medically most important *Klebsiella spp*, *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).

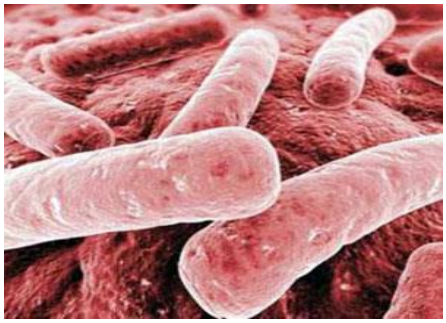

Figure 1.5: *Klebsiella spp.*

1.6.3.1 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).

1.6.3.2 Pathogenesis and Clinical Features

Nosocomial *Klebsiella spp*. infections most commonly involve the urinary and respiratory tracts. Typical *Klebsiella pneumonia*, which mostly affects those with weakened immune systems and tends to cause nosocomial infections (Li et al., 2014).

1.6.3.3 Isolation and Identification

Klebsiella spp. 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 (Podschun & Ullmann, 1998).

1.6.3.4 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 Vegetables are known as source of contamination with *K. pneumonia.* Raw vegetables are usually consumed in salads and other dishes (Puspanadan et al., 2012)

1.6.4 *Vibrio spp.*

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 12holera* and cholera when working in Egypt in 1886.

1.6.4.1 Characteristics

Vibrio spp. are Gram-negative, short rods which are motile. Catalase and oxidase-positive cells are 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%.

1.6.4.2 Pathogenesis and Clinical Features

Cholera usually has an incubation period of between one and three days and can vary from mild, self-limiting diarrhoea to a severe, life threatening disorder. The infectious dose in normal healthy individuals is large when the organism is ingested without food but is considerably reduced if consumed with food which protects the bacteria from stomach acidity. 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.

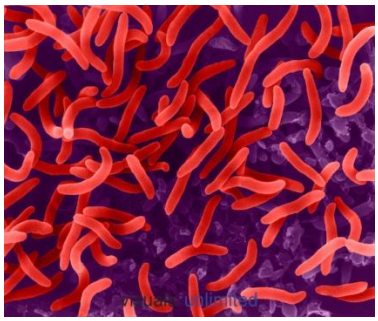

Figure 1.6: *Vibrio spp.*

1.6.4.3 Isolation and Identification

The enrichment media used for *vibrio spp.* 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 *vibrio spp.* by other organisms. Bile salt broth (pH 9.0 –9.2) is a more selective enrichment medium and can be incubated overnight.

1.6.4.4 Association with Foods

Cholera is regarded primarily as a water-borne 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 (Adams & Moss, 2008).

1.6.5 *Shigella spp.*

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. *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.

Fig 1.7: *Shigella spp.*

1.6.5.1 Characteristics

Shigella spp. are members of the family *Enterobacteriaceae*. They are non-motile, non-spore forming, Gram-negative rods which are catalase positive (with the exception of *S.bacillus, S. dysenteriae* serotype 1), oxidase-negative, and facultative anaerobes. They produce acid but usually no gas from glucose. 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.

1.6.5.2 Pathogenesis and Clinical Features

Shigella spp. 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, 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.

1.6.5.3 Isolation and Identification

Lack of interest in *Shigella spp.* as a food-borne pathogen has meant that laboratory protocols for its isolation and identification from foods are relatively underdeveloped. Selective plating media used are generally those employed for enumerating the *Enterobacteriaceae* although neither are entirely satisfactory.

1.6.5.4 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 spp.* In food-borne cases, the source of the organism is normally a human carrier involved in preparation of the food (Adams & Moss, 2008).

CHAPTER 2

OBJECTIVE

2.1 Research Objective

The Objective of this study was,

To find out the presence of enteric bacteria specially *Escherichia coli, Shigella, Vibrios, Klebsiella* and *Salmonella* in different food samples.

CHAPTER 3

METHODOLOGY

3.1 Study Area

10 private universities of Dhaka city which are North South University (NSU), BRAC University (BU), 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 of Bangladesh (ULAB), Stamford University (SU), 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.

3.3Bacteriological 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 in different sealed poly bags to prevent their contact with any other source that can contaminate the samples.

Table 3.1: Category of Foods

Deep Fried and Fried Items (n=15)	Spicy Preparations (n=6)	Baked Items (n=5)	Rice and Noodles (n=2)	Sweet Items (n=2)
Singara, Samucha, Aluchop, Chicken ball, Roll, Egg chop	Chhola, Velpuri, Pani fuchka	Cake, Butter bun, Biscuit	Noodles	Mishti

3.3.2 Sample Processing

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

3.3.3 Enrichment of the Organisms

3.3.3.1 Enrichment of *Salmonella Species* 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.

Figure 3.1: Pre-Enrichment of the organisms

3.3.3.2 Enrichment of *E. coli Species* 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.

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 Species* 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 Species* 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.

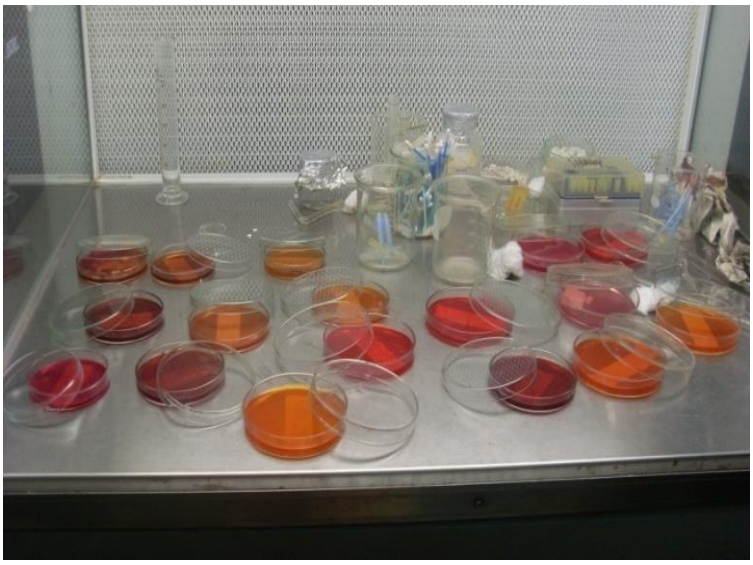

Figure 3.4: Petri dishes preparation

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 3.2: 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
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.

3.4.6 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

3.4.7 Standard Biochemical Test results of Suspected Organism

Table 3.3: 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
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. Where, K indicates acid and A indicates alkali. It can be K/A, A/K, K/K or even A/A for slant/butt.

CHAPTER 4

RESULTS

4.1 Bacterial colony morphology

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

Name of Universitiy	Sample	Agar Plates				
		MacConkey	TBX	BGA	XLD	TCBS
North South University (NSU)	Singara 1	Pink	Blue	No growth	No growth	No growth
	Samucha 1	Pink	No growth	White	No growth	No growth
	Cake 1	Flat pink	No growth	yellow	No growth	No growth
BRAC University (BU)	Singara 2	Pink centered	No growth	No growth	No growth	Yellow
	Chicken ball	No growth	No growth	Yellowish	Yellow	No growth
	Velpuri	Pink	Blue	White	Yellowish	No growth
East West University (EWU)	Singara 3	Pink	No growth	Whitish	No growth	Yellow
	Biscuit 1	No growth	No growth	No growth	No growth	No growth
	Butter bun	No growth	No growth	white	Yellowish	No growth
South East University (SEU)	Singara 4	Mucoid Pink	No growth	No growth	No growth	No growth
	Chhola 1	Pink	No growth	Yellow	No growth	No growth
	Egg chop	Pink	No growth	No growth	No growth	No growth
United International University (UIU)	Singara 5	Flat Pink	White	No growth	No growth	Yellow
	Samucha 2	Pink	Blue	No growth	No growth	No growth
	Pani Fuchka 1	No growth	No growth	Yellowish	Yellow	Yellow

Table 4.1 shows bacterial colony morphology isolated from different street vended food samples. 15 food samples were collected from the area around five different private

universities in Dhaka city. In total 14 samples show growth of different pathogenic or non pathogenic microorganisms. Of which 12 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.

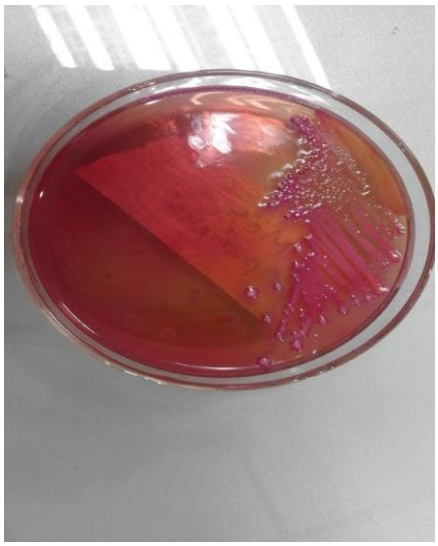

Figure 4.1: Bacterial colony (pink) on MacConkey agar plate

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

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

Name of Universitiy	Sample	Agar Plates				
		MacConkey	**TBX**	**BGA**	**XLD**	**TCBS**
Independent University Bangladesh (IUB)	Singara 6	Colorless	Colorless	No growth	No growth	Yellow
	Chhola 2	Pink	Blue	No growth	No growth	No growth
	Pani Fuchka 2	No growth	white	yellow	No growth	No growth
University of Asia Pacific (UAP)	Roll	No growth	No growth	No growth	No growth	No growth
	Cake 2	No growth	No growth	Yellow	Yellow	Yellow
	Noodles 1	No growth	Whitish	No growth	Yellowish	No growth
University of Liberal Arts of Bangladesh (ULAB)	Singara 7	No growth	White	No growth	No growth	Yellow
	Mishti 1	No growth	No growth	No growth	No growth	No growth
	Noodles 2	No growth	Colorless	Yellow	Yellow dot	No growth
Stamford University (SU)	Singara 8	No growth	No growth	No growth	No growth	No growth
	Biscuit 2	No growth	No growth	No growth	Yellow	No growth
	Aluchop 1	Pink flat dot	No growth	White	No growth	No growth
American International university, Bangladesh (AIUB)	Chhola 3	Flat pink dot	Blue	No growth	No growth	No growth
	Cake 3	No growth	No growth	No growth	No growth	No growth
	Mishti 2	No growth	white	White	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 12 samples show growth of different pathogenic or non pathogenic microorganisms. Of which 9 samples show positive growth of our suspected

organisms (*E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp.*) and 3 samples show no growth in these agar media.

4.2 Suspected organisms from different biochemical test

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	3	3	0	0	0
BRAC	2	2	1	0	0
EWU	1	1	1	0	0
SEU	3	3	0	0	0
UIU	2	2	2	0	0
IUB	2	2	1	0	0
UAP	0	0	1	0	0
ULAB	0	0	1	0	0
SU	1	1	0	0	0
AIUB	1	1	1	0	0

Among 30 samples were collected from street vendors in the area around 10 private universities of Dhaka city. About 26 (87%) food samples were contaminated with pathogenic or non pathogenic microorganisms (Table 4.1 and Table 4.2). Of which 21 (70%) 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 15 samples were suspected to be contaminated with either *E.coli* or *Klebsiella spp.*, 8 samples were suspected to be contaminated with *Vibio spp.*

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

Samples	Plates	Colony mor-phology	M	I	O	Citrate	Urease	Oxidase	KIA			Organism
									Slunt /butt	H_2S	Gas	
Singara 1	Mac Conkey	Pink	-	+	-	+	-	-				*Klebsiella spp.*
Singara 2	Mac Conkey	Pink centered	-	+	-	+	-	-				
Singara 4	Mac Conkey	Mucoid pink	-	+	-	+	-	-	K/A	-	+	
Velpuri	Mac Conkey	Pink	-	+	-	+	-	-				
Samucha 1	Mac Conkey	Pink	-	+	-	+	-	-	K/A	-	+	
Chhola 1	Mac Conkey	Pink	-	+	-	+	-	-				

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

Table 4.4 shows identification of the suspected organism (*Klebsiella spp.*) from different biochemical test. In total 6 (14%) food samples were identified to be contaminated with our suspected organism *Klebsiella spp.* from these biochemical tests.

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

Sample	Plates	Colony mor-phology	M	I	O	Citrate	Urease	Oxidase	KIA			Organism
									Slunt /butt	H_2S	Gas	
Singara3	Mac Conkey	Pink	+	+	-	+	-	-				*E.coli*
Singara5	Mac Conkey	Flat pink	+	+	-	+	-	-	K/A	_	+	
Chhola2	Mac Conkey	Pink	+	+	-	+	-	-				
Chhola3	Mac Conkey	Flat pinkdot	+	+	-	+	-	-	K/A	_	+	
Cake1	Mac Conkey	Flat pink	+	+	-	-	-	-				
Samucha2	Mac Conkey	Pink	+	+	-	+	-	-	K/A	-	+	
Singara6	TCBS	Yellow	+	+	-	+	-	-	K/A	_	+	*Vibrio spp.*

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

Table 4.5 shows identification of the suspected organism (*E.coli*, *Vibio spp. and Shigella spp.*) from different biochemical test. In total 6 (14%) food samples were identified to be contaminated with *E.coli* and 1 (3%) food sample was identified to be contaminated with *Vibio spp.* from these biochemical tests.

Figure 4.3: Different Biochemical test

Table 4.6: 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	1	2	0	0	0
BU	0	2	0	0	0
EWU	1	0	0	0	0
SEU	0	2	0	0	0
UIU	2	0	0	0	0
IUB	1	0	1	0	0
AIUB	1	0	0	0	0

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

In NSU, 3 food samples were suspected to be contaminated with *Klebsiella spp*. and *E.coli*. respectively.

In BU, 2 food samples were suspected to be contaminated with *Klebsiella spp*.

In EWU, 1 food sample was suspected to be contaminated with *E.coli*.

In SEU, 2 food samples were suspected to be contaminated with *Klebsiella spp*.

In UIU, 2 food samples were suspected to be contaminated with *E.coli*.

In IUB, 2 food samples were suspected to be contaminated with *E.coli*. and *Vibio spp* respectively.

In AIUB, 1 food sample was suspected to be contaminated with *E.coli*.

Table 4.7: Incidence of food-borne pathogens in various street vended food samples (n=30)

Pathogens	Food Categories					
	Deep Fried and Fried Items (n=15)	Spicy Preparations (n=6)	Baked Items (n=5)	Rice and Noodles (n=2)	Sweet Items (n=2)	Total (n=30)
E.Coli	3(20%)	2(33%)	1(20%)	Nd	Nd	6 (20%)
Klebsiella spp.	4(27%)	2(33%)	Nd	Nd	Nd	6 (20%)
Vibrio spp.	1(7%)	Nd	Nd	Nd	Nd	1 (3%)

Nd; Not detected

Table 4.7 shows the presence of pathogens in various street vended food samples. Here, 4 *Klebsiella spp.* were found in deep fried and fried items and 2 from spicy preparations. 1 *vibrio spp.* was found in deep fried and fried items. 3 *E.Coli* were found in deep fried and fried items, 2 *E.Coli* were found in spicy preparations and 1 *E.coli* was found in baked items. From total 30 samples, 14% *klebsiella spp.* 14% *E.Coli* and 3% *Vibro spp.* were obtained.

CHAPTER 5
DISCUSSION

5.1 Discussion

Although there is a growing demand for street vended food products, not enough information is available regarding the microbiological quality of these products in Dhaka city, Bangladesh.

This study was conducted to find out the enteric bacteria specially *E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp.* In this study, 30 different food samples were collected from 10 private universities in 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 certain microorganisms in food items. MacConkey and TBX agar are used for the identification and isolation of *E. coli* and *Klebsiella.* MacConkey agar is highly selective for gram negative bacteria. Those who ferment lactose give pink colonies and those who are non-fermenting give colorless colony on plate. On TBX agar *E. coli* gives blue green colored colony. For these agars, samples are enriched in yeast extract and TSB (trypticase soy broth) broth and then inoculated on plate. TCBS Agar is highly selective for *Vibrio* species isolation. They generally give yellow colony on the plate. In this study, most of the colonies on TCBS were yellow and brownish-yellow. Some green colonies were also found. After biochemical tests, we can say that yellow colonies were may be of Vibrio species. Before inoculation, specimen is enriched in APW (alkaline peptone water) broth. XLD and BGA are used for isolation of *Salmonella* and *Shigella* species from food specimen. *Salmonella* gives red colonies and some with black centers. *Shigella* species gives red colonies. Here samples are enriched in BPW (buffered peptone water) broth before inoculation.

A study revealed that 95(72%) of the food samples had pathogenic bacterial contaminations in Jigjiga City, Eastern Ethiopia. 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'. This study revealed that there is a reasonable gap on food safety knowledge among street food venders. The microbial profile was also higher compared to standards set by the World Health Organization (Nguyen et al., 2014).

A Study was conducted on 37 street vended food samples which were examined for bacterial identification. The isolates were identified as *Escherichia coli* (37.5%), *Pseudomonas aeruginosa* (3.57%), *Staphylococcus aureus* (14.20%), *Salmonella sp.* (5.36%), *Klebsiella sp.* (10.71%), *Shigella sp.* (19.64%) and *Enterobacter sp.* (8.93%) respectively. These findings demonstrated that the ready-to-eat foods vended in Silchar city constitute an important potential hazard to human health and provision of health education to the vendors would improve quality of street foods (Sharma & Mazumdar, 2014).

A study was conducted on 60 food samples in some minimally and fully processed ready - to - eat foods in Kano metropolis, Nigeria, *Escherichia coli* recorded the highest frequency of occurrence of 24 (46.6%), followed by *V. cholerae* with 15 (25.0%) while *Salmonella typhi* recorded the least occurrence rate of 6 (10.0%). Overall, the fully processed foods were observed to be less contaminated with *enteropathogenic* bacteria than the minimally processed foods. The results indicated that most of the ready – to – eat food samples examined in this study did not meet bacteriological quality standards (Bukar et al., 2010).

In our present study, we found 3 suspected organisms *E.coli, Klebsiella spp.* and *Vibio spp.* were found from 13 (43%) samples. From the biochemical test results of the colonies of MacConkey, XLD and TCBS agar media. 6 (20%) food samples were suspected to be contaminated with *E.coli*, 6 (20%) food samples were suspected to be contaminated with *Klebsiella spp.*, and 1 (3%) food sample was suspected to be contaminated with *Vibrio spp.* from different university. *shigella spp.* and *Salmonella spp*. were not 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.

The findings of this study indicate that the foods sold by street vendors in Dhaka city were contaminated with pathogenic bacterial organisms, which are likely to pose a potential hazard to 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. By providing 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.

5.2 Conclusion

In Bangladesh the frequency of food-borne illness increases day by day because of the availability of large number of street vended foods. The present study showed that street vended foods in Dhaka city plays an important potential hazard to human health. In this study there only five organisms (*E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp*.) responsible for food-borne illness were to be identified, due to limitations of facilities other tests were not performed. Therefore, to reduce the frequency of food-borne illness future studies will be needed to determine the presence of various microorganisms responsible for food-borne illnesses. There is also 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. So it is very important to maintain the personal hygiene of the street food vendors to reduce the rate of food-borne illness. Government can also play an important role to create consciousness on food management and individual hygiene among street food vendors as well as consumers.

CHAPTER 6

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