Identification of Gram Negative Bacteria in Different Ready-to-Eat Foods Vended in Different Institutional Premises Situated in Dhaka City

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

Submitted by

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I, Tasnim Binte Shahjahan, hereby declare that the dissertation entitled "Identification of Gram Negative Bacteria in Different Ready-to-Eat Foods Vended in Different Institutional Premises Situated in Dhaka City" submitted by me to the Department of Pharmacy, East West University and in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, work carried out by me during the period 2016 of my research in the Department of Pharmacy, East West University, under the supervision and guidance of Ms. Nafisa Tanjia, Lecturer, Department of Pharmacy, East West University. The thesis paper has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Table of Content

List of Content	Page No.
List of Table	I
List of Figures	II
List of Abbreviations	III
Abstract	IV

Serial No.	Title	Page No.
CHAPTER 01	INTRODUCTION AND LITERATURE REVIEW	1-20
1.1	Street Food in Bangladesh	1
1.1.1	Benefits of Street Foods	1-2
1.1.2	Risks of Street Foods	2
1.1.3	Street Food Sector in Bangladesh	2
1.1.4	Types of Street Food Available in Bangladesh	2-3
1.2	Foodborne Illness	3-4
1.3	Diseases Causes Due to Consumption of Street Food	4
1.3.1	Epidemiology	4
1.4	Common Causes of Fodborne Disease	5-6
1.5	Populations at Risk for Foodborne Disease	6
1.6	Hazard Analysis of Street Vended Foods	6-7
1.7	Factors Affecting Growth of Microorganisms in Food	7-9
1.8	Microorganisms Responsible for Foodborne Diseases:	9-18
1.8.1	*Escherichia coli*	9
1.8.1.1	Pathogenesis	10-12
1.8.2	*Klebsiella spp.*	12
1.8.2.1	Pathogenesis	13-14
1.8.3	*Salmonella spp.*	14
1.8.3.1	Pathogenesis	15

1.8.4	*Shigella spp.*	16
1.8.4.1	Pathogenesis	16-17
1.8.5	*Vibrio spp.*	17
1.8.5.1	Pathogenesis	17-18
1.9	Foodborne Disease Outbreaks Around the World	18-20
CHAPTER 02	RESEARCH OBJECTIVE	21
CHAPTER 03	METHODOLOGY	22-34
3.1	Study Area	22
3.1.1	Study Duration	22
3.2	Bacteriological Subculture	22
3.2.1	Sample Collection	22
3.2.2	Sample Category	22
3.2.3	Sample Processing	22
3.2.4	Enrichment of the Organisms	23
3.2.4.1	Enrichment of *Salmonella* and *Shigella spp*	23
3.2.4.2	Enrichment of *E.coli* and *Klebsiellas spp*	23
3.2.4.3	Enrichment of *Vibrio spp*	23
3.2.5	Selective Growth of the Organisms	23
3.2.5.1	Selective Growth of Salmonella and *Shigella spp*	23
3.2.5.2	Selective Growth of *E.coli* and *Klebsiella spp*	24
3.2.5.3	Selective Growth of *Vibrio spp*	24
3.2.6	Sterilization Procedure	24
3.2.7	Preparation of Petri dishes	25
3.2.8	Incubation	26

3.2.9	Standard Colony Morphology of Suspected Organism in Different Media	26-27
3.3	Apparatus and Reagent used for Isolation and Identification of Specific Organism	27
3.4	Biochemical Tests	28
3.4.1	Kliglar Iron Agar Test (KIA Test)	28
3.4.1.1	Test Tub Preparation for KIA Test	28
3.4.1.2	Inoculation for KIA Test	28
3.4.2	MIO Test	28
3.4.2.1	Test Tube Preparation for MIO test	28
3.4.2.2	Inoculation for MIO Test	28
3.4.3	Citrate Test	29
3.4.3.1	Test Tube Preparation for Citrate Test	29
3.4.3.2	Inoculation for Citrate Test	29
3.4.4	Urease Test	30
3.4.4.1	Test Tube Preparation for Urease Test	30
3.4.4.2	Inoculation for Urease Test	30
3.4.5	Oxidase Test	30
3.4.6	Standard Biochemical Test Results of Suspected Organism	31
3.4.7	Apparatus and Reagent used for Biochemical Tests	31
3.5	Cell Counting and Serial Dilutions	32
3.5.1	Theory	32
3.5.2	Materials Required	33
3.5.3	Procedure	33
3.5.4	Preparation of Serial Dilutions	33
3.5.5	Mixing the Dilutions into Agar Plates	34

CHAPTER 04	RESULT	35-44
4.1.1	Bacterial Colony Morphology	35
4.2	Suspected Organism from Different Biochemical Test	39
4.3	Counting Bacterial Colonies	42
CHAPTER 05	DISCUSSION AND CONCLUSION	45-47
5.1	Discussion	45-46
5.2	Conclusion	47
CHAPTER 06	REFERENCE	48-49

List of Table

Table No.	Name of Table	Page No.
Table 1.1	Common Causes of Foodborne Diseases	5-6
Table 1.2	Hazard Analysis of Street Vended Foods	6
Table 3.1	Standard Colony Morphology of Suspected Organism in Different Media	26-27
Table 3.2	Biochemical Test Observation	31
Table 3.3	Preparation of Serial Dilutions	34
Table 3.4	Bacterial Colony Morphology Isolated From Different Street-vended Food Samples	35
Table 3.5	Bacterial Colony Morphology Isolated From Different Street-vended Food Samples	36
Table 3.6	Number of Food Samples with Growth of Suspected Organisms Determined by Colony Morphology (n=30)	37
Table 3.7	Identification of the Suspected Organisms (*Klebsiella spp*) from Different Biochemical Tests	39
Table 3.8	Identification of the Suspected Organisms (*E.coli and Vibrio spp*) from Different Biochemical Tests	40
Table 3.9	Presence of Suspected Organisms in No. of Food Samples from Different university (n=7)	41
Table 3.9.1	Incidence of Foodborne Pathogens in Various Street vended Food Samples	42
Table 3.9.2	Colony Counting of Various Samples	43
Table 3.9.3	Number of Colonies per ml of Sample	44

List of Figures

Figure No.	Name of Figure	Page No.
Figure 1.1	Street Food in Bangladesh	01
Figure 1.2	Different Types of Street Food Available in Bangladesh	03
Figure 1.3	*Escherichia Coli (E.coli)*	10
Figure 1.4	Klebsiella spp.	13
Figure 1.5	Salmonella spp.	14
Figure 1.6	Shigella spp.	16
Figure 1.7	Vibrio spp.	17
Figure 3.1	Enrichment for Targeted Organisms	23
Figure 3.2	Autoclave and Hot Air Oven	24
Figure 3.3	Laminar Air Flow Cabinet	25
Figure 3.4	Petri Dishes Preparation	25
Figure 3.5	Incubator	26
Figure 3.6	Preparation of Test Tubes for KIA Test	28
Figure 3.7	Preparation of Test Tubes for MIO Test	29
Figure 3.8	Preparation of Test Tubes for Citrate Test	29
Figure 3.9	Preparation of Test Tubes for Urease Test	30
Figure 4.1	Bacterial Colony (pink) on MacConkey Agar Plate	38
Figure 4.2	Bacterial Colony (blue) on TBX Agar Plate	38
Figure 4.3	Different Biochemical Test	40

List of Abbreviations

ETEC	Enterotoxigenic E. coli
EIEC	Enteroinvasive E. coli
EPEC	Enteropathogenic E. coli
EHEC	Enterohaemorrhagic E. coli
VTEC	Verotoxin-producing E. coli
HUS	Haemolytic Uraemic Syndrome
CFU	Colony Forming Unit
TSB	Trypticase Soya Broth
YE	Yeast Extract
BPW	Buffered Peptone Water
APW	Alkaline Peptone Water
TSB	Tryptone Bile X-Glucoronide
BGA	Brilliant Green Agar
XLD	Xylose Lysine Deoxycholate
TCBS	Thiosulfate citrate-bile salts sucrose
KIA	Kliglar Iron Agar

Abstract

Ready to eat foods vended on the streets are foods and beverages that are prepared and sold for immediate consumption by vendors on the street or other public places. These types of foods are an affordable source of nourishment for the students, low income workers and others. The objective of this study was to identify presence of gram negative bacteria in different ready-to-eat foods vended in different institutional premises situated in Dhaka City. From the premises, 30 food samples were collected and tested to find out the presence of suspected enteric bacteria *E.coli*, *Vibrio, Klebsiella*, *Salmonella* and *Shigella spcies*. Five different agar media were used to observe the growth of the microorganisms. Later several different biochemical tests were performed to find out the presence of bacteria in the samples. Among 30 samples, 17 (56.6%) samples were observed to be contaminated by different bacteria. Among 17, after biochemical tests were performed, 7 (23.3%) samples were observed to be contaminated with the suspected microorganisms. Among 7 (23.3%) samples, 3 (10%) samples were suspected to contain *Klebsiella spp*., 2(6.66%) samples were suspected to contain *E.coli* and 2 (6.66%) samples were suspected to contain *Vibrio spp.* We have also performed colony counting of additional 6 ready to eat food samples by standard method. The samples were Nargis kabab, Chola, Laddu, Boroi achar, Alur chop and Tikia. Out of 6 samples, Colony Forming Units (CFUs) have been observed and maximum concentrated colonies were found in Laddu, Tikia, Nargis kabab and Chola. All these enteric bacteria can be the potential cause of foodborne illness and so they pose as a great hazard to the students. Therefore, providing appropriate education and training to the street vendors has become an important necessity to ensure good public health.

Keywords: Street food, Gram negative bacteria, Colony forming units, E.coli, *Vibrio, Klebsiella*, *Salmonella*, *Shigella,* Agar media, Foodborne illness.

CHAPTER 01

INTRODUCTION AND LITERATURE REVIEW

1.1 Street Food in Bangladesh

Street foods are referred to as those types of foods and beverages which are prepared and/or sold by street vendors on streets and other public places like market places, parks, zoos, tourist spots, or in front of schools, colleges and universities. These foods are prepared for immediate consumption or for consumption at a later time but without any further processing or preparation.

Street foods which are seen generally to be sold include fresh fruits, vegetables, snack items, different types of beverages etc.

Figure 1.1: Street Food in Bangladesh

1.1.1 Benefits of Street Foods

These street vended foods are consumed due to a lot of purposes such as they are a source of inexpensive, convenient ready-to-eat foods which are also often source of nutrition for urban and rural poor people. They are also a source of attractive and varied food for tourists and also the economically advantaged people. Moreover, these street vending has provided for a source of income for a vast number of people especially for women and an opportunity for

self employment through which people can develop their business skills with low capital investment.

1.1.2 Risks of Street Foods

In spite of all these benefits there are some potential risks which are associated with these types of foods. Usually street vendors are poor, uneducated or little educated with lack of knowledge about how to prepare food in a hygienic way and steps for safe food handling. They also lack general factual knowledge about the microbiological status or the precise epidemiological significance of many street-vended foods. As a result, due to inadequate public awareness of hazards posed by certain street foods, they are perceived to be a major health risk for people of all ages.

Generally, the vending facilities of street vendors vary from mobile carts to food stalls and food centers. As the nature of street vendors is to move from one place to another, they lack basic infrastructure and services like potable water supplies. Moreover, there is restricted access to toilets, refrigeration, washing and waste disposal facilities. These factors also contribute to the quality of the street foods.

1.1.3 Street Food Sector in Bangladesh

In the past few decades, the street food sector has experienced immense growth because of the socioeconomic changes in our country. In developing countries like Bangladesh, urbanization and population growth is increasing in a continuous streak, which demands that street food sector will also expand into the next century.

(WHO, 1996)

1.2 Types of Street Food Available in Bangladesh

There are different types of street food available at different places in Bangladesh. The types of street food found depend on the culture, the surrounding environment, the food habit and the demand of the people at that specific area. Such as, the most important places the street vendors usually sell foods are public places, where people assemble in great numbers, in particular markets, bus and train terminals, university campuses, in front of school grounds and hospitals, and nearby clusters of working places. So in front of schools, colleges and

universities, food items like chotpoti, fuchka, velpuri, achar items, potato chops, egg chops, different kabab items, fruits sliced, noodles, burgers, jhal muri, shingara, samucha, chola, sweets, cake and different types of beverages like lemonade are found. So according to their location different food items are available.

Figure 1.2: Different types of Street Food Available in Bangladesh

1.3 Foodborne Illness

There are inadequate data to confidently declare that street foods significantly contribute to major food poisonings. However, there are some documented findings which suggest thatstreet foods have resulted in food poisoning outbreaks.

Foodborne illness also known as Foodborne disease or Foodborne infection or Food Poisoning is a common but costly and yet preventable public health problem. This disease is causes due to consumption of contaminated foods or beverages. Many different disease-causing microbes, or pathogens, can contaminate foods, so there are many different types of foodborne infections. In addition, poisonous chemicals, or other harmful substances can cause foodborne diseases if they are present in food.

More than 250 different types of foodborne diseases have been described to be caused by consumption of contaminated food items. Most of these diseases are infections, caused by a variety of bacteria, viruses, and parasites that can be foodborne. Other diseases are

poisonings, caused by harmful toxins or chemicals that have contaminated the food, for example, poisonous mushrooms.

These different diseases have many different symptoms, so there is no one "syndrome" that is foodborne illness. However, the microbe or toxin present in food enters the body through the gastrointestinal tract, and often causes the first symptoms there, so nausea, vomiting, abdominal cramps and diarrhea are common symptoms in many foodborne diseases. However, such diseases can also have neurological, gynaecological, immunological and other symptoms. Multiorgan failure and even cancer may result from the ingestion of contaminated foodstuffs, thus representing a considerable burden of disability as well as mortality.

1.3.1 Diseases Caused Due to Consumption of Street Foods

People, who patronize street food, have been reported to suffer from food borne diseases like diarrhea, cholera, typhoid fever, jaundice and food poisoning (CDC, 2016).

1.4 Epidemiology

Foodborne diseases can be caused due to various infectious agents such as bacteria, parasites, toxins and viruses. Although efforts have been made to investigate foodborne diseases, less than 50 % of all outbreaks caused are identified only, because of limited diagnostic capabilities.

Viruses are likely the most common cause of foodborne disease but are seldom investigated and confirmed because of the short duration and self-limited nature of the illness. In addition, the inherent difficulty of laboratory investigation and subsequent cost of viral studies lead to a lack of clinician investigation and therefore overall underreporting. Bacteria are the most common documented cause.

Cultural and demographic factors, as well as increased mobility, have resulted in major epidemiologic shifts in foodborne disease during recent decades. Previous outbreaks of foodborne disease were smaller and limited in scope, more often originated in the home, and were associated with *Staphylococcus* or *Clostridium* spp. Family picnics or dinners and

home-canned foods were the typical sources for the outbreaks. Today, many more people dine outside the home and travel more extensively. As a result, more than 80% of foodborne disease cases occur from exposures outside the home (Cleveland Clinic, 2004).

1.5 Common Causes of Foodborne Diseases (Table 1.1):

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

Miscellaneous	Niacin Monosodium glutamate

1.6 Populations at Risk for Foodborne Diseases:

Any population of people can be affected by foodborne diseases, but some consumers are more susceptible to such diseases and may experience more severe symptoms. This group of consumers includes young children, older adults, pregnant women, and persons with illnesses that affect the immune system.

Young Children: Young children are more likely to be affected by foodborne diseases since they tend to eat according to their desire without any knowledge about food hygiene or safety.

Individuals with Immune Deficiencies: Individuals who are in diseased condition like cancer, diabetes, hypertension, HIV, or have undergone transplant have compromised immune system. So they are more prone to fall sick due to foodborne pathogen.

Older Adults: Older adult group of people are also at risk due to weak immune system.

Pregnant Women: Pregnant women require special attention because they are at a highly sensitive stage where any pathogen ingested via food can cause diseases to the mother and the baby (UC Food Safety, 2016).

1.7 Hazard Analysis of Street Vended Foods (Table 1.2):

Analysis should be performed to understand how hazardous street foods are to the people. In this analysis some very important factors must be considered. In the table below, the source and type of hazard and the microbial risk involved are shown:

S.No.	Source	Hazard	Risk involved
1	Vendor location	Improper food handling	Transfer of pathogens like *Salmonella* and *E. coli*, *S. aureus* from human body and environment into foods
		Improper waste disposal	Transmission of enteric pathogens like *Salmonella*, *Shigella* and *E. coli* via vectors
2	Raw materials	Water	Passage of pathogens like *E. coli*, fecal streptococci, *Salmonella* and *Vibrio cholerae*
		Vegetables and spices	Introduction spore formers like Bacilli and Clostridium and pathogens like *L. monocytogenes*, *Shigella*, *Salmonella*, etc.
3	Utensils and equipments	Chemical contaminants	Leaching of chemical leading to poisoning
		Microbial contaminants	Cross contamination of food with *Staphylococcus aureus*, *E. coli* and *Shigella* due to contaminated water, dish cloth, handler
4	Storage and reheating	Improper storage temperature and reheating of food	Likelihood of heat stable toxins produced by pathogens like *C. perfringens* and *B. cereus*
5	Personal hygiene of vendors	Biological hazards	Introduction of *Staphylococcus*, *Salmonella* and *Shigella* via carriers

(Rane, 2011).

1.8 Factors Affecting Growth of Microorganisms in Food:

There are important factors which directly or indirectly affect the growth of microorganisms in food products. When microorganisms grow in food they cause varying degrees of change in the food's characteristics as a result of metabolic activity. Some of these changes, like those taking place during fermentation, are desirable, while others, like those resulting in food spoilage and food poisoning are undesirable.

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

1.8.1 Intrinsic factors:

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

- Nutrient content,
- Water activity,
- pH value,
- Redox potential, and
- The presence of antimicrobial substances and mechanical barriers to microbial invasion;

1.8.2 Extrinsic Factors:

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

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

1.8.3 Implicit Factors:

Factors related to the microorganisms themselves, the "implicit factors," include:

- Interactions between the microorganisms contaminating the food and between these microorganisms and the food, e.g., their abilities to utilize different nutrient sources, tolerate stresses, and produce promoters or inhibitors of growth of other microorganisms, etc.

1.8.4 Processing Factors:

Processing factors, which include treatments such as heating, cooling, and drying that affect the composition of the food and also affect the types and numbers of microorganisms that remain in the food after treatment

1.8.5 Interactions:

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

1.9 Microorganisms Responsible for Foodborne Diseases:

1.9.1 Escherichia Coli:

Escherichia coli also known as ***E. coli*** is a gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, and preventing colonization of the intestine with pathogenic bacteria. *E. coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards.

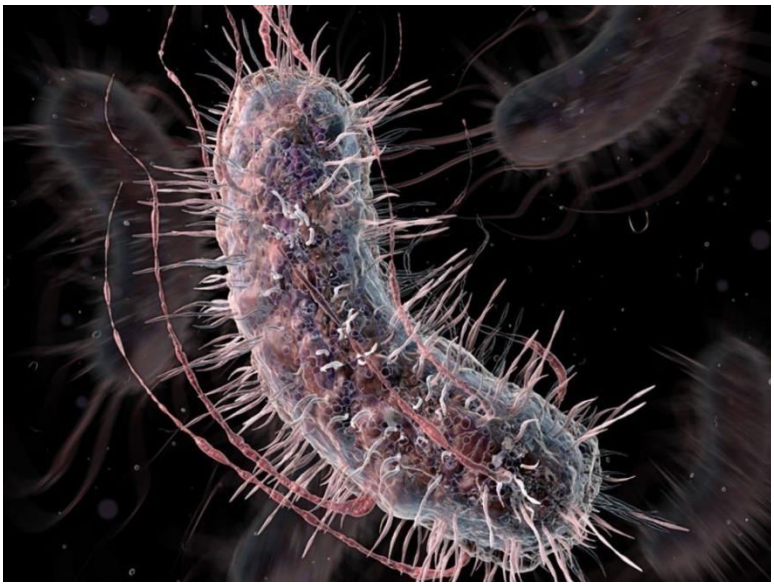

Figure 1.3: *Escherichia Coli*

1.9.1.1 Pathogenesis:

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

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

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

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

The key virulence factor for EHEC is Stx, which is also known as verocytotoxin (VT). Stx consists of five identical B subunits that are responsible for binding the holotoxin to the glycolipid globotriaosylceramide (Gb3) on the target cell surface, and a single A subunit that cleaves ribosomal RNA, causing protein synthesis to cease12. The Stx family contains two

subgroups — Stx1 and Stx2 — that share approximately 55% amino acid homology. Stx is produced in the colon and travels by the bloodstream to the kidney, where it damages renal endothelial cells and occludes the microvasculature through a combination of direct toxicity and induction of local cytokine and chemokine production, resulting in renal inflammation. This damage can lead to HUS, which is characterized by haemolytic anaemia, thrombocytopoenia and potentially fatal acute renal failure. Stx also induces apoptosis in intestinal epithelial cells — a process that is regulated by the Bcl-2 family44.

- **Enterotoxigenic *E. coli* (ETEC):**

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

- **Enteropathogenic *E. coli* (EPEC):**

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

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

- **Enteroaggregative *E. coli* (EAEC)**
- **Enteroinvasive *E. coli* (EIEC):**

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

- **Diffusely adherent *E. coli* (DAEC):**

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

1.9.2 Klebsiella Species:

Klebsiella is a type of Gram-negative bacteria that can cause different types of healthcare-associated infections, including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis.

Increasingly, *Klebsiella* bacteria have developed antimicrobial resistance, most recently to the class of antibiotics known as Carbapenems. *Klebsiella* bacteria are normally found in the human intestines (where they do not cause disease). They are also found in human stool (feces). In healthcare settings, *Klebsiella* infections commonly occur among sick patients who are receiving treatment for other conditions. Patients whose care requires devices like ventilators (breathing machines) or intravenous (vein) catheters, and patients who are taking long courses of certain antibiotics are most at risk for *Klebsiella*infections. Healthy people usually do not get *Klebsiella* infections.

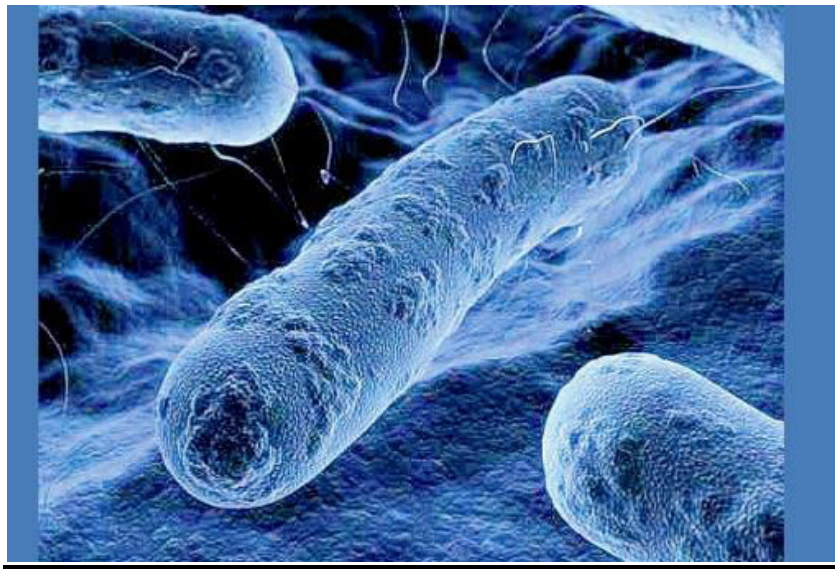

Figure 1.4: Klebsiella Species

1.9.2.1 Pathogenesis:

The symptoms of a *K. pneumoniae* infection differ depending on where the infection is located, and are similar to symptoms of the same diseases caused by other microbes. For instance, meningitis from *K. pneumoniae* produces the hallmark symptoms of bacterial meningitis, which include:

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

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

- Fever,
- Chills,
- Rash,
- Light-headedness, and
- Altered mental states.

Pneumonia from K. pneumoniae can result in:

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

1.9.3 Salmonella species:

Salmonella species are ubiquitous human and animal pathogens, and salmonellosis, a disease that affects an estimated 2 million Americans each year, is common throughout the world. Salmonellosis in humans usually takes the form of a self-limiting food poisoning (gastroenteritis), but occasionally manifests as a serious systemic infection (enteric fever) which requires prompt antibiotic treatment. In addition, salmonellosis causes substantial losses of livestock.

Figure 1.5: Salmonella Species

1.9.3.1 Pathogenesis:

Salmonellosis includes several syndromes (gastroenteritis, enteric fevers, septicemia, focal infections, and an asymptomatic carrier state). Particular serovars show a strong propensity to

produce a particular syndrome (*S typhi*, *S paratyphi-A*, and *S schottmuelleri* produce enteric fever; *S choleraesuis* produces septicemia or focal infections; *S typhimurium* and *S enteritidis* produce gastroenteritis); however, on occasion, any serotype can produce any of the syndromes. In general, more serious infections occur in infants, in adults over the age of 50, and in subjects with debilitating illnesses.

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

After invading the epithelium, the organisms multiply intracellularly and then spread to mesenteric lymph nodes and throughout the body via the systemic circulation; they are taken up by the reticuloendothelial cells. The reticuloendothelial system confines and controls spread of the organism. However, depending on the serotype and the effectiveness of the host defenses against that serotype, some organisms may infect the liver, spleen, gallbladder, bones, meninges, and other organs. Fortunately, most serovars are killed promptly in extraintestinal sites, and the most common human *Salmonella* infection, gastroenteritis, remains confined to the intestine (NCBI, 1996).

1.9.4 Shigella Species:

Shigellas are members of the family Enterobacteriaceae. They are nonmotile, non-sporeforming, Gram-negative rods which are catalasepositive (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.

Figure 1.6: Shigella Species

1.9.4.1 Pathogenesis:

Shigella causes bacillary dysentery in humans and other higher primates. Studies with human volunteers have indicated that the infectious dose is low; of the order of 10–100 organisms. The incubation period can vary between 7 h and 7 days although foodborne 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 250 Bacterial Agents of Foodborne Illness 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 and Moss, 2008).

1.9.5 Vibrio Species:

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.

Figure 1.7: Vibrio Species

1.9.5.1 Pathogenesis:

Cholera usually has an incubation period of between one and three days and can vary from mild, self-limiting diarrhoea to a severe, lifethreatening disorder. The infectious dose in normal healthy individuals is large when the organism is ingested without food or buffer, of the order of 1010 cells, but is considerably reduced if consumed with food which protects the bacteria from stomach acidity. Studies conducted in Bangladesh indicate that 103 –104 cells may be a more typical infectious dose. Individuals with low stomach acidity (hypochlorohydric) are more liable to catch cholera.

Cholera is a non-invasive infection where the organism colonizes the intestinal lumen and produces a potent enterotoxin. In severe cases, the hypersecretion 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. The diarrhoea, which can be up to 20 l day1 and contains up to 108 vibrios ml1 , is accompanied by vomiting, but without any nausea or fever. Unless the massive losses of fluid and electrolyte are replaced, there is a fall in blood volume and pressure, an increase in blood viscosity, renal failure, and circulatory collapse. In fatal cases death occurs within a few days. In untreated outbreaks the death rate is about 30–50% but can be reduced to less than 1% with prompt treatment by intravenous or oral rehydration using an electrolyte/glucose solution (Adams and Moss, 2008).

1.9.6 Foodborne Disease Outbreaks around the World:

Expression of the similar symptoms or sickness by two or more of the individuals after consumption of the same contaminated food is labeled as an outbreak of food-borne illness. The description of outbreak includes time, place, and person distribution (Jahan, 2012).

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

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

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).

CHAPTER 02

RESEARCH OBJECTIVE

2.1 Research objective

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

- To identify the presence of gram negative bacteria especially *E. coli, Klebsiella, Salmonella, Shigella* and *Vibrio* species from different types of ready-to-eat foods vended in different institutional premises situated in Dhaka city, Bangladesh.

CHAPTER 03

METHODOLOHY

3.1 Study Area

10 private universities of Dhaka city which are East West University (EWU), BRAC University, United International University (UIU), University of Liberal Arts Bangladesh (ULAB), Prime Asia University (PAU), Bangladesh University of Professionals (BUPS), Government Titumir College (GTC), Ahsanullah University of Science and Technology (AUST), Daffodil University (DU) and Dhaka International University (DIU).

3.1.1 Study Duration

This study was carried out over a period of 9 months from February 2016 to October 2016.

3.2 Bacteriological Subculture

3.2.1 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.

3.2.2 Sample Category

Five different categories of food samples were collected. They were deep fried and fried items (Singara, Samucha, Dimchop, Nargis kabab, Kathi kabab, Chicken toast, Porota,), spicy items (Chola), baked items (Vanilla cake, Chocolate cake, Danish, Bun, Toast biscuit, Cream roll), sweet items (Laddu, Misty, Patishapta pitha, Jilapi) and achar items (Boroi achar, Amrar achar, Amer morobba).

3.2.3 Sample Processing

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

3.2.4 Enrichment of the Organisms

3.2.4.1 Ennrichment of *Salmonella and Shigella* Species

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

3.2.4.2 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 37℃ for 18-24 h.

Figure 3.1: Enrichment for targeted organisms

3.2.4.3 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 37℃ for 18-24 h.

3.2.5 Selective Growth of the Organisms

3.2.5.1 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 ℃ for 18-24 h for selective growth of the organism.

3.2.5.2 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.

3.2.5.3 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.

3.2.6 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

3.2.7 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.

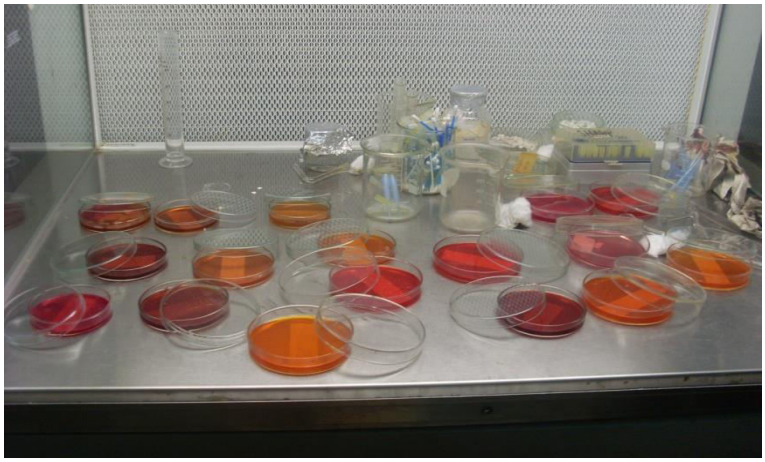

Figure 3.4: Petri dishes preparation

3.2.8 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

3.2.9 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 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.

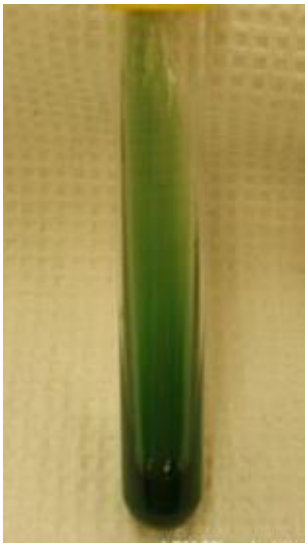

Figure 3.8: Preparation of test tubes for Citrate test

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 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 to identify acid and alkali. K indicates acid and A indicates alkali. It can be K/A, A/K, K/K or even A/A for slant/butt.

3.4.7 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

3.5 Cell Counting and Serial Dilutions

3.5.1 Theory:

In quantitative microbiology, we are concerned with determining the concentration of colony forming units (CFUs) in our sample – i.e., the number of CFUs per ml or per gram of the sample. More realistically, the concentration of CFUs in the sample could have been considerably greater. Counting the colonies on a plate inoculated with one ml of sample may be impossible. It is desirable to have "countable" plates – containing between 30 and 300 colonies. If fewer than 30, we run into greater statistical inaccuracy. If greater than 300, the colonies would be tedious to count and also would tend to run together.

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

3.5.2 Materials Required:

1. Tubes
2. Micropipette with tips

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

3.5.3 Procedure:

There are four major steps in the procedure:

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

3.5.4 Preparation of Serial Dilutions

1. A sample was taken containing the bacteria to be counted.
2. Four test tubes were taken and labeled them 10^{-1} to $10^{-4.}$
3. Nine mL of distilled water was pipette into each of the tubes.
4. One gm of the undiluted sample was given into the tube marked 10^{-1}. The contents were mixed and using a new pipette 1 mL from the 10^{-1} tube was pipette into the 10^{-2} tube.
5. This was continued until transfers had been completed to the 10^{-4} tube.
6. Therefore the following dilutions of the original sample were obtained.

Table 3.3: Preparation of Serial Dilutions

Tubes	Dilution	Dilution	Dilution Factor
1	10^{-1}	1/10	10^{1}

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

3.5.5 Mixing the dilutions into agar plates

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

CHAPTER 04

RESULT

4.1 Result

4.1.1 Bacterial colony morphology

Table 3.4: Bacterial colony morphology isolated from different street-vended food samples

Name of Universitiy		Agar Plates				
	Sample	**MacConk**	**TBX**	**BGA**	**XLD**	**TCBS**
Prime Asia University (PAU)	Laddu 7	Pink, Flat Pink	Blue	No growth	No growth	No growth
	Nargis Kabab	Colorless	No growth	No growth	No growth	No growth
	Kathi Kabab	No Growth	No growth	No growth	No growth	No growth
BRAC University (BU)	Chola 1	Flat Pink	Blue	No growth	No growth	No growth
	Dimchop 2	Pink	No growth	No growth	No growth	No growth
	Vanilla Cake	No growth	No growth	No growth	No growth	No growth
East West University (EWU)	Dimchop 1	Colorless	No growth	No growth	No growth	No growth
	Boroi Achar Amrar	No growth	No growth	No growth	No growth	No growth
	Amrar Achar	No growth	No growth	No growth	No growth	No growth
Bangladesh University of Professional (BUP)	Samucha 4	No growth	No growth	No growth	No growth	Yellow
	Jilapi 1	Flat pink	No growth	No growth	No growth	No growth
	Porota	No growth	No growth	No growth	No growth	No growth
United International University (UIU)	Samucha 7	No growth	No growth	No growth	No growth	Yellow
	Shingara 7	Mucoid pink	No growth	No growth	No growth	No growth
	Chicken Toast	No growth	No growth	No growth	No growth	No growth

Table 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 9 samples show growth of different pathogenic or non

pathogenic microorganisms. Of which 4 samples show positive growth of our suspected organisms (*E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp.)* and 6 samples show no growth in these agar media. The reason for observing no growth in sample may include the following:

a) sometimes fresh foods were collected early in the morning so no contamination occurred yet, b) sometimes food were hot which prevented growth of bacteria.

Table 3.5: Bacterial colony morphology isolated from different street vended food samples

Name of University		Agar Plates				
	Sample	MacConkey	TBX	BGA	XLD	TCBS
Government Titumir University (GTU)	Dimchop 7	Mucoid pink	No growth	No growth	No growth	Yellow
	Laddu 6	Colorless	No growth	No growth	No growth	No growth
	Patishapta pitha	No growth	No growth	No growth	No growth	No growth
Ahsanullah University of Science and Technology (AUST)	Dimchop 4	No growth	Blue	No growth	No growth	No growth
	Jilapi 2	Colorless	No growth	No growth	No growth	No growth
	Bun	No growth	No growth	No growth	No growth	No growth
University of Liberal Arts Bangladesh (ULAB)	Dimchop 5	Mucoid pink, Pink	No growth	No growth	No growth	Yellow
	Chocolate cake	Colorelss	No growth	No growth	No growth	No growth
	Amer morobba	No growth	No growth	No growth	No growth	No growth
Daffodil University (DU)	Dimchop 3	No growth	No growth	No growth	No growth	Yellow
	Danish	No growth	No growth	No growth	No growth	No growth
	Toast Biscuit	No growth	No growth	No growth	No growth	No growth
Dhaka International University (DIU)	Shingara 4	Pink	No growth	No growth	No growth	No growth
	Misty	No growth	No growth	No growth	No growth	No growth
	Cream roll	No growth	No growth	No growth	No growth	No growth

Table 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 8 samples show growth of different pathogenic or non

pathogenic microorganisms. Of which 3 samples show positive growth of our suspected organisms (*E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp.*) and 7 samples shows no growth in these agar media. The reason for observing no growth in sample may include the following:

a) sometimes fresh foods were collected early in the morning so no contamination occurred yet, b) sometimes food were hot which prevented growth of bacteria.

Table 3.6: 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 spp*	No. of samples with +ve growth by *Vibrio spp*	No. of samples with +ve growth by *Shigella spp*	No. of samples with +ve growth by *Salmonella spp*
PAU	2	1	0	0	0
BU	2	2	0	0	0
EWU	1	0	0	0	0
BUP	1	0	1	0	0
UIU	1	0	1	0	0
GTU	2	1	1	0	0
AUST	2	0	0	0	0
ULAB	3	0	1	0	0
DU	0	0	1	0	0
DIU	1	0	0	0	0

30 food samples were collected from street vendors in the area around 10 private universities of Dhaka city. About 17 (56.66%) food samples were contaminated with pathogenic or non pathogenic microorganisms. Of which 7 (23.33%) samples were suspected to be contaminated with our targeted organisms (*E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp.)*

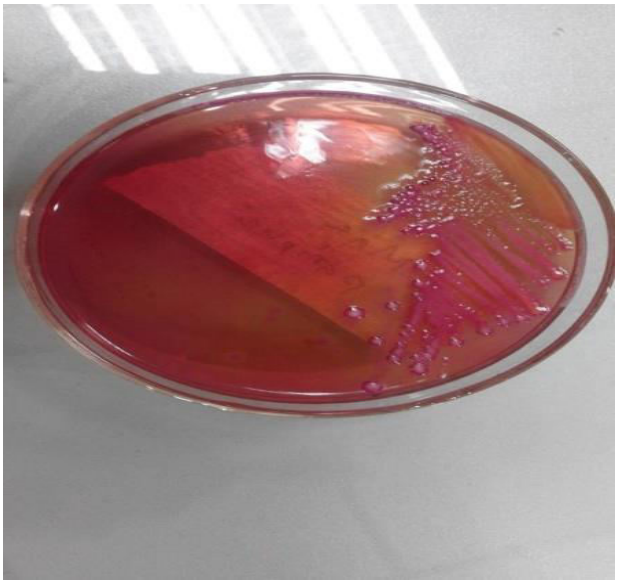

Figure 4.1: Bacterial colony (pink) on MacConkey agar plate

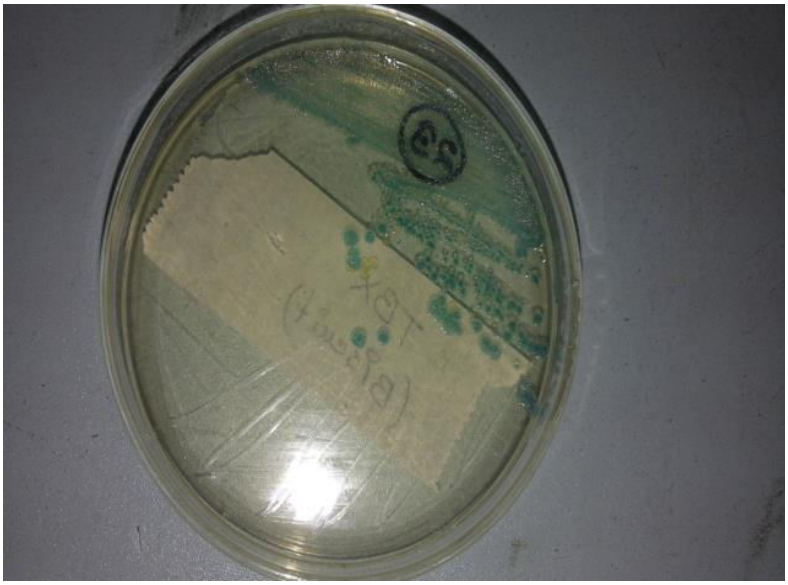

Figure 4.2: Bacterial colony (blue) on TBX agar plate

4.2 Suspected organism from different biochemical test

Table 3.7: Identification of the suspected organism (*Klebsiella* species) from different biochemical tests

Samples	Plates	Colony Morpho-Logy	M	I	O	Ci-trate	Urea se	Oxi-Dase	KIA			
									Slunt/butt	H_2S	Gas	
Laddu 7	Mac Conkey	Pink	_	+	_	+	_	_	A/A	_	+	***Klebsiella* spp.**
	Mac Conkey	Flat pink	_	+	_	+	_	_	A/A	_	+	
	TBX	Blue	_	+	_	+	_	_	A/A	_	+	
Chola 1	TBX	Blue	_	+	_	_	_	_	A/A	_	+	
Dimchop 4	TBX	Blue	_	+	_	+	_	_	A/A	_	+	
Dimchop 7	Mac Conkey	Mucoid Pink	_	_	_	+	_	_	K/A	_	+	

Among 17 (56.66%) food samples were subjected for different biochemical test to identify our targeted organisms. Biochemical test results of about 7 (23.33%) 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 shows identification of the suspected organism (*Klebsiella spp.*) from different biochemical test. In total 4 (13.33%) food samples were identified to be contaminated with our suspected organism *Klebsiella spp.* from these biochemical tests.

Biochemical test results for the samples Laddu 7, Chola 1, Dimchop 4, and Dimchop 7 matched with the standard results for *Klebsiella* species. So, we can say that the samples may contain the *Klebsiella* species.

Table 3.8: Identification of the suspected organism (*E.coli* and *Vibio spp.*) from different biochemical test

Sample	Plates	Colony mor-phology	M	I	O	Cit-rate	Ure-ase	Oxi-dase	KIA			Organism
									Slunt /butt	H_2S	Gas	
Chola 1	Mac Conkey	Flat Pink	+	+	–	–	–	–	A/A	–	+	*E. coli*
Dimchop 5	Mac Conkey	Flat pink	+	+	–	–	–	–	A/A	–	+	
Samucha 4	TCBS	Yellow	+	+	–	+	–	–	K/A	–	+	*Vibrio spp.*
Samucha 7	TCBS	Yellow	+	+	–	+	–	–	K/A	–	+	

Among 17 (56.66%) food samples were subjected for different biochemical test to identify our targeted organisms. Biochemical test results of about 7 (23.33%) 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 shows identification of the suspected organism (*E.coli and Vibio spp.*) from different biochemical test. In total 2 (6.6%) food samples were identified to be contaminated with E.coli and 2 (6.6%) food samples were identified to be contaminated with *Vibio spp.* from these biochemical tests.

Figure 4.3: Different Biochemical test

Table 3.9: Presence of suspected organisms in no of food samples from different university (n=7)

Name of University	*E.coli*	*Klebsiella spp*	*Vibrio spp*	*Shigella spp*	*Salmonella spp*
PAU	0	3	0	0	0
BU	1	1	0	0	0
EWU	0	0	0	0	0
BUP	0	0	1	0	0
UIU	0	0	1	0	0
GTU	0	1	0	0	0
AUST	0	1	0	0	0
ULAB	1	0	0	0	0
DU	0	0	0	0	0
DIU	0	0	0	0	0

Table shows presence of suspected organisms in number of food samples from different university. In total 7 (23.3%) food samples from different university were suspected to be contaminated with our targeted organisms *E.coli, Klebsiella spp. and Vibio spp.*.

Among them, from PAU we got 3 *Klebsiella*, from BRAC 1 *E. coli* and 1 *Klebsiella*, from BUP 1 *Vibrio*, from UIU 1 *Vibrio*, from GTU 1 *Klebsiella,* from AUST 1 *Klebsiella,* from ULAB 1 *E.coli.* In total, we got 4 (13.3%) *Klebsiella*, 2 (6.6%) *Vibrio and* 2 (6.6%) *E. coli* species.

Table 3.9.1: Incidence of food borne pathogens in various street vended food samples

Pathogen	Food Categories					
	Deep fried and fried items (n=14)	Spicy items (n=1)	Achar items (n=3)	Baked items (n=6)	Sweet items (n=4)	Total (n=30)
E.coli	1 (7.14%)	1 (100%)	Nd	Nd	Nd	2 (6.66%)
Klebsiella spp	2 (14.28%)	1 (100%)	Nd	Nd	1(25%)	4 (13.33%)
Vibrio spp.	2 (14.28%)	Nd	Nd	Nd	Nd	2 (6.66%)
Shigella spp	Nd	Nd	Nd	Nd	Nd	Nd
Salmonella spp	Nd	Nd	Nd	Nd	Nd	Nd

Table shows the incidence of food borne pathogens in various street vended food samples. Among 14 deep fried and fried items, 1 (7.14%) sample was suspected to contain *E.coli,* 2 (14.28%) samples were suspected to contain *Klebsiella spp* and 2 (14.28%) samples were suspected to contain *Vibrio spp*. Among 1 spicy item, 1 (100%) sample was suspected to contain *E.coli*. and *Klebsiella spp.* Among 4 sweet items, 1 (25%) sample was suspected to contain *Klebsiella spp*.

4.3 Counting bacterial colonies

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

Table 3.9.2: Colony counting of various samples

Sample Name	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Nargis Kabab	Uncountable	Uncountable	35	27
Chola	Uncountable	Uncountable	33	8
Laddu	Uncountable	Uncountable	56	38
Boroi achar	Uncountable	36	22	13
Alur Chop	Uncountable	31	18	7
Tikia	Uncountable	Uncountable	42	31

For nargis kabab plate 3 was selected as it has between 30-300 colonies. The other plates cannot be used because they are either too thick with colonies or too thin. The calculation of cell concentration in the original culture is:

35 colonies on plate 3 x dilution factor of 1000 = 35,000 cells/ml.

For chola plate 3 was selected as it has between 30-300 colonies. The other plates cannot be used because they are either too thick with colonies or too thin. The calculation of cell concentration in the original culture is:

33 colonies on plate 3 x dilution factor of 1000 = 33,000 cells/ml.

For laddu plate 3 was selected as it has between 30-300 colonies. The other plates cannot be used because they are either too thick with colonies or too thin. The calculation of cell concentration in the original culture is:

56 colonies on plate 4 x dilution factor of 1000 = 56,000 cells/ml.

For boroi achar plate 2 was selected as it has between 30-300 colonies. The other plates cannot be used because they are either too thick with colonies or too thin. The calculation of cell concentration in the original culture is:

36 colonies on plate 2x dilution factor of 100 = 3,600 cells/ml.

For alur chop plate 2 was selected as it has between 30-300 colonies. The other plates cannot be used because they are either too thick with colonies or too thin. The calculation of cell concentration in the original culture is:

31 colonies on plate 2 x dilution factor of 100 = 3,100 cells/ml.

For tikia plate 2 was selected as it has between 30-300 colonies. The other plates cannot be used because they are either too thick with colonies or too thin. The calculation of cell concentration in the original culture is:

42 colonies on plate 3 x dilution factor of 1000 = 42,000 cells/ml.

Table 3.9.3: Number of colonies per ml of sample

Sample Name	Nargis Kabab	Chola	Laddu	Boroi Achar	Alur Chop	Tikia
Colony forming unit (CFU) (cells/ml)	35,000	33,000	56,000	3,600	3,100	42,000

CHAPTER 05

DISCUSSION AND CONCLUSION

5.1 Discussion

At present time, foodborne illness has become major international health problem and an important cause of reduced economic growth. The rate of foodborne illness occurring around the world is increasing day by day, mostly in developing countries like Bangladesh. The street food sector has become an important component of food distribution system in many cities in both developing and industrialized countries, particularly for midday meals. However, certain street-vended food can pose significant risk to consumers due to microbiological contamination and this contamination is causing increased outbreaks of foodborne illness. The factors responsible are mainly lack of proper education and knowledge regarding safe handling of foods, preparing food in unhygienic conditions, lack of supervision of street vended foods and lack of awareness of general people about street vended food.

Therefore this research project was undertaken to identify the microbial contamination of street vended foods and isolate enteric bacteria specially *E.coli*, *Klebsiella spp*., *Vibrio spp*., *Salmonella spp.* and *Shigella spp.* from street vended food items collected around 10 private university premises in Dhaka city. A total of 30 samples were collected from different street vendors around 10 universities. Five different agar media were used for identification and isolation of our suspected microorganisms from the food samples. The agar media used were MacConkey, TBX or Tryptone Bile X-glucoronide agar, BGA or Brilliant Green Agar, XLD or Xylose Lysine Deoxycholate and TCBS or Thiosulfate Citrate-Bile Salts Sucrose agar. Microbial growth was observed in different media plates.

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 in Amravati city, India to identify microbiological hazard of ready to eat foods sold on the streets in 2008. A total of 55 samples were collected from around the Amravati city and analyzed for contamination by microorganisms. The bacterial pathogens identified were *P. aeruginosa* (39%), *E.coli* (21%), *S. aureus* (16%), *Salmonella spp.* (12%) and *Proteus spp.* (12%). The highest frequencies of occurrence of bacterial pathogens were *P. aeruginosa* in samosa (25%), *E. coli* in kachori (32%), *S. aureus* in kachori (27%), *Proteus spp.* in palakwada (45%) and (36%) *Salmonella spp.* in samosa. Food contamination in Amravati City streets was

mainly due to poor water quality and hygiene during food preparation, washing of utensils, poor personal and domestic hygiene, peeling of fruits long before consumption, and crowded, dusty and poorly maintained shopping areas (Tambekar et al, 2008).

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

In our present research study, 30 food samples were collected from private university premises and analyzed for microbial contamination. Among 30 samples, contamination was present in 17 samples (56.66%). In this 17 samples, 7 samples were suspected to contain our targeted microorganisms (*E.coli*, *Klebsiella*, *Vibrio*, *Shigella* and *Salmonella* species). In total 7 samples, 3 amples were suspected to be contaminated with Klebsiella spp., 2 samples were suspected to be contaminated with E.coli and 2 samples were suspected to be contaminated with Vibrio species. No Salmonella and Shigella species were found in the food samples. Several biochemical tests were performed for characterization of the microorganisms but PCR was not done. So it cannot be said confidently that colonies of the media plates are the claimed ones.

Street vended food has now become a major source of health problems and the cause of foodborne illness outbreaks. So more research work should be performed in this sector to correctly identify and isolate the enterobacteria responsible for causing these diseases. And street food vendors should be monitored so that they maintain proper hygiene and safety in handling and preparing food.

5.2 Conclusion

Street food has become an important part of diet for many people as such food is easily accessible and affordable. It also plays an important role in providing employment opportunities

for millions of men and women with limited education or skills, especially as the initial investment is low. In contrast to its benefits, it is also recognized that street-food vendors are often poor and uneducated and lack appreciation for safe food handling.

In this present study, we have found that among 30 samples, contamination was present in 17 samples (56.66%). In these 17 samples, 7 (23.3%) samples were suspected to contain our targeted microorganisms (*E.coli*, *Klebsiella*, *Vibrio*, *Shigella* and *Salmonella* species). In total 7 samples, 3 (10%) samples were suspected to be contaminated with Klebsiella spp., 2 (6.6%) samples were suspected to be contaminated with E.coli and 2 (6.6%) samples were suspected to be contaminated with Vibrio species. No Salmonella and Shigella species were found in the food samples.

So it can be concluded that street foods are a major public health risk. If a community is to have the full benefits of street vended foods with minimal risk of foodborne disease, government intervention is required to ensure that the standard of safety for such foods is the best attainable in the context of the prevailing local situation.

So, education and training of street-food vendors may offer the most cost-effective way to reduce the incidence of foodborne disease. While some countries have conducted training programmes to educate vendors on food safety, the development of training materials for vendors has to be tailored to meet their needs and situations.

CHAPTER 06

REFERENCE

Reference List:

Adams, M.R. and Moss, M.O., (2008) *Food microbiology Cambridge, RSC Publ*. [Online] Available at: http://197.14.51.10:81/pmb/AGROALIMENTAIRE/Food%20Microbiology.pdf (Accessed 20th November, 2016)

Centre for Disease Control and Prevention (2015) New CDC Data on Foodborne Disease Outbreaks [Online] Available at: http://www.cdc.gov/features/foodborne-diseases-data/

(Accessed 21st November, 2016)

Centre for Disease Control and Prevention (2016) *Foodborne Germs and Illness*

Available at: http://www.cdc.gov/foodsafety/foodborne-germs.html

(Accessed 2nd November, 2016)

Centre for Disease Control and Prevention (2016) *Klebsiella pneumoniae* in Healthcare Settings Available at: http://www.cdc.gov/hai/organisms/klebsiella/klebsiella.html (Accessed 18th November, 2016)

Cleveland Clinic (2004) *Foodborne Disease* Available at: http://www.clevelandclinicmeded.com/medicalpubs/diseasemanagement/infectious-disease/foodborne-disease/ (Accessed 3rd November, 2016) (Accessed 4th November , 2016)

Kaper, J.B., Nataro, J.P. and Mobley, H.L., (2004) Pathogenic escherichia coli. *Nature Reviews Microbiology*, *2*(2), pp.123-140. Available at: http://www.tcd.ie/academicunits/schools/medicine/clinical_microbiology/assets/docs/MSC/nrmicro818.pdf (Accessed 4th November, 2016)

Li, B., Zhao Y., Liu C., Chen Z. & Zhou D. (2014) Molecular pathogenesis of Klebsiella pneumoniae. *Future Microbiology*, 9(9), 1071-81.

Lynch, M. F., Tauxe, R. V., & Hedberg, C. W. (2009). The Growing Burden of Foodborne Outbreaks Due to Contaminated Fresh Produce: Risks and Opportunities. *Epidemiology and Infection*, 137, 307-315.

Mahmoud, B. S. M. (ed.) (2011) *Salmonella – A Dangerous Foodborne Pathogen.* [Online] Croatia, InTech. Available from: www.intechopen.com [Accessed 29th April 2016].

Monday, I. E., Francis, J.I. & Mohammad, S.U. (2014) Microbiological Quality of Ready-To-Eat Foods (Rice and Moimoi) Sold By Food Vendors in Federal Polytechnic Bali,Taraba State Nigeia. *Journal of Environmental Science, Toxicology and Food Technology*, 8(2), 145-149.

NCBI (1996) Salmonella Available at: https://www.ncbi.nlm.nih.gov/books/NBK8435/ (Accessed 20th November, 2016)

Rane, S., (2011) Street vended food in developing world: hazard analyses *Indian journal of microbiology* [Online] *51*(1), pp.100-106. Available at: http://link.springer.com/article/10.1007/s12088-011-0154-x (Accessed 3rd November, 2016)

UC Food Safety (2016) *Populations at Risk for Foodborne Illness* Available at: http://ucfoodsafety.ucdavis.edu/Populations_at_Risk_for_Foodborne_Illness/ (Accessed 3rd November, 2016)

Wiley Online Library (2012) Factors Affecting the Growth of Microorganisms in Food Available at: http://onlinelibrary.wiley.com/doi/10.1002/9781119962045.ch20/summary

World Health Organization (1996) *Essential safety requirements for street-vended foods*. Available at: http://apps.who.int/iris/bitstream/10665/63265/1/WHO_FNU_FOS_96.7.pdf (Accessed 2nd November, 2016)