Identification of microbiological contamination of ready to eat food vended in streets of different institutions in Dhaka city, Bangladesh

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

Submitted by

Arzu Arafin Lisa

ID: 2013-1-70-069

Department of Pharmacy

East West University

Declaration by the Candidate

I, Arzu Arafin Lisa, hereby declare that the dissertation entitled "Identification of microbiological contamination of ready to eat food vended in streets of different institutions of Dhaka city, Bangladesh" submitted by me to the Department of Pharmacy, East West University and in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, work carried out by me during the period 2016 of my research in the Department of Pharmacy, East West University, under the supervision and guidance of Ms. Nafisa Tanjia, Senior Lecturer, Department of Pharmacy, East West University. The thesis paper has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

Arzu Arafin Lisa

ID: 2013-1-70-069

Department of Pharmacy

East West University, Dhaka.

Certificate by the Supervisor

This is to certify that the thesis entitled “Identification of microbiological contamination of ready to eat food vended in streets of different institutions of Dhaka city, Bangladesh” submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by Arzu Arafin Lisa, ID: 2013-1-70-069, during the period 2016 of her research in the Department of Pharmacy, East West University, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

_____

Nafisa Tanjia

Senior Lecturer

Department of Pharmacy

East West University, Dhaka.

Certificate by the Co-Supervisor

This is to certify that the thesis entitled "Identification of microbiological contamination of ready to eat food vended in streets of different institutions of Dhaka city, Bangladesh" submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by Arzu Arafin Lisa, ID: 2013-1-70-069, during the period 2016 of her research in the Department of Pharmacy, East West University, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

_____

Dr. Sufia Islam

Professor

Department of Pharmacy

East West University, Dhaka.

Certificate by the Chairperson

This is to certify that the thesis entitled “Identification of microbiological contamination of ready to eat food vended in streets of different institutions of Dhaka city, Bangladesh” submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by Arzu Arafin Lisa, ID: 2013-1-70-069, during the period 2016 of her research in the Department of Pharmacy, East West University

_____

Dr. Shamsun Nahar Khan

Associate Professor & Chairperson

Department of Pharmacy

East West University, Dhaka

ACKNOWLEDGEMENT

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

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

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

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

Abstract

Street vended foods are easily available sources of meals for many people but the biological safety of this type food is invariably questionable. The aim of this study was to isolate and identify the existence of enteric bacteria in various street vended foods collected from different private universities in Dhaka city. From established and moving vendors thirty food samples were collected from area around 10 private universities in Dhaka city. Food samples that were taken for bacteriological tests were shingara, alur chop, laddu, pakora, beguni, samaucha, kabab e.t.c. About three different food samples were collected from each university using cleaned polythene bags each day. These food samples were tested for the presence of presence enteric microorganisms using standard microbiological methods. For the confirmation of *Escherichia coli, Klebsiella* spp, *Shigella* spp, *Salmonela* spp, *Vibrio* spp biochemical tests were done. Among the thirty food samples four (13.3%) were contaminated with *E. coli,* one food sample (3.33%) was suspected to be contaminated with *Klebsiella* spp and two food samples(6.67%) were suspected to be contaminated with *Vibrio* spp. We have also performed colony counting of additional six food samples by standard methods. The samples were fuchka, noodles, cake, butter nun, vhelpuri, ghugni. Out of six samples maximum uncountable colonies were found in vhelpuri. These enteric pathogens present in street vended foods can be a potential cause of foodborne illnesses. Therefore the vendors need hygiene education for the handling and preparation of these street vended foods.

Key Words: Street foods, *Escherichia coil, Klebsiella* spp, *Vibrio* spp, Enteric pathogen, Private Universities, Dhaka city.

List of Contents

List of Tables

List of Figures

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 Syndome
CFU	Colony Forming Unit
TSB	Trypticase Soy Broth
YE	Yeast Extract
BPW	Buffered Peptone Water
APW	Alkaline Peptone Water

TBX	Tryptone Bile X-glucoronide
BGA	Brilliant Green Agar
XLD	Xylose Lysine Deoxycholate
TCBS	Thiosulfate Citrate Bile salts Sucrose
KIA	Kliglar Iron Agar

Chapter-01

Introduction & Literature Review

1.1: Street Vended Foods:

Street vended foods are ready to-eat foods and beverages arranged or potentially sold by street vendors and peddlers particularly in streets and other comparable open spots. Street vended foods are not just refreshing for their one of a kind flavor, mouth watering taste; they have additionally gotten to be critical and crucial for keeping up the healthful status of the populaces. Other than offering business open doors for creating business people, the offer of street foods can make a sizeable commitment to the economies of developing nations. In India, the National Policy for Urban Street Vendors/Hawkers expressed that road sellers constitute around 2% of the number of inhabitants in a city. The street vended foods are prepared under unhygienic conditions and displayed openly leading to a high degree of contamination. Thus, from the health point of view, the microbiological quality of street vended foods becomes important as food can act as a major source for transmission of food borne infections and intoxications (Rane, 2016).

In developing nations food sold by road sellers is the significant wellspring of food borne disease. Despite the fact that foods from these outlets are acknowledged for the most part for their one of a kind flavor and for their benefit, their microbiological wellbeing is not generally certain (Islam, Sufia, et al, 2015). The conventional preparing techniques that are utilized as a part of the preparation, wrong holding temperature and poor individual cleanliness of food handlers are a portion of the fundamental driver of tainting of prepared to eat foods. These types of foods are also not secured from dust, soil and flies. Street foods are seen to be a noteworthy general wellbeing hazard because of absence of essential framework and administrations, trouble in controlling the vast quantities of street food distributing operations in view of their differing qualities, versatility and transitory nature. The street food industry plays a crucial part in meeting food necessities of workers and urban occupants in numerous urban communities and towns of developing countries. It feed a huge number of individuals every day with a wide range of food that is generally low in price and easily available (Feglo and Sakyi, 2012).

1.2: Varieties of Street Vended Foods:

A wide variety of street vended foods are available in different countries. Street vended foods varies in different countries based on the cultural norms, geography etc. Some common stuff like salads, seafood's, chicken items, gravy etc are available in every country. In Bangladesh there are a wide variety of street vended foods are available. Similar to other developing countries in Bangladesh street vended food is a movement that gives work to numerous at the same time giving ready to eat and low price foods to many workers and low salary bunches. Street food shops are very small so they can place it on a moving small vehicle and set it out anywhere ranging from in the premises of nursery schools, colleges, and universities, various corporate offices, markets, shopping malls, in the footpath etc. Some of the popular food items in our country are:

Pitha – In the winter season vapa pitha is an extremely regular road sustenance thing. Vapa pitha seller or vendors are regularly ladies. At night they get ready vapa pitha. furthermore, Vapa pitha is an extremely well known night and morning breakfast menu.

Chotpoti and puchka – Chotpoti and puchka are extremely prominent among youthful natives. Chotpoti and phuchka shops are accessible anyplace nearby and urban areas.

Jhal Muri – Jhalmuri is generally accessible and a top pick among youngsters. It is typically served in cone formed paper.

Badam vaja – Badam vaja (singed peanuts) is a period pass sustenance thing and exceptionally mainstream.

Vhelpuri – Vhelpuri is also a common type of food items that are found on streets and they have wide amount of popularity among young people, children, workers etc.

Fruit juices- Fruit juices are well-known beverages. They are sold in stalls that are located in the roadside.

Others – Other prominent street foods are puri, somucha, singara, beguni, alu chop, dim chop, different types of kabab and so on these are fried (Rahman, Rahman & Ansary, 2014).

1.3: Food Borne Diseases:

Food borne diseases result in considerable morbidity, mortality and economic costs. So it becomes globally important. Food borne maladies are in charge of a large burden of illness (morbidity) and demise (mortality) in both asset rich and asset poor nations. Food borne illness is caused by consuming food or beverages that are contaminated by disease causing microbes (pathogen). More than 200 ailments can be transmitted to individuals through the ingestion of food debased with microorganisms (microscopic organisms, infections, and parasites) or with chemicals. Contamination of food can happen at any phase of the food processing—on farms where crops are grown and animals raised, in industrial facilities where food is handled, and amid food stockpiling and readiness in shops, eateries and the home. Numerous food borne sicknesses (for instance, norovirus, Escherichia coli, and campylobacter diseases) give gastrointestinal indications—stomach spasms, looseness of the bowels, and spewing. Be that as it may, some foodborne ailments cause manifestations influencing different parts of the body and some have genuine sequelae (unusual substantial conditions or illnesses emerging from a previous malady). For instance, contamination with a few strains of E. coli can prompt to kidney impairment (Kirk et al., 2015). The orofecal route has been perceived as the most essential method of transmission for pathogenic organisms from food handlers to food. The potential for the sullying of street food with pathogenic microorganism has been well documented and several disease outbreaks have been followed to administration of street foods. The street foods are contaminated with enteropathogens, for example, Escherichia, Salmonella, Shigella and Enterobacter alongside poison delivering microscopic organisms, for example, Staphylococcus and Clostridium species. Aside from these, foods may likewise be contaminated with Pseudomonas, Bacillus, Vibrio and Klebsiella sp. Around 250 distinctive foodborne maladies have been portrayed by Center for Disease Control in 2011. Most foodborne illnesses are acute, meaning they happen suddenly and last a short time, and most people recover on their own without treatment. Rarely, food borne illnesses may lead to more serious complications (Sharma & Mazumdar, 2014).

1.4: Who Gets Food Borne Illnesses

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

- infants and children
- pregnant women and their fetuses
- older adults
- people with weak immune systems

These groups also have a greater risk of developing severe symptoms or complications of foodborne illnesses (Niddk.nih.gov, 2016).

1.5: Causes of Food Borne Diseases:

The dominant parts of food borne diseases are brought on by destructive microscopic organisms and viruses. Some parasites and chemicals additionally cause food borne sicknesses.

1.5.1 Microorganisms

Microorganisms are small life forms that can bring about contaminations of the GI tract. Not all microscopic organisms are destructive to people.

Some unsafe microorganisms may as of now be available in foods when they are purchased. Crude nourishments including meat, poultry, fish and shellfish, eggs, unpasteurized milk and dairy items frequently contain microorganisms that cause food borne ailments. Microbes can contaminate food—making it unsafe to eat—whenever amid development, harvesting, handling, processing, storage and shipping.

Foods may likewise be polluted with microscopic organisms during food preparation in restaurant or home kitchen. In the event that food preparers don't altogether wash their hands, kitchen utensils, cutting sheets, and other kitchen surfaces that come into contact with crude food, cross-contamination—the spread of microscopic organisms from contaminated food to uncontaminated food may happen.

In the event that hot food is not kept sufficiently hot or cool food is not kept sufficiently cold, microbes may increase. Microscopic organisms duplicate immediately when the temperature of food is somewhere around 40 and 140 degrees. Cold has to be kept beneath 40 degrees and hot food should be kept above 140 degrees. When the food is refrigerated, bacteria multiply more slowly and freezing foods can assist moderate or even stop the spread of microbes. In any case, microscopic organisms in refrigerated or solidified nourishments get to be dynamic again when food is conveyed to room temperature. Properly cooking foods eliminates bacteria.

Numerous sorts of bacteria cause foodborne ailments. Example incorporates:

- *Salmonella*, a bacterium found in numerous foods, including crude and undercooked meat, poultry, dairy items, and fish. Salmonella may likewise be available on egg shells and inside eggs.
- *Campylobacter jejuni (C. jejuni),* found in crude or undercooked chicken and unpasteurized milk.
- *Shigella*, a bacterium spread from individual to individual. These microscopic organisms are available in the stools of individuals who are contaminated. In the event that individuals who are infected don't wash their hands properly after using bathrooms, they can contaminate food that they handle or prepare.
- *Escherichia coli (E. coli),* which incorporates a few unique strains, just a couple of which cause disease in people. E. coli O157:H7 is the strain that causes the most extreme ailment. Common sources of E. coli incorporate crude or undercooked cheeseburger, unpasteurized fruit juices, milk.
- *Listeria monocytogenes (L. monocytogenes),* which has been found in crude and undercooked meats, unpasteurized milk, soft cheeses, and prepared to-eat shop meats and sausage.
- *Vibrio,* a bacterium that may contaminate fish or shellfish.
- *Clostridium botulinum (C. botulinum),* a bacterium that may contaminate not properly canned foods and smoked and salted fish.

1.5.2 Viruses

Viruses are the smallest of the human infectious agents, ranging in size from approximately by 20-300 nm in diameter much smaller than bacteria. They contain one kind of nucleic acid, either RNA or DNA as their entire genome. Viruses cause infections that can lead to sickness. Individuals can pass viruses to each other. Viruses are available in the stool or vomit of individuals who are infected. Individuals who are infected with a virus may contaminate foods and beverages, particularly in the event that they don't wash their hands altogether subsequent to utilizing the washroom. Some common sources of food borne viruses are:

- Foods that are made by a person who are infected with a virus.

- Shellfish from polluted water
- Produce crop with contaminated water

Common food borne viruses are:

- *Norovirus*, which cause inflammation of the stomach and digestion systems
- *Hepatitis A*, which causes inflammation of the liver

1.5.3 Parasites

Parasites are small organisms that live inside another living organism. In developed countries, for example, the United States, parasitic contaminations are generally uncommon. *Cryptosporidium parvum* and *Giardia intestinalis* are parasites that are spread through water contaminated with the stools of individuals or animals that are infected. Foods that come into contact with contaminated water during growth or preparation can get to be polluted with these parasites. Food preparers who are infected with these parasites can also contaminate foods if they don't altogether wash their hands after utilizing the washroom and before handling of foods. *Trichinella spiralis* is a kind of roundworm parasite. Individuals might be contaminated with this parasite by expending crude or undercooked pork or wild amusement.

1.5.4 Chemicals

Unsafe chemicals that cause ailment may contaminate foods, for example,

- Fish or shellfish, which may feed on algae that produce toxins, prompting to high convergences of poisons in their bodies. A few sorts of fish, including tuna and mahi mahi, might be defiled with microscopic organisms that create toxins if the fish are not appropriately refrigerated before they are cooked or served.
- Certain sorts of wild mushrooms.
- Unwashed leafy foods that contain high groupings of pesticides (Niddk.nih.gov, 2016).

Table 1.5: Foodborne Illness Chart

Pathogen	Sign & Symptoms	Food involved
Salmonella (infection)	abdominal pain; diarrhea; chills; fever; nausea; vomiting	Poultry; meat and meat products; eggs and egg products; other food contaminated by the feces of infected humans and other animals.
Shigella (infection)	abdominal pain; diarrhea (sometimes bloody); chills; fever; dehydration	Moist prepared foods, especially salads such as potato, tuna and macaroni salads; raw fruits and vegetables; unpasteurized milk and dairy products; poultry
Staphylococcus (intoxication)	nausea; vomiting; abdominal pain; diarrhea	ham; meat; poultry; cream-filled pastry; food mixtures; leftover foods
Trichinella (infection)	abdominal pain; vomiting; nausea; fever; muscle pain.	pork; bear meat; walrus flesh
Yersinia (infection)	watery diarrhea; vomiting; abdominal pain; fever; headache; sore throat; may mimic appendicitis	Meats (especially pork, beef and lamb); tofu; oysters; fish; ice cream; powdered mild; unpasteurized milk; raw vegetables; soy products
Anisakis simplex (infection)	abdominal pain; vomiting; coughing	Salt water fish
Bacillus cereus (toxico-infection)	nausea; abdominal pain; diarrhea; vomiting	cereal products; rice; custards and sauces; meatloaf
Campylobacter jejuni (infection)	Diarrhea (sometimes bloody); severe abdominal pain; fever; anorexia.	Raw milk; poultry; beef liver; raw clams; contaminated water

Pathogen	Sign & symptoms	Food Involved
Clostridium botulinum (intoxication)	vertigo; double vision; difficulty in swallowing, speaking and breathing; weak muscles; respiratory paralysis. Frequently fatal.	home-canned low-acid food; garlic and oil mixtures; vacuum packed fish; fermented fish eggs; fish; marine
Clostridium perfringens (toxico-infection)	abdominal pain; diarrhea	cooked meat; poultry; gravy; sauces; soups
Cryptosporidium (infection)	severe diarrhea; lowgrade fever and severe intestinal distress	any food product that comes into contact with a contaminated person or contaminated water
Escherichia coli O157:h7 (E.coli) (toxico-infecton)	severe abdominal pain; diarrhea (sometimes bloody); nausea; vomiting; fever; chills; headache; muscular pain; bloody urine	soft unpasteurized cheese; contaminated water; any undercooked animal-source foods, especially hamburger
Giardiasis lamblia (infection)	abdominal pain; diarrhea; fever; cramps	water; raw vegetables and fruits
Hepatitis A (infection) 15 to 50 days	fever; anorexia; nausea, abdominal pain; jaundice	shellfish; contaminated water; any food contaminated by the feces, urine or blood of infected humans and other primates
Listeria monocytogenes (infection) 1 to 70 days	nausea; vomiting; stomach cramps; diarrhea; headache; constipation; fever	Unpasteurized milk; soft cheeses; undercooked poultry; prepared meats; unwashed raw vegetables
Norovirus (infection)	Nausea; vomiting; diarrhea; abdominal pain	Contaminated water, food, or food contact surfaces

(Foodsafe.ca, 2016)

1.6: Significant Factors Contributing to Microbial Contamination of Street-Vended Foods:

1.6.1. Sell out Location: Food Handling and Waste Disposal

The conditions under which some street vendors work are accounted for to be unsatisfactory for the making and vending of foods. Preparation of the food occurs either at home or at shops, which are situated in the road side and are comprised of wood, polythene packs, tin, and so on. The place of preparation is not generally neat and clean, sufficiently bright and not a long way from originating of contamination. Preparation surfaces utilized by a few vendors have stays of food arranged before that can advance cross contamination. A large portion of these foods are not secured and are presented to flies and dirt, which may harbor foodborne pathogens. Presences of animals, insects and liquid wastes have been accounted for in 70-90% of the cases. The two noteworthy sources from where the contaminants can enter the preparation area are: Inappropriate food handling and waste transfer.

1.6.2. Food Handling

The most frequently found source of contamination has been the unsanitary handling of street foods by the some of the vendor. Pathogens like *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter* and *S. aureus* can be carried by the vendors who consequently transfer these food borne hazards to the consumers. The most important medium for the transfer of organisms from faeces, nose, and skin to the food are the hands of the food handlers. Salmonella, non-typhi salmonellae, *Campylobacter* and *E. coli* can survive on finger tips and other surfaces for varying extents of times and in some cases even after washing are the findings that supports the reports of contamination of street vended food with toxigenic *S. aureus*, the major being suppurative lesions of human beings and the environment (Rane, 2011).

1.6.3 Waste Disposal

Few vendors gather in overcrowded places where there are high numbers of potential customers, which frequently provide narrow approach to fundamental sanitary facilities. Moreover, the waste which is generated by food processing is many times associated with the contamination of street food, which is usually dumped near the vending site. Wastes to be thrown into nearby streets and gutters are encouraged by the deficiencies of facilities for

liquid drainage and wastewater and garbage disposal. Habitats for rodents, breeding points for flies and media for growth of microorganisms can be acted by such areas. 85% of the vendors made foods like fish, fruit salads, roasted maize and chips in unhygienic conditions, provided that garbage and dirty waste were conspicuously near to the stalls is the revelation of the study conducted in Africa. In these areas bulk amount of garbage gather together which provide shelter for insects and animal pests that are connected to enteric disease transmission (*Shigella*, *Salmonella* and *E. coli*) (Rane, 2011).

1.6.4. Quality of Raw Materials: Water and Other Material

A very important factor is the quality of raw materials utilized in the making of street foods as their contamination can persist through preparation and or cooking.

1.6.4.1 Water

A critical raw material in many street-vended operations is water. Public health risk can be created by contaminated water when it is used for drinking, washing of foods, added in the food as an ingredient and utilized in the processing of food or utilized for washing equipment, utensils and hands. It is a well known carrier for enteropathogens such as *E. coli*, *Salmonella* spp. and *Campylobacter* spp. amongst others. The inaccessibility of potable water for various activities at the vending site is frequently pointed as a major concern in studies carried out in different regions of Asia, Africa, and South America. Especially for cleaning utensils and used dishes, many vendors tend to use the same water repeatedly due to the deficiency of clean potable water.

Frequent contaminations with coliforms and fecal coliforms of water have been revealed by the study which was carried out to find out the bacteriological quality of the utilized by some street vendors. It was reported found that 35% of foods were contaminated by *E. coli* while 57.5% of water utilized by vendors were contaminated by coliforms, when the street foods in Trinidad and Tobago were assayed. The findings that the stored water used by consumers and vendors, at the vending site, showed heavy bacteriological contamination of faecal origin which is analogous with these reports. A primary source of diarrheal diseases to the street food consumers is such heavily contaminated water. When water samples from storage tanks used by some vendors were examined at different domains in Pune, India, it was disclosed that 29.6% of the water samples were not conforming to the WHO standards of potability and had coliform counts of more than 16/100 ml, while fecal coliform counts were more than

16/100 ml in 15.5% of water samples, 4.5% of samples were positive for *E. coli* and 2.7% for enteropathogenic *E. coli*. Similarly, the water which is used by vendors for dishwashing pathogens such as *Salmonella* and *Shigella* has been identified (Rane, 2011).

1.6.4.2 Other Raw Materials

Other raw materials are also important to the safety of the street vended foods along with water because of the biological, chemical and physical hazards that they might acquaint. In order to keep prices down, some vendors buy low priced or adulterated ingredients containing unpermitted chemical additives from unauthorized suppliers which may further increase the risks associated with the food so prepared. Raw meat, poultry and vegetables are commonly contaminated with large numbers of bacteria, including potential foodborne pathogens such as *B. cereus*, *C. perfringens*, *C. jejuni*, *E. coli*, *L. monocytogenes*, *Salmonella* and *S. aureus*. A large number of microorganisms are harbored by spices which include members of the genus *Bacillus*, anaerobic sporeformers, enterococci, and members of Enterobacteriaceae, a variety of yeast and mould and pathogens like coagulase positive staphylococci. Foods contaminated by spices which act as spore carriers has been reported to give rise to food spoilage and can even give rise to food poisoning. Food spoilage may be lead by sporeformers in spices, when they survive the cooking process and multiply under favorable conditions.

In 30 of the 50 samples, unauthorized food additives were detected in 30 out of the 50 samples which were suspected of adulteration in a study done in Calcutta. Similarly, in raw chicken, salad and gravy raw materials pathogens like *B. cereus*, *S. aureus*, *C. perfringens*, *V. metschnikovii* and *E. coli* were reported. These organisms were believably present in these foods either before to purchase by vendors or may have been introduced by cross contamination during food handling or during preparation (Rane, 2011)

1.6.5. Utensils and Equipments: Chemical and Microbial Contaminants

A critical factor to the safety of street vended foods is often use of proper utensils for cooking and storage of prepared food. Toxin formation, pathogen growth or recontamination can be caused by poor quality of material coupled with inappropriate practices. A very important factor to food safety is the design, construction and maintenance of equipments, as their poor maintenance may lead to the inability to effectively clean and sanitize surfaces. This may then result in the buildup of residues of food, facilitating microbial growth, leading to an

increased liability of contamination. The proper use of equipment is also crucial to prevent the cross contamination from raw materials (Rane, 2011).

1.6.6. Chemical Contaminants

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

1.6.7. Microbial Contaminants

Utensils that are used to serve foods at the vending site are often contaminated with *Micrococcus* spp. and *Staphylococcus* spp. which may have originated from the vendors hands when they touched the food preparation areas, dishcloths, or the water during dish washing or hand washing which denotes cross contamination between dishwater, food preparation surfaces, and the food itself.

It is reported that utensil surface can contain bacteria from dirty dish washing water and other sources and can constitute a risk during the food vending process. Presence of *Salmonella* and *Shigella* has shown on microbiological analysis of utensils surface and knives. It is also reported that the raw material is cut and chopped using the same knife without in between cleaning during the preparation of food and such knives are often invaded by flies (Cardinale et al, 2005).

1.6.8. Food Preparation: Storage and Reheating

Food storage temperature is an important issue effecting food contamination and devoting to further increase in contamination. The key factors that devote to food poisoning outbreaks are the preparation of food long before its consumption, storage at room temperature, insufficient cooling and reheating, contaminated processed food, and undercooking.

1.6.9 Storage

A major contributor to the occurrence of food poisoning outbreaks is the keeping foods at high ambient temperatures for large extent of time have been reported. High microbial populations are harbored as foods are frequently kept for couple of hours after cooking and this incorporates overnight holding at ambient temperatures, until sold. Besides, some of the

foods are held in the pans in which they are cooked, until sold or reheated, which results in longer holding time, hence creating favorable conditions are further created for the growth of foodborne pathogens. The counts of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium perfringens* counts are reported to be high in such foods.

42 (26.3%) samples of fried fish, tuwo, soup, boiled rice and moin moin were analyzed in a study in India and they found that *B. cereus* is present which suggest that their spores survived the cooking process. The storage of these foods at ambient temperatures for several hours under high temperature and high relative humidity and the presence of this bacterium can be linked together which showed that the product could be potentially unsafe. When foods are held under conducive conditions for several hours *B. cereus* generates heat stable (emetic) and heat sensitive (diarrheal) toxins that why this bacterium has been considered to be responsible for foodborne illness outbreaks.

A number of pathogens, such as *E. coli*, *Salmonella typhimurium*, *Salmonella gallinarum*, *Shigella dysentriae*, *Pseudomonas fluorescens* and *Klebsiella pneumoniae* were also found to be present in the fruit chat sold by a street vendor in Chandigarh, India a study carried out by Kaul and Agarwal (Rane, 2011).

1.6.10. Reheating

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

1.6.11. Personal Hygiene of the Vendors or Food Handlers

WHO indicates that, in ensuring food safety throughout the chain of food production, processing, storage and preparation food handling personnel play an important role. Food vendors mishandling and omission of hygienic measures may enable pathogens to come into contact with food and in some cases to survive and multiply in sufficient numbers to cause illness in the consumer.

Cross contamination after handling raw materials can occur when the food handlers suffer from specific diseases may initiate biological hazards and physical hazards by careless food handling practices. Polythene bags are used by the most of the vendors to pack the food for their customers. They blow air into the polythene bags to open them during packing of these foods, in this process a number of pathogens can be passed on to the consumer.

Pathogenic microorganism including *Salmonella typhi*, *Staphylococcus aureus*, *Salmonella enteritidis*, and *Shigella* were found in over 30% of a group of food handlers whom were examined in a study carried out in Santa Fe de Bogota, Colombia (Ohiokephai, 2003).

1.7: Description of Some Common Microorganism Contributing to Foodborne Diseases:

1.7.1 *E. coli*

Escherichia coli (or *E. coli*) are the most prevalent infecting organism in the family of gram-negative bacteria known as enterobacteriaceae. 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. *E. coli* is very closely related to the genus *Shigella* genitically, although characteristically it ferments the sugar lactose and is otherwise far more active biochemically than *Shigella* spp. Late lactose fermenting, non-motile, biochemically inert strains of *E. coli* can however be difficult to distinguish from *Shigella. E. coli* 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. Generally a harmless commensal, it can be an opportunistic pathogen causing a number of infections such as Gram-negative sepsis, urinary tract infections, pneumonia in immunosuppressed patients, and meningitis in neonates. Its common occurrence in faeces, ready culturability, generally non-pathogenic character, and survival characteristics in water led to the adoption of *E. coli* as an indicator of faecal contamination and the possible presence of enteric pathogens such as S. Typhi in water. This usage has been transferred to foods where greater circumspection is required in interpreting the significance of positive results.

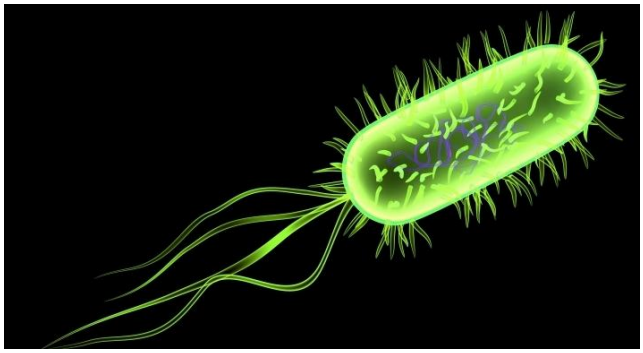

Fig. 1.1: ***Escherichia coli (E. coli)***

Strains producing diarrhoea are classified into three types based on their virulence properties:

1. enteropathogenic *E. coli* (EPEC),
2. enteroinvasive *E. coli* (EIEC),
3. enterotoxigenic *E. coli* (ETEC).

They are not very common causes of foodborne illness in developed countries, but an important cause of childhood diarrhoea in less developed countries.

ETEC is also frequently associated with so-called traveller's diarrhoea. Enterohaemorrhagic *E. coli* (EHEC) particularly associated with serotype O157:H7 has been recognized as the cause of a number of outbreaks of haemorrhagic colitis and haemolytic uraemic syndrome, particularly in North America, where foods such as undercooked ground meat, raw milk and fresh produce have been implicated. Two further types of *E. coli* are recognized as causes of diarrhoea, primarily in children. Termed enteroaggregative *E. coli* (EaggEC) and diffusely adherent *E. coli* (DAEC), they have characteristic patterns of adherence to Hep-2 cells in culture. The emergence of these numerous pathotypes of *E. coli* is thought to reflect the plasticity of the organism's genome. The acquisition, loss or rearrangement of genetic elements introduces new pathogenicity and virulence characterisitics and the different pathotypes represent strains sharing common virulence determinants.

1.7.1.1 Pathogenesis:

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

Illness caused by ETEC usually occurs between 12 and 36 h after ingestion of the organism. Symptoms can range from mild afebrile 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. The ingested organism resists expulsion from the small intestine with the rapidly flowing chyme by adhering to the epithelium through attachment or colonization factors in the form of fimbriae on the bacterial cell surface. Two toxin types are produced: the heat-stable toxins (ST), which can withstand heating at 100 1C for 15 min and are acid resistant, and the heat-labile toxins (LT) which are inactivated at 60 1C after 30 min and at low pH. LTI bears a strong similarity to cholera toxin; it consists of five B subunits (Mr 11.5 kDa) which are responsible for binding of the toxin to the epithelial cells and an A subunit (Mr 25 kDa) which is translocated into the epithelial cell where it activates adenylate cyclase. The subsequent increase in cAMP levels then inhibits Na^+, Cl^- and water absorption by the villus cells and stimulates their loss from intestinal crypt cells thus leading to profuse watery diarrhoea. LTII toxin produced by certain ETEC strains has similar biological activity to LTI but does not cross react with antiserum to LTI or cholera toxin.

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

Infection by EIEC results in the classical symptoms of an invasive bacillary dysentery normally associated with *Shigella*. Like *Shigella*, EIEC invades and multiplies within the epithelial cells of the colon causing ulceration and inflammation, though EIEC strains do not produce Shiga toxin. Clinical features are fever, severe abdominal pains, malaise and often watery diarrhoea which precede the passage of stools containing blood, mucus, and faecal leukocytes. The infective dose of EIEC appears to be greatly higher than for *Shigella* and this is thought to be a reflection of the organism's greater sensitivity to gastric acidity.

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

Pathogenesis is related to the ability of EPEC strains to adhere closely to the enterocyte membrane and produce the so-called attaching and effacing lesions. This is a complex and fascinating process mediated by the genes encoded on a 35 kb pathogenicity island called the locus of enterocyte effacement (LEA). Binding to the enterocytes occurs in three stages: non-intimate association mediated by pili, attachment or signal transduction, and then intimate contact. During this process the bacteria facilitate their own binding by producing a series of changes in the underlying enterocytes. A bacterial type III secretion system translocates another LEA encoded protein, Tir, into the enterocyte where it is incorporated into the cell's membrane. There it acts as a receptor for an outer membrane bacterial protein, intimin, which mediates close contact. The attachment stage is accompanied by increased levels of intracellular Ca21, release of inositol phosphates and activation of tyrosine kinase, an enzyme which phosphorylates tyrosine residues on intracelluar proteins. Following this the enterocytes accumulate filamentous actin as they form pedestal-like surface structures on which the bacteria rest. This results in deformation and loss of some microvilli; events which are thought to cause diarrhoea by disrupting the balance between absorption and secretion in the small intestine.

Symptoms of EPEC infection, malaise, vomiting and diarrhoea with stools containing mucus but rarely blood, appear 12–36 h after ingestion of the organism.

- ***Enterohaemorrhagic E. coli* (EHEC):**

EHEC, sometimes also known as Verotoxin-producing *E. coli* (VTEC), was first described in Canada where in some areas it competes with Campylobacter and Salmonella as the most frequent cause of diarrhoea. *E. coli* O157:H7 is the most common EHEC serotype reported, although others do occur. Nonmotile O111 and O157 are more common in Australia for example. EHEC has attracted attention not only because foodborne transmission is more common than with other diarrhoeagenic *E. coli*, but because the illness it causes can range from a non-bloody diarrhoea, through haemorrhagic colitis, to the life threatening conditions haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP).

Attachment is an important factor in virulence and O157:H7 strains possess the LEA pathogenicity island and adhere by a mechanism similar to EPEC, characterized by

intimate attachment of the bacteria to the epithelial cells and effacement of the underlying microvilli. EHEC strains produce the cytotoxin Verotoxin (so-called because of its ability to kill Vero (African Green Monkey Kidney) cells). Studies have revealed the presence of at least two toxins VTI and VTII (Adams & Moss, 2008).

1.7.2. *Salmonella spp.*

Salmonellas are members of the Enterobacteriaceae. They are Gramnegative, non-sporeforming rods (typically 0.5 mm by 1–3 mm) which are facultatively anaerobic, catalase-positive, oxidase-negative, and are generally motile with peritrichous flagella. Growth has been recorded from temperatures just above 5°C up to 47°C with an optimum at 37°C Salmonellas are heat sensitive and are readily destroyed by pasteurization temperatures.

Most *salmonellas* are regarded as human pathogens, though they differ in the characteristics and the severity of the illness they cause. Typhoid fever is the most severe and consequently was the earliest salmonella infection to be reliably described. *Salmonellas* are now established as one of the most important causes of foodborne illness worldwide (Adams & Moss, 2008).

Fig 1.2: *Salmonella spp.*

1.7.2.1 Pathogenesis:

Salmonellas are responsible for a number of different clinical syndromes grouped here as enteritis and systemic disease.

- ***Enteritis***

Ingested organisms, which survive passage through the stomach acid, adhere to the epithelial cells of the ileum via mannose-resistant fimbriae. They are then engulfed by the cells in a

process known as receptor mediated endocytosis. The ability of salmonellas to enter non-phagocytic cells is a property essential to their pathogenicity. Endocytosed *salmonellas* pass through the epithelial cells within a membrane-bound vacuole, where they multiply and are then released into the lamina propria via the basal cell membrane. This prompts an influx of inflammatory cells leading to the release of prostaglandins which activate adenylate cyclase producing fluid secretion into the intestinal lumen.

- ***Systemic Disease.***

Host-adapted serotypes are more invasive and tend to cause systemic disease in their hosts; a feature which is linked to their resistance to phagocytic killing. In humans, this applies to the typhoid and paratyphoid bacilli, *S. Typhi*, and *S. Paratyphi* A, B, and C, which cause the septicaemic diseases, enteric fever. Typhoid fever has an incubation period of anything from 3 to 56 days, though it is usually between 10 and 20 days. Invasive salmonellas penetrate the intestinal epithelium and are then carried by the lymphatics to the mesenteric lymph nodes. After multiplication in the macrophages, they are released to drain into the blood stream and are then disseminated around the body. They are removed from the blood by macrophages but continue to multiply within them. This eventually kills the macrophages which then release large numbers of bacteria into the blood stream causing a septicaemia. In this, the first phase of the illness, the organism may be cultured from the blood. There is a slow onset of symptoms including fever, headache, abdominal tenderness and constipation and the appearance on the body of rose red spots which fade on pressure. During the second stage of the illness, the organism reaches the gall bladder where it multiplies in the bile. The flow of infected bile reinfects the small intestine causing inflammation and ulceration. The fever persists but with the onset of a diarrhea in which large numbers of the bacteria is excreted with the characteristic ‘pea soup’ stools and, to a lesser extent, with the urine. In more serious cases, haemorrhage of the ulcers may occur and perforation of the intestine leading to peritonitis. In milder cases, the ulcers heal and fever falls with recovery after 4–5 weeks.

1.7.3 *Shigella spp.*

Shigellas are members of the family Enterobacteriaceae. They are nonmotile, non-sporeforming, Gram-negative rods which are catalase positive (with the exception of Shiga’s bacillus, *S. dysenteriae* serotype 1), oxidase-negative, and facultative anaerobes. They produce acid but usually no gas from glucose and, with the exception of some strains of *S. sonnei*, are unable to ferment lactose; a feature they share with most *salmonellas*. *Shigellas*

are generally regarded as rather fragile organisms which do not survive well outside their natural habitat which is the gut of humans and other primates. They have not attracted the attention that other foodborne enteric pathogens have, but such evidence as is available suggests that their survival characteristics are in fact similar to other members of the Enterobacteriaceae (Adams & Moss, 2008).

Fig 1.4: *Shigella spp.*

1.7.3.1 Pathogenesis:

Shigellas cause bacillary dysentery in humans and other higher primates. Studies with human volunteers have indicated that the infectious dose is low; of the order of 10–100 organisms. The incubation period can vary between 7 h and 7 days although 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 develop which can persist for several months. Milder forms of the illness are self-limiting and require no treatment but *Sh. dysenteriae* infections often require fluid and electrolyte replacement and antibiotic therapy. Shigellosis is an invasive infection where the organism's invasive property is encoded on a large plasmid (Adams & Moss, 2008).

1.7.4 *Vibrio spp.*

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

stimulates the growth of all species and is an obligate requirement for some. The optimum level for the growth of clinically important species is 1–3%. *V. parahaemolyticus* grows optimally at 3% NaCl but will grow at levels between 0.5% and 8% (Adams & Moss, 2008). The genus Vibrio consists of at least 28 species, and 3 that are often associated with *V. parahaemolyticus* in aquatic environments and seafood are *V. vulnificus*, *V. alginolyticus*, and *V. cholera* (Jay, Loessner & Golden, 2005).

Fig 1.5: *Vibrio spp.*

1.7.4.1 Pathogenesis:

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 day^{-1} and contains up to 108 vibrios ml^{-1} 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.

The reported incubation period for V. paraha emolyticus food poisoning varies from 2 h to 4 days though it is usually 9–25 h. Illness persists for up to 8 days and is characterized by:

- vomiting and
- profuse watery diarrhoea free from blood or mucus,
- abdominal pain,

- fever

V. parahaemolyticus is more enteroinvasive than *V. cholerae*, and penetrates the intestinal epithelium to reach the lamina propria. A dysenteric syndrome has also been reported from a number of countries including Japan. Pathogenicity of *V. parahaemolyticus* strains is strongly linked to their ability to produce a 22 kDa, thermostable, extracellular haemolysin. When tested on a medium known as Wagatsuma's agar, the haemolysin can lyse fresh human or rabbit blood cells but not those of horse blood, a phenomenon known as the Kanagawa reaction. The haemolysin has also been shown to have enterotoxic, cytotoxic and cardiotoxic activity. Most (96.5%) strains from patients with *V. parahaemolyticus* food poisoning produce the haemolysin and are designated Kanagawa positive (Ka+) while 99% of environmental isolates are Ka-(Adams & Moss, 2008).

V. vulnificus is a highly invasive organism that causes a primary septicaemia with a high fatality rate. This organism is found in seawater and some seafoods. It is isolated more often from oysters and clams than from crustacean shellfish products (Jay, Loessner & Gold, 2005). Most of the cases of foodborne transmission identified occurred in people with pre-existing liver disease, diabetes or alcoholism. Otherwise healthy individuals are rarely affected and, when they are, illness is usually confined to gastroenteritis. In foodborne cases, the symptoms of malaise followed by fever, chills and prostration appear 16–48 h after consumption of the contaminated food, usually seafoods, particularly oysters. Unlike other vibrio infections, *V. vulnificus* infections require treatment with antibiotics such as tetracycline (Adams & Moss, 2008).

1.7.5 *Klebsiella spp.*

Klebsiella pneumoniae (K. pneumonia) is a rod shaped non motile, Gram negative, lactose fermenting and facultative anaerobic bacterium. It is frequently found in the normal flora of skin, mouth, and intestines. One of the most important members of *Klebsiella* genus in Enterobacteriacae family is *K. pneumoniae*, which is account for pneumonia (the destructive lung inflammation disease). Besides urinary and lower biliary tract infections are also caused by *Klebsiella*. *Klebsiella* is diplomatic pathogen that predominantly attacks immunocompromised individuals and hospitalized patients. 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 (Puspandan et al, 2012).

Klebsiella spp. has been identified as important common pathogens for nosocomial pneumonia (7 to 14% of all cases), septicaemia (4 to 15%), urinary tract infection (UTIs; 6 to 17%), wound infections (2 to 4%), intensive care unit (ICU) infections (4 to 17%), and neonatal septicaemias (3 to 20%). *Klebsiella* spp. can also cause bacteremias and hepatic infections, and have been isolated from a number of unusual infection, including endocarditis, primary gas-containing mediastinal abscess, peritonitis, acute cholecystitis, crepitant myonecrosis, pyomyositis, necrotizing fasciitis, psoas muscle abscess, fascial space infections of the head and neck, and septic arthritis (Phac-aspc.gc.ca, 2016).

Increasingly, antimicrobial resistance has occured to *Klebsiella* bacteria, 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). *Klebsiella* infections frequently occur among sick patients in healthcare settings who are receiving treatment for other conditions. *Klebsiella* infections are most prone to occur in patients whose care requires devices like ventilators (breathing machines) or intravenous (vein) catheters, and patients who are taking long courses of certain antibiotics. Healthy people usually do not get *Klebsiella* infections (CDC, 2016).

Figure 1.5: *Klebsiella spp*

1.7.5.1 Pathogenesis:

Host defense against bacterial invasion relies on phagocytosis by polymorphonuclear granulocytes and the bactericidal impact of serum, mediated in vast part by complement proteins. Alternate-pathway complement activation which does not require the presence of immunoglobulins directed against bacterial antigens seems to be the more active pathway in *K pneumoniae* infections.

Neutrophil myeloperoxidase and lipopolysaccharide-binding protein has a role in host defense against *K pneumonia* infection suggested by a recent data from preclinical studies. Neutrophil myeloperoxidase is thought to mediate oxidative inactivation of elastase, an enzyme implicated in the pathogenesis of different tissue-destroying diseases. Lipopolysaccharide-binding protein aids in the transfer of bacterial cell wall components to inflammatory cells. Investigators showed higher rates of infection in trial mice lack in the genes that control expression of these 2 agents.

The bacteria defeat innate host immunity through several means. They possess a polysaccharide capsule, which is the principle determinant of their pathogenicity. The capsule is composed of complex acidic polysaccharides. Its enormous layer shields the bacterium from phagocytosis by polymorphonuclear granulocytes. Also the capsule avoids bacterial death caused by bactericidal serum factors. This is achieved principally by inhibiting the activation or uptake of complement components, particularly C3b. The bacteria additionally generate multiple adhesins. These may be fimbrial or nonfimbrial, each with particular receptor specificity. These help the microorganism to adhere to host cells, which is critical to the infectious process.

Another bacterial pathogenicity factors are Lipopolysaccharides (LPS). They have the capability to activate complement, which causes selective deposition of C3b onto LPS molecules at sites far from the bacterial cell membrane. This suppresses the development of the membrane attack complex (C5b-C9), which averts membrane damage and bacterial cell death.

Availability of iron increases host susceptibility to *K pneumoniae* infection. Bacteria are able to compete effectively for iron bound to host proteins because of the secretion of high-affinity, low molecular weight iron chelators known as siderophores. Most host iron is bound to intracellular and extracellular proteins so this is essential. In order to deprive bacteria of iron, the host also secretes iron-binding proteins (Emedicine.medscape.com, 2016).

1.8. Frequency of foodborne illness in different countries:

1.8.1 Foodborne illness outbreaks in United States:

Each year in the United States, 31 pathogens caused 37.2 million (90%) illnesses, of which 36.4 million (90%) were domestically acquired; of these, 9.4 million (90%) were foodborne. It was estimated that 5.5 million (59%) foodborne illnesses were caused by viruses, 3.6 million (39%) by bacteria, and 0.2 million (2%) by parasites. The pathogens that caused the most illnesses were norovirus (5.5 million, 58%), nontyphoidal Salmonella spp. (1.0 million, 11%), C. perfringens (1.0 million, 10%), and Campylobacter spp. (0.8 million, 9%). These 31 pathogens caused 228,744 (90%) hospitalizations annually, of which 55,961 (90%) were caused by contaminated food eaten in the United States. Of these, 64% were caused by bacteria, 27% by viruses, and 9% by parasites. The leading causes of hospitalization were nontyphoidal Salmonella spp. (35%), norovirus (26%), Campylobacter spp. (15%), and T. gondii (8%). These 31 pathogens caused 2,612 deaths (90%), of which 1,351 (90%) were caused by contaminated food eaten in the United States. Of these, 64% were caused by bacteria, 25% by parasites, and 12% by viruses. The leading causes of death were nontyphoidal Salmonella spp. (28%), T. gondii (24%), L. monocytogenes (19%), and norovirus (11%) (Scallan et al, 2011).

1.8.2. Foodborne illness outbreaks in Australia:

In Australia among the 16 known pathogens were an estimated 4.6 million (95%) cases of gastroenteritis due to all modes of transmission. Of these, an estimated 1.6 million (95%) were due to bacterial infections, 2.3 million (95%) were due to viral infections, and 0.70 million (95%) were due to parasites.

Among known pathogens, 1.5 million (95%) cases were acquired through food. Enteropathogenic *E. coli*, noroviruses, *Campylobacter* spp. and *Salmonella* spp. accounted for 88% of all foodborne disease in this group of pathogens. The proportion of gastroenteritis due to foodborne transmission was estimated at 32%. The product of the total number of cases of gastroenteritis (17.2 million; 95%) multiplied by the proportion that was foodborne (0.32, 95%) produced an estimate of 5.4 million cases of foodborne gastroenteritis in 1 year in Australia, with a 95% CrI of 4.0–6.9 million cases.

Hospitalizations

Among hospitalizations for gastroenteritis due to the 14 known pathogens were 10,070 diagnoses of gastroenteritis; an estimated 3,640 (95%) of these cases were due to eating contaminated food. The overall proportion of hospitalizations estimated to be from foodborne gastroenteritis was 0.36 (95%).

The total number of hospital diagnoses for gastroenteritis was estimated at 41,000 (95%). The number due to foodborne transmission was 14,700 (95%).

Deaths

The national hospital morbidity database (NHMD) 1993/1994 to 1998/1999 showed 1,302 deaths (157–311 per year) in patients with a code for a principal or additional diagnosis of infectious gastroenteritis in the 6 years. The average was 217 per year. Of these 1,302 deaths, 287 occurred in patients with a principal diagnosis of infectious gastroenteritis. Application of the proportion of hospital diagnoses due to foodborne gastroenteritis (36%, 95% CrI 30%–41%) to the number of deaths in which the diagnosis included infectious gastroenteritis (217, 95% CrI 120–320) provided an estimate of 76 (95% CrI 41–120) deaths due to foodborne gastroenteritis each year (Hall, 2005).

1.8.3. Foodborne illness outbreaks in Japan and Korea:

The average prevalence of reported foodborne illness from 1981 to 1995 was 2.44 per 100,000 population in Korea, and 28.01 in Japan. The mean case fatality rate in Korea was 0.74% and in Japan, 0.03%. When both prevalence and case fatality rates in Korea and Japan were compared during the same period, the prevalence in Japan was much higher than that in Korea. However, the case fatality rate of patients in Korea was much higher than that in Japan. The distribution of monthly and seasonal patterns of foodborne illness outbreaks strongly indicates the outbreaks may be associated with climatic conditions, frequencies of national holidays, and vacation seasons. Comparison study indicates that the foodborne illness outbreaks in Korea most frequently involved homemade foods (47% of the total cases); in Japan, restaurants accounted for 31.3%. Foodborne illness cases of bacterial origin in Korea were 59.3% of the total and included *Salmonella* spp.(20.7%), *Vibrio* (17.4%), *Staphylococcus* (9.7%), pathogenic *Escherichia coli* (2.4%), and other species (9.1%); in Japan, 72.8% of the total cases and the majority of the bacterial foodborne illness was caused

by *Vibrio* (32.3%), *Staphylococcus* (15.9%), *Salmonella* (14.2%), pathogenic *E. coli* (3.0%), and other species (7.2%). In conclusion, the outbreaks of foodborne illness in Korea and Japan may be mainly caused by improper food handling, and their occurrences may be differentiated according to food sources (Lee, 2001).

1.8.4. Foodborne illness outbreaks in India

Foodborne diseases cause morbidity and mortality in the general population and they have emerged as a growing public health and economic problem in many countries during the last 2 decades. The global burden of foodborne disease is currently unknown but the World Health Organization (WHO) has responded to this data gap by launching a new initiative to provide better estimates. In 2005 it was reported that 1.8 million people died from diarrheal diseases largely due to contaminated food and water. The scientific investigations/reports on outbreak of foodborne diseases in India for the past 29 (1980–2009) years indicated that a total of 37 outbreaks involving 3,485 persons have been affected due to food poisoning. In India foodborne diseases are not categorized separately in the Health Information of India. For example, in the official document of health information, Government of India for 2004, 9575112 cases of acute diarrhoel diseases including gastroenteritis with 2855 deaths have been recorded and cases of foodborne disease may have been categorized under gastroenteritis (Sudershan, 2014).

1.8.4. Street food condition in Dhaka city

Bangladesh is populated with numerous vendors of street foods of various types. Like all developing countries, street food preparation and selling in Bangladesh provides a regular source of income for millions of men and women with limited education or skills. In front of every school, university, office, footpaths street food shops are available, and they are very popular. Especially the rickshaw puller, laborers eat street foods just to satisfy their hunger. There are many varieties of street foods found in Bangladesh. A large part of the urban population in Bangladesh, particularly from the lower income groups, meets a substantial part of its dietary and nutritional needs through meals and beverages offered by outdoor vendors, but such “street foods” are often contaminated with bacteria and other germs, making them dangerous for the health of consumers. The presence of coliform, and salmonellae, shigellae, staphylococci or Enteropathogenic Escherichia coli (EEC) in street foods is evidenced. Vendors generally prepare the food with their bare hands, passing on germs to what they have on offer. The water used to prepare foods and to clean cooking and eating utensils – and

sometimes even the swab cloths used for drying – are another source of contamination. The government and the public together need to maintain certain standards so that consumers are satisfied with what they consumed in terms of their quality, standard and hygiene. The concerned authority should formulate realistic laws, rules and regulations on street-food vending, establish adequate infrastructure facilities and develop plans of action for implementation of street food vending and take policy to make awareness of hygiene, health science among the vendors and consumers. Arrangement can also be made for free training of hygiene and health science for the street vendors to make them conscious.

Chapter-02

Research Objective

Objective of this study

- Isolation and identification of the presence of enteric bacteria (*Escherichia coli, Klebsiella* spp, *Shigella* spp, *Shigella* spp, *Salmonella* and *Vibrio* spp) in different street vended foods collected from different private universities.
- Counting of colonies in different street vended foods collected from private universities.

Chapter-03

Methodology

3.1 Study Area

10 private universities of Dhaka city which are Ahsanullah University of Science and Technology (AUST), BRAC University, East West University (EWU), Safena Dental College (SDC), United International University (UIU), Green University of Bangladesh (GU), University of Asia Pacific (UAP), Bangladesh Islamic University (BIU), Stamford University (SU) and Primeasia University (PU).

3.2 Study Duration

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

3.3 Bacteriological Subculture

3.3.1 Sample Collection

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

3.3.2 Sample Processing

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

3.3.3 Enrichment of the Organisms

3.3.3.1 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 ºC for 18-24 h.

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

5 gm solid sample mixed well with 45 ml of Trypticase Soy Broth (TSB) + 0.3% yeast extract (YE) and then transferred them to conical flasks. The open mouths of the flasks were covered with foil paper and incubated at 37ºC for 18-24 h.

Figure 3.1: Enrichment for targeted organisms

3.3.3.3 Enrichment of *Vibrio* Species

5 gm solid sample mixed well with 45 ml of APW (Alkaline Peptone Water) broth, then transferred them to conical flasks. The open mouths of the flasks were covered with foil paper and incubated at 37ºC for 18-24 h.

3.3.4 Selective Growth of the Organisms

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

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

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

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

3.3.4.3 Selective Growth of *Vibrio* Species

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

3.3.5 Sterilization Procedure

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glassware were sterilized by autoclaving at a temperature of 121°C and a pressure of 15-lbs. /sq. inch for 20 minutes. Screw cap test tubes, conical flasks, prepared media etc. were also sterilized.

Figure 3.2: Autoclave and Hot air Oven

Figure 3.3: Laminar Air Flow Cabinet

3.3.6 Preparation of Petri dishes

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

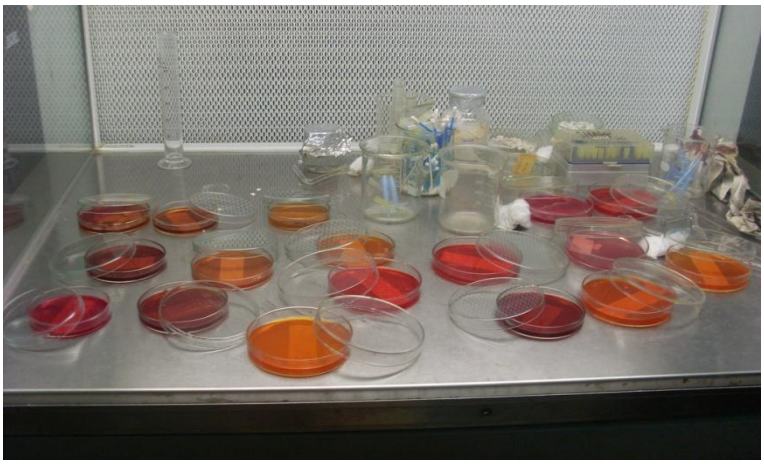

Figure 3.4: Petri dishes preparation

3.3.7 Incubation

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

Figure 3.5: Incubator

3.3.8 Standard Colony Morphology of Suspected Organism in Different Media

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

Table 3.1: Standard Colony Morphology of Suspected Organism

Organism	Media	Appearance
E. coli	MacConkey	Lactose fermenting pink colonies Non-lactose fermenting colorless colonies
	TBX	Blue colonies
Salmonella	BGA	Typical red colonies
	XLD	Red or clear colonies with black centers
Vibrio	TCBS	Large yellow colonies
Shigella	XLD	Typical red colonies
	MacConkey	Smooth non-lactose fermenting transparent colony
Klebsiella	MacConkey	Pink colonies

3.3.9 Apparatus & Reagent used for Isolation and Identification of Specific Organism

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

- Trypticase Soy Broth (TSB)
- 0.3% yeast extract (YE)
- BPW (Buffered Peptone Water) broth
- APW (Alkaline Peptone Water) broth

- Inoculating loop
- Spirit burner
- Hand gloves
- Mortar and pestle
- Incubator
- Measuring Cylinder (100ml)
- Distilled water
- Analytical balance
- Media preparation bottle

3.4 Biochemical Tests

3.4.1 Kliglar Iron Agar Test (KIA Test)

3.4.1.1 Test Tube Preparation for KIA Test

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

3.4.1.2 Inoculation for KIA Test

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

Figure 3.6: Preparation of test tubes for KIA test

3.4.2 MIO Test

3.4.2.1 Test Tube Preparation for MIO Test

For motility test, about 5 ml of MIO agar medium was poured into screw cap test tubes and kept straight. 100 µl of Kovac's reagent was added for indole test.

3.4.2.2 Inoculation for MIO Test

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

Figure 3.7: Preparation of test tubes for MIO test

3.4.3 Citrate Test

3.4.3.1 Test Tube Preparation for Citrate Test

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

3.4.3.2 Inoculation for Citrate Test

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

Figure 3.8: Preparation of test tubes for Citrate test

3.4.4 Urease Test

3.4.4.1 Test Tube Preparation for Urease Test

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

3.4.4.2 Inoculation for Urease Test

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

Figure 3.9: Preparation of test tubes for Urease test

3.4.5 Oxidase Test

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

3.4.6 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. A indicates acid and K 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. Colony 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. About 9 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.

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}

3.5.6 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

3.5.7. Counting bacterial colonies

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

Chapter-4

Result

4.1 Bacteriological colony morphology

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

Name of University	Sample	Plates				
		MacConkey	TBX	BGA	XLD	TCBS
East West University (EWU)	Singara 1	No growth	No growth	No growth	No growth	Yellow
	Laddu 1	Colorless	No growth	No growth	No growth	Yellow
	Misty Singara	No growth	No growth	No growth	Red	No growth
Safena Dental College	Laddu 2	Colorless	No growth	No growth	No growth	Yellow
	Pakora	No growth	No growth	No growth	No growth	No growth
	Singara 2	Mucoid pink, Pink	No growth	No growth	No growth	No growth
Stamford University (SU)	Laddu 3	Pink	No growth	No growth	No growth	No growth
	Patis	No growth	No growth	No growth	No growth	No growth
	Singara 3	Pink	No growth	No growth	No growth	Yellow
Green University	Singara 4	Pink	No growth	No growth	No growth	No growth
	Chom chom	No growth	No growth	No growth	No growth	No growth
	Roll	No growth	No growth	No growth	No growth	No growth
Ahsanullah University (AUST)	Singara 5	Pink	No growth	No growth	No growth	No growth
	Samaucha	No growth	No growth	No growth	No growth	No growth
	Beguni	No growth	No growth	No growth	No growth	No growth

Table 4.1 shows bacterial colony morphology isolated from different street vended food samples. An estimate of 15 food samples was collected from the area around five different private universities in Dhaka city. In total 11 samples show growth of different pathogenic or non pathogenic microorganisms. Of which 5 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.

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

Name of University	Sample	Plates				
		MacConkey	**TBX**	**BGA**	**XLD**	**TCBS**
University of Asia Pacific (UAP)	Singara 6	Pink	No growth	No growth	No growth	No growth
	Teheri	No growth	No growth	No growth	No growth	No growth
	Alur chop	No growth	No growth	No growth	No growth	No growth
Bangladesh Islamic University	Singara 7	Mucoid pink	No growth	No growth	No growth	No growth
	Samaucha	No growth	No growth	No growth	No growth	No growth
	Biscuit	No growth	No growth	No growth	No growth	No growth
United International University	Laddu 4	Colorless	No growth	No growth	No growth	Yellow
	Kabab	No growth	No growth	No growth	No growth	Yellow
	Jhal Singara	Mucoid pink	No growth	No growth	No growth	No growth
Brac University (BU)	Laddu 5	Colorless	Blue	No growth	No growth	No growth
	Nimki	No growth	No growth	No growth	No growth	No growth
	Vanilla cake	No growth	No growth	No growth	No growth	No growth
Primeasia University (PAU)	Jhal Singara	Mucoid pink	No growth	No growth	No growth	No growth
	Laddu 6	Colorless	No growth	No growth	No growth	Yellow
	Singara 8	Flat pink,	Blue	No growth	No growth	Yellow
		Pink	Blue	No growth	No growth	Yellow

Table 4.2 shows bacterial colony morphology isolated from different street vended food samples. An estimate of 15 food samples was collected from the area around five different private universities in Dhaka city. In total 8 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 shows no growth in these agar media.

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*
EWU	1	0	2	1	0
SDC	2	1	1	0	0
SU	2	0	1	0	0
GU	1	0	1	0	0
AUST	1	2	2	0	0
UAP	1	0	0	0	0
BIU	0	0	0	0	0
UIU	1	1	2	0	0
BU	2	0	0	0	0
PAU	3	0	1	0	0

Among 30 samples were collected from street vendors in the area around 10 private universities of Dhaka city. About 18 (60%) food samples were contaminated with pathogenic or non pathogenic microorganisms (Table 5.1 and Table 5.2). Of which 8 (27%) samples were suspected to be contaminated with our targeted organisms (*E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp.).*

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

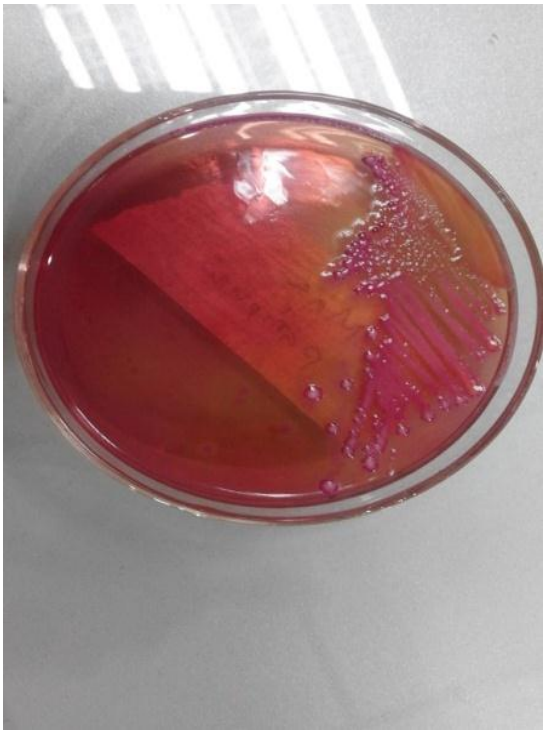

Figure 4.1: Bacterial colony (pink) on MacConkey agar plate

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

4.2 Suspected Organisms from Biochemical Tests

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

Sample	Plates	Colony Morphology	M	I	O	Citrate	Urease	Oxidase	KIA			Suspected organism
									Slunt/ Butt	H_2S	Gas	
Shingara1	TCBS	Yellow	+	+	-	+	-	-	K/A	-	+	*Vibrio spp*
Shingara2	MacConkey	Pink	-	+	-	+	-	-	A/A	-	+	*Klebsiella pneumonia*
Shingara3	TCBS	Yellow	+	+	-	+	-	-	K/A	-	+	*Vibrio spp*
	MacConkey	Pink	+	+	-	+	-	-	A/A	-	+	*E.coli*
Shingara5	MacConkey	Pink	+	+	-	+	-	-	A/A	-	+	*E.coli*
Shingara6	MacConkey	Pink	+	+	-	+	-	-	A/A	-	+	*E.coli*
Shingara8	MacConkey	Flat Pink	+	+	-	+	-	-	A/A	-	+	*E.coli*
	MacConkey	Pink	+	+	-	+	-	-	A/A	-	+	*E.coli*
	TBX	Blue	+	+	-	+	-	-	A/A	-	+	*E.coli*
	TCBS	Yellow	+	+	-	+	-	-	A/K	-	-	*Vibrio spp*

Among 30 samples were collected from street vendors in the area around 10 private universities of Dhaka city. About 18 (60%) food samples were contaminated with pathogenic or non pathogenic microorganisms (Table 5.1 and Table 5.2). Of which 8 (27%) samples were suspected to be contaminated with our targeted organisms (*E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp.).*

Table 4.4 shows identification of the suspected organism (*Klebsiella spp., Vibrio, E. coli*) from different biochemical test. In total 6 (20%) food samples were identified to be contaminated with our suspected organism (*Klebsiella spp., Vibrio, E. coli*) from these biochemical tests.

Table 4.5: Presence of suspected organisms in no of food samples from different Universities (n=10)

Name of University	*E.coli*	*Klebsiella spp.*	*Vibrio spp.*	*Shigella spp.*	*Salmonella spp.*
EWU	0	0	1	0	0
SDC	0	1	0	0	0
SU	1	0	1	0	0
AUST	1	0	0	0	0
UAP	1	0	0	0	0
PAU	3	0	1	0	0

Table 4.5 shows presence of suspected organisms in number of food samples from different university. In total 6 (20%) food samples from different university were suspected to be contaminated with our targeted organisms *E. coli*, *Klebsiella* spp, *Vibrio* spp and except *Shigella* spp, and *Salmonella* spp.

In EWU, 1 food sample was suspected to be contaminated with *Vibrio* spp. In SDC, 1 food sample was suspected to be contaminated with *Klebsiella* spp. In SU, 1 food sample was suspected to be contaminated with *Klebsiella* spp. In AUST, 1 food sample was suspected to be contaminated with *E, coli.* In PAU, 1 food sample was suspected to be contaminated with *E. coli* and *Vibrio* spp.

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

Pathogen	Food Categories					
	Deep fried and fried items (n=18)	Rice items (n=1)	Noodles (n=1)	Baked items (n=3)	Sweet items (n=7)	Total (n=30)
E.coli	6 (33%)	Nd	Nd	Nd	Nd	6(20%)
Klebsiella spp.	1 (5%)	Nd	Nd	Nd	Nd	1 (3%)
Vibrio spp.	3 (16%)	Nd	Nd	Nd	Nd	3(10%)
Shigella spp.	Nd	Nd	Nd	Nd	Nd	Nd
Salmonella spp.	Nd	Nd	Nd	Nd	Nd	Nd

Table 4.6 shows the incidence of food borne pathogens in various street vended food samples. Among 18 deep fried and fried items, 6 (33%) samples were suspected to contain *E. coli*, 1 (5%) sample was suspected to contain *Klebsiella pneumonia* and 3 (16%) sample was suspected to contain *Vibrio* spp.

4.3. Colony Forming Unit (CFU) from Colony Counting

Table 4.7: Colony counting of various samples

Sample Name	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Fuchka	Uncountable	Uncountable	Uncountable	53
Noodles	Uncountable	Uncountable	31	9
Cake	33	10	8	6
Butter bun	Uncountable	Uncountable	Uncountable	45
Vhelpuri	Uncountable	Uncountable	Uncountable	61
Ghugny	Uncountable	Uncountable	59	24

For fuchka plate 4 was selected as it has between 30-300 colonies. The other plates cannot be used because they are either too thick with colonies or too thin. The calculation of cell concentration in the original culture is:

53 colonies on plate 4 x dilution factor of 10,000 = 530,000 cells/ml.

For noodles 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:

31 colonies on plate 3 x dilution factor of 1000 = 31000 cells/ml.

For cake plate 1 was selected as it has between 30-300 colonies. The other plates cannot be used because they are either too thick with colonies or too thin. The calculation of cell concentration in the original culture is:

33 colonies on plate 1 x dilution factor of 10 = 330 cells/ml.

For butter bun plate 4 was selected as it has between 30-300 colonies. The other plates cannot be used because they are either too thick with colonies or too thin. The calculation of cell concentration in the original culture is:

45 colonies on plate 4 x dilution factor of 10,000 = 450,000 cells/ml.

For vhelpuri plate 4 was selected as it has between 30-300 colonies. The other plates cannot be used because they are either too thick with colonies or too thin. The calculation of cell concentration in the original culture is:

61 colonies on plate 4 x dilution factor of 10,000 = 610,000 cells/ml.

For ghugni 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:

59 colonies on plate 4 x dilution factor of 1000 = 59000 cells/ml.

4.7.1. Table: Number of colonies per ml of sample

Sample Name	Fuchka	Noodles	Cake	Butter bun	Vhelpuri	Ghugni
Colony forming unit (CFU) (cells/ml)	530,000	31,000	330	450,000	610,000	59,000

Chapter-5

Discussion

5.1 Discussion

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

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

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

A study was conducted on 132 samples of beef, chicken, salad, and gravy from two street vendors in Johannesburg, South Africa. The predominant population isolated from the aerobic plate counts was *Bacillus* spp., *Staphylococcus* spp., Enterobacteriaceae and *Alcagines* spp., *Bacillus cereus* was detected in 17%, *Clostridium perfringens* in 1% *Staphylococcus aureus* in 3% and *Vibrio metchnikovii* in 2% of food samples. *Campylobacter jejuni, Listeria monocytogenes,* and *Escherechia coli.* O157:H7 were not detected. Non-pathogenic *E. coli* was detected in 13% of food samples, in 86 and 36% of dish water samples collected from vendors 1 and 2, respectively, and in 36% of surface swab samples from vendor 2. Inappropriate food handling practices contributed to the development of this out break (Christison, Lindsay & Holy, 2008).

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

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

A study was conducted in Amravati, India. Forty water sample of panipuri were aseptically collected from eleven locations of Amravati City. Analysis of the food samples revealed that 93% of panipuri water samples had high loads of bacterial pathogens such as Escherichia coli (41%), Staphylococcus aureus (31%), Klebsiella spp. (20%), Pseudomonas spp. (5%) and yeast (3%). It is suggested that regular monitoring of the quality of street foods must be practiced to avoid any food-borne infection in future (Tambekar et al., 2011).

In this study, 30 different food samples were collected from 10 private universities. Among them, we found contamination in 18 (60%) samples. Of which, 6 (20%) samples were suspected to be contaminated with our targeted organisms (*E coli, Klebsiella, Shigella, Salmonella* and *Vibrio* species). In total 6 samples, 4 (13.3%) samples were suspected to be contaminated with *E coli*, 1 (3.33%) with *Klebsiella*, 3 (10%) with *Vibrio.* From the results of biochemical test we got 10 of our suspected bacteria from 6 different samples.

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

Chapter-06

Reference

6.2 Conclusion

Hygiene in handling and cooking of street foods is very essential. Human are the largest source of contamination which emphasis importance on personal hygiene. So it is very important to maintain cleanliness. The present study revealed that street vended foods in Dhaka city constitute an important potential hazard to human health which needs to be addressed. Among the 30 different food samples collected from 10 private universities, we found contamination in 18 (60%) samples. Of which, 6 (20%) samples were contaminated with our targeted organisms (*E coli, Klebsiella, Shigella, Salmonella* and *Vibrio* species). In total 6 samples, 4 (13.3%) samples were contaminated with *E coli*, 1 (3.33%) with *Klebsiella*, 3 (10%) with *Vibrio*. From the results of biochemical test we got 10 of our suspected bacteria from 6 different samples. Because of lack of methods and facilities only five microorganisms were to be identified. From this study, it clear that all the samples are microbiologically unacceptable to eat. Strict public health regulations should be established to control the situation. The maintenance of these street vended foods should be monitored cautiously. The government should take necessary steps to provide regular training and to create consciousness on food management and individual hygiene among street food vendors as well as consumers.

References:

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

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

CDC. (2016) *CDC – Klebsiella pneumonia in Healthcare Settings- HAI.* [Online] Available at: http://www.cdc.gov/HAI/organisms/klebsiella/klebsiella.html. [Accessed 9 Nov. 2016]

Emedicine.medscape.com. (2016). *Klebsiella Infections: Background, Pathophysiology, Epidemiology of Klebsiellae*. [online] Available at: http://emedicine.medscape.com/article/219907-overview#a5 [Accessed 10 Nov. 2016].

Feglo, P. and Sakyi, K. (2016). *Bacterial contamination of street vending food in Kumasi, Ghana*. [online] Ajol.info. Available at: http://www.ajol.info/index.php/jmbs/article/view/77101 [Accessed 5 Nov. 2016].

Foodsafe.ca. (2016). *FOODSAFE Home*. [online] Available at: http://www.foodsafe.ca/resources/Foodborne_Illness_Chart.pdf [Accessed 5 Nov. 2016].

Hall, G., Kirk, M.D., Becker, N., Gregory, J.E., Unicomb, L., Millard, G., Stafford, R., Lalor, K. and OzFoodNet Working Group, 2005. Estimating foodborne gastroenteritis, Australia. *Emerg Infect Dis*, *11*(8), pp.1257-1264.

Islam, S., Nasrin, N., Rizwan, F., Nahar, L., Bhowmik, A., Esha, S.A., Talukder, K.A., Akter, M., Roy, A. and Ahmed, M., 2015. Microbial contamination of street vended foods from a university campus in Bangladesh. *Southeast Asian Journal of Tropical Medicine and Public Health*, *46*(3), p.480.

Jay, J., Loessner, M. & Golden, D. (2005) *Modern food microbiology*. 7th ed. New York: Springer.

Kirk, M., Pires, S., Black, R., Caipo, M., Crump, J., Devleesschauwer, B., Dopfer, D., Fazil, A., Fischer-Walker, C., Hald, T., Hall, A., Keddy, K., Lake, R., Lanata, C., Torgerson, P., Havelaar, A. & Angulo, F. (2005) World Health Organization Estimates of the Global and

regional Disease Burden of 22 Food borne Bacterial, Protozoal and Viral Diseases, 2010: A Data Synthesis. *Plos Med.* 12 (12). p.21

Lee, W.C., Lee, M.J., Kim, J.S. and Park, S.Y., 2001. Foodborne illness outbreaks in Korea and Japan studied retrospectively. *Journal of Food Protection®*, *64*(6), pp.899-902.

Niddk.nih.gov. (2016). *Foodborne Illnesses*. [online] Available at: https://www.niddk.nih.gov/health-information/health-topics/digestive-diseases/foodborne-illnesses/Pages/facts.aspx#top [Accessed 5 Nov. 2016].

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

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

Phac-aspc.gc.ca. (2016). *Klebsiella spp. - Pathogen Safety Data Sheets*. [online] Available at: http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/klebsiella-eng.php [Accessed 10 Nov. 2016].

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

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

Rane, S. (2011) Street vended Food in Developing World: Hazard Analyses. *Indian Journal of Microbiology.* 51 (1). P. 100-106. [Online] SpringerLink. Available at: http://link.springer.com/article/10.1007/s12088-011-0154-x [Accessed 5 Nov. 2016].

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.l. and Griffin, P.M., 2011. Foodborne illness acquired in the United States- major pathogens. *Emerg Infect Dis, 17(1).*

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

(2). p. 169.

Sudershan, R.V., Naveen Kumar, R., Kashinath, L., Bhaskar, V. and Polasa, K., 2014. Foodborne infections and intoxications in hyderabad India. *Epidemiology Research International*, *2014*.