Isolation and identification of different foods by microbiological method collected from different university cafeterias 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

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Declaration by the Candidate

I, kamrunnaher Esha , hereby declare that the dissertation entitled “Isolation and identification of different foods by microbiological method collected from different university cafeterias in 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 2017 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.

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Certificate by the Supervisor

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This is to certify that the thesis entitled "Isolation and identification of different foods by microbiological method collected from different university cafeterias in Dhaka city, Bangladesh" submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by kamrunnaher Esha, ID: 2013-3-70-015, during the period 2017 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.

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List of Abbreviations:

Abbreviations	Full Name
ETEC	Enterotoxigenic *E. coli*
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
EHEC	Enterohaemorrhagic *E. coli*
VTEC	Verotoxin-producing *E. coli*
HU	Haemolytic Uraemic Syndrome
CFU	Colony Forming Unit
TSB	Trypticase Soy Broth
TBX	Tryptone Bile X-glucuronide
APW	Alkaline Peptone Water
BPW	Buffered Peptone Water
XLD	Xylose lysine deoxycholate
TCBS	Thiosulfate citrate-bile salts sucrose
BGA	Brilliant Green Agar
KIA	Kliglar Iron Agar

Abstract

Foodborne illnesses are infections or irritations of the gastrointestinal tract (GIT) caused by food or beverages that contain harmful bacteria, parasites, viruses, or chemicals. Common symptoms of foodborne illnesses include vomiting, diarrhea, abdominal pain, fever, chills and dehydration. Anyone who eats contaminated food can be affected by foodborne illness. This illness may lead to more serious complications; range from mild to severe and death. Many enteric pathogens are responsible for foodborne illnesses. They are *Salmonella***,** *Campylobacter jejuni, Vibrios, Shigella, Escherichia coli (E. coli)* etc. Food handlers play a major role in the transmission of food borne pathogens via hands. Food in the student cafeteria can be a source of contamination that affects the safety and well-being of the students. This study was carried out to identify and isolate the causative enteric bacteria in foods from different university cafeterias in Dhaka city, Bangladesh. The identified causative organisms included *Escherichia coli*, *Shigella* spp, *Salmonella spp.* and *Vibrio* spp. The tested samples were typical native foods and they were singara, somucha, noodles, hot dogs, egg curry, egg vorta, vegetable roll, egg chop, etc. Sterile polythene bags were used to collect different samples from each university cafeteria. After collection of the cafeterias foods, they were analyzed through serial dilution, inoculation, incubation, subculture and biochemical tests. All food samples were tested for the presence of microorganisms following conventional microbiological method. Biochemical tests were done for the confirmation of *Escherichia coli*, *Shigella* spp, *Salmonella* and *Vibrio* spp. Out of 35 food samples, 12 (63%) were suspected to contain *Vibrio spp*, 8 (42%) were suspected to contain *Escherichia coli.* All these enteric pathogens could be the potential cause for food-borne illnesses. Future study is needed for further confirmation of enteric pathogenic bacteria by the use of serology and PCR technology.

Key Words: Foodborne pathogens, Contamination, *Escherichia coli*, *Shigella* spp, *Salmonella spp*, *Vibrio* spp, Isolation, Identification.

Chapter 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Cafeteria Food

A cafeteria is a type of food service location in which there is little or no waiting staff table service, whether a restaurant or within an institution such as a school, college or university. Cafeteria food is often inexpensive, bought in bulk, high in calories, delicious, and easily available. The demand for food service has constantly developed particularly in universities, as there are a continuously increasing number of students. As a result, these increased demands on university food service operations are putting an amplified pressure on operators to satisfy students' needs, due to intense competition. However, as a consequence of inappropriate manipulation and storage conditions, both pathogenic and deteriorative microorganisms may contaminate a food product, thus increasing the risk of microbial diseases and cases of foodborne illness due to consumption of different cafeteria's foods. Even though the sources of food contamination are diverse, food handlers serve as important source of food contamination either as carriers of pathogens or through poor hygienic practices. It will have an important implication for future development of hygiene legislations.

Figure 1.1: Some cafeteria foods

Food-borne illness is a major international health problem with consequent economic reduction.Food borne bacterial pathogens commonly detected in cafeteria foods are *Bacillus cereus* causes vomiting and diarrhea, *Clostridium perfringen* these bacteria are very much harmful and causes abdominal cramps and diarrhea. *Staphylococcus aureus* causes vomiting, diarrhea, loss of appetite, severe abdominal cramps and mild fever and *Salmonella* species causes typhoid fever (Sharma et al., 2015). The most frequently contaminated foods are meats 64 (30%), fish 34 (16%), seafood 13 (6%), salad 12 (6%), sandwiches 11 (5%) and eggs 9 (4%). Chicken, the most frequently implicated meat, was associated with 27 (13%) (Gould, D, 2016). Outbreaks of food-borne diseases can be

reduced if the food supervisors understand the role of the hands in disease transmission and the importance of hand hygiene in controlling infection in the food establishment are well established.

In Bangladesh the demand of cafeteria food is mounting gradually among the adolescent. There is no recent information is available regarding the microbiological quality of the cafeteria products in Dhaka city, Bangladesh. The present study was conducted to determine the microbiological quality of some cafeteria food collected from different universities of Dhaka city, Bangladesh.

1.2 Categories of Cafeteria Food

In cafeteria we can have the benefits of tasty foods. Often in cafeteria we get three types of food items.

Breakfast items: sabzi **(**mixed vegetables), Dal Paratha (lentils), Naan Roti**.**

Snacks and Breads: Singara, Samosa, Fried roti stuffed with egg & onions,plan cake, ovaltine cake, Noodles, Dim vorta, Sandwich, Jambu barger, beguni , Chicken roll ,pizza etc

Launch items: Biriani, Koal Pakhir torkari, Khicuri, Goru torkari, Alu vorta, Egg curry etc

1.3 Foodborne Illnesses

Food-borne illnesses have a dramatic impact in both developing and developed countries. The health status of the food handlers, their personal hygiene, knowledge and practice of food hygiene play an important role of food contamination. Foodborne diseases can be defined as diseases commonly transmitted through food. Foodborne diseases comprise a broad group of illnesses caused by microbial pathogens, parasites, chemical contaminants and biotoxins. The burden of disease can be defined as the incidence and prevalence of morbidity, disability, and mortality associated with acute and chronic manifestations of diseases.

The World health organization (WHO) estimated that in developed countries, up to 30% of the population suffers from food borne diseases each year, whereas in developing countries up to 2 million deaths are estimated per year. It is estimated that 3.5 billion people are affected and that 450 million people are ill as a result of intestinal parasites and

protozoan infections, majority of being children. Common symptoms of foodborne illnesses include vomiting, diarrhea, abdominal pain, fever, and chills. Most foodborne illnesses are acute, meaning they happen suddenly and last a short time, and most people recover on their own without treatment. CDC estimates that the most common foodborne illnesses are caused by norovirus.

1.4 Variables of The Foodborne Illness

Dependent variables

- Bacterial hand contamination
- Types of bacterial hand contaminants

Independent variables

- Hand washing habit
- Fingernail status
- Presence of jewelry on fingers
- Regular medical checkup
- Hygiene training
- Outer protective coat, and hair cover

1.5 Causes of Foodborne Illnesses

The majority of foodborne illnesses are caused by harmful bacteria and viruses. Some parasites and chemicals also cause foodborne illnesses.

1.5.1 Bacteria:

Bacteria are tiny organisms that can cause infections of the GI tract. Not all bacteria are harmful to humans. Some harmful bacteria may already be present in foods when they are purchased. Many types of bacteria cause foodborne illnesses. Examples include:

Salmonella, a bacterium found in many foods, including raw and undercooked meat, poultry, dairy products, and seafood.

Campylobacter jejuni (C. jejuni), found in raw or undercooked chicken and unpasteurized milk.

Shigella, a bacterium spread from person to person. These bacteria are present in the stools of people who are infected

Escherichia coli (E. coli), which cause several illness in humans.Common sources of *E. coli* include raw or undercooked hamburger, unpasteurized fruit juices and milk, and fresh produce.

Listeria monocytogenes (L. monocytogenes), which has been found in raw and undercooked meats, unpasteurized milk, soft cheeses

Vibrio, a bacterium that may contaminate fish or shellfish (Jay, Loessner & Golden, 2005).

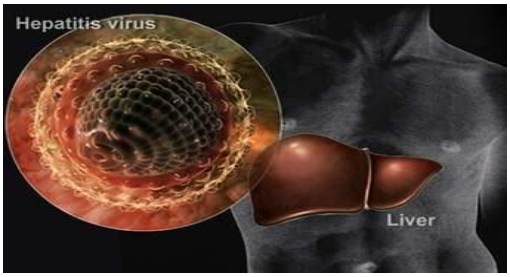
Staphylococcus aureus , it is important pathogen responsible for a variety of diseases ranging from mild skin and soft tissue infections, food poisoning to highly serious diseases such as osteomyelitis, endocarditis, and toxic shock syndrome (Soriano JM ,e.2017).

Clostridium perfringens (C. perfringens), which *is* an anaerobic, Gram-positive, spore-forming bacillus and a natural inhabitant of soil and the intestinal tracts of humans and other warm-blooded mammals.It causes an estimated one million illnesses each year, making it the second most common bacterial cause of food-borne illness (Gormley, ,2011).

Figure 1.5.1: Picture of bacteria

1.5.2 Viruses

Viruses are tiny capsules, much smaller than bacteria that contain genetic material. People who are infected with a virus may contaminate food and drinks, Common foodborne viruses include,Norovirus, which causes inflammation of the stomach and intestines (Jay, Loessner & Golden, 2005).Hepatitis a, which causes inflammation of the liver.



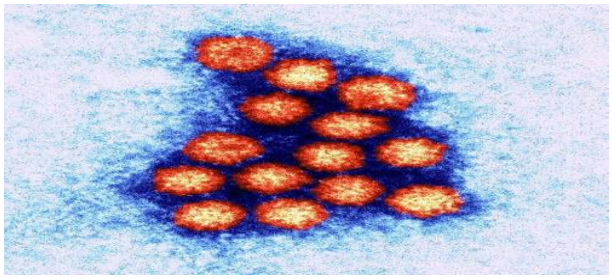


Figure 1.5.2: Hepatitis A virus **Figure1.5.2: Norovirus**

1.5.3 Parasites

Parasites are tiny organisms that live inside another organism.some common parasite are included-

Cryptosporidium parvum and *Giardia intestinalis* are parasites that are spread through water contaminated with the stools of people or animals that are infected (Quiroz ES,e, 2017).

Trichinella spiralis is a type of roundworm parasite. People may be infected with this parasite.

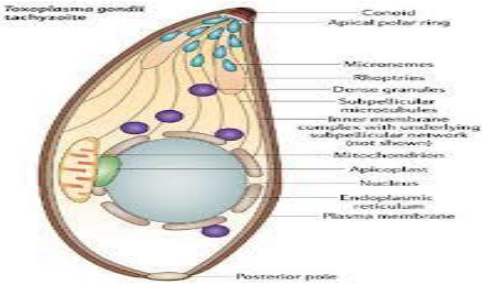


Figure 1.5.3: *Trichinella spiralis* **Figure 1.5.3:** *Cryptosporidium parvum*

1.5.4 Fungi

Food-borne pathogens are ongoing problems, and new pathogens are emerging. The impact of fungi, however, is largely underestimated. This study demonstrates that *Mucor circinelloides* can spoil food products and cause gastrointestinal illness in consumers and may pose a particular risk to immune compromised patients (Jay, Loessner & Golden, 2005).

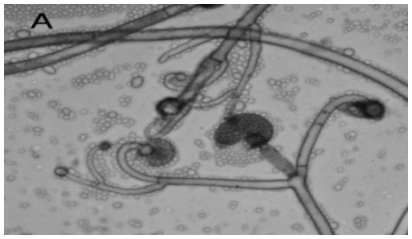


Figure 1.5.4: *Mucor circinelloidesp* structure

1.5.5 Chemicals

Harmful chemicals that cause illness may contaminate foods such as some types of fish, including tuna may be contaminated with bacteria that produce toxins if the fish are not properly refrigerated before they are cooked or served. As some containers will leach hazardous chemicals like copper, lead and cadmium into food, use of equipment and utensils incompatible with the food being handled, should be avoided. This has been observed particularly with acidic food and beverages.

1.6 Complications of Foodborne Illnesses

Foodborne illnesses may lead to dehydration, hemolytic uremic syndrome (HUS), and other complications. Acute foodborne illnesses may also lead to chronic or long lasting health problems.

- **Dehydration:** When someone does not drink enough fluids to replace those that are lost through vomiting and diarrhea, dehydration can result.
- **HUS (Hemolytic uremic syndrome):** Hemolytic uremic syndrome is a rare disease that mostly affects children younger than 10 years of age. HUS develops when *E. coli* bacteria lodged in the digestive tract make toxins that enter the bloodstream. The toxins start to destroy red blood cells, which help the blood to clot, and the lining of the blood vessels.
- **Reactive arthritis:** A type of joint inflammation that usually affects the knees, ankles, or feet. Some people develop this disorder following foodborne illnesses caused by certain bacteria, including **c.** *Jejuni* and *salmonella spp.*
- **Irritable bowel syndrome:** A disorder of unknown cause that is associated with abdominal pain, bloating, and diarrhea
- **Guillain-barré syndrome:** A disorder characterized by muscle weakness or paralysis that begins in the lower body and progresses to the upper body. This

syndrome may occur after foodborne illnesses caused by bacteria, most commonly *C. Jejuni.*

1.7 Food Poisoning

It is a common and distressing problem of today's generation, which results when the people get infection with the foodborne diseases. The common illness associated with food poisoning is dehydration and bloody diarrhea. It is sometimes life threatening. Food preparation at unhygienic conditions can also lead to food poisoning. Infectious organisms including bacteria, viruses and parasites or their toxins are the most common causes of food poisoning. Infectious organisms or their toxins can contaminate food at any point of processing, production, growing, harvesting, storing, shipping or preparing (Kirk et al., 2015). The distinction matters should be identified because public health authorities need to know how a particular disease is spreading and the appropriate steps should be taken to stop it.

1.7.1 Types of Diseases Caused By Food Poisoning

Diseases caused due to viable pathogenic bacteria cells, spores in the case of infant botulism or food containing toxins produced by toxigenic bacteria and molds. On the basis of mode of illnesses, these can be arbitrarily divided into three groups: intoxication or poisoning, infection, and toxiccoinfection More than 250 different diseases can cause food poisoning**.** Some of the most common diseases are infections caused by bacteria, such as *Campylobacter, Salmonella, Shigella, E. coli O157:H7, Listeria***,** *Botulism*, and *Norovirus*.

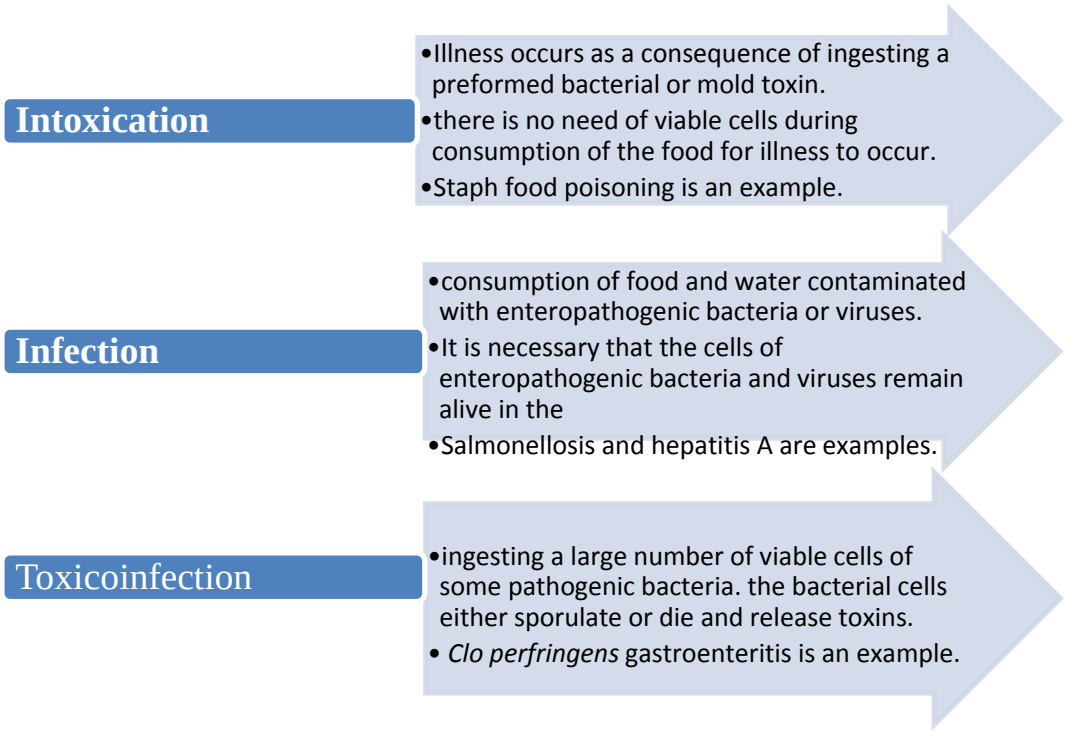


Figure 1.7.1: Types of diseases microbes can cause food poisoning.

1.7.2 Persons Susceptible for Food Poisoning

Pregnant Women: Foodborne illness during pregnancy is serious and can lead to miscarriage, premature delivery, stillbirth, sickness or the death of a newborn baby.

Young Children: Young children are more at risk for foodborne illness because their immune systems are still developing.

Older Adults: As people age, their immune system and other organs become sluggish in recognizing and ridding the body of harmful bacteria and other pathogens that cause infections, such as foodborne illness.

People with chronic disease*:* Having a chronic condition such as diabetes, liver disease or AIDS or receiving chemotherapy or radiation therapy for cancer reduces immune response (Niddk.nih.gov, 2016).

1.8 Microorganisms Causing Foodborne Illnesses

1.8.1 *Escherichia coli*

Escherichia coli (or E. coli) are the most prevalent infecting organisms in the family of gram-negative bacteria known as enterobacteriaceae. Most *E. coli* are harmless and actually are an important part of a healthy human intestinal tract. However, some *E. coli* are pathogenic, meaning they can cause illness, either diarrhea or illness outside of the intestinal tract. The types of *E. coli* that can cause diarrhea can be transmitted through contaminated water or food, or through contact with animals or persons.

Figure 1.8.1: *Escherichia coli*

1.8.1.1 Pathogenesis

E. coli bacteria escape the intestinal tract through a perforation (for example from an ulcer, a ruptured appendix, or due to a surgical error and enter the abdomen, they usually cause peritonitis that can be fatal without prompt treatment. However, *E. coli* are extremely sensitive to such antibiotics as streptomycin or gentamicin. Recent research suggests treatment of enteropathogenic *E. coli* with antibiotics may not improve the outcome of the disease four pathotypes (ETEC, EPEC, EIEC, and EHEC) are associated with diarrhea and collectively are referred to as diarrheagenic *E. coli*. They are:

Name	Hosts	Description
Enterotoxigenic E. coli (ETEC)	causative agent of diarrhea (without	ETEC uses fimbrial adhesin**s** (projections from the bacterial cell
Enteropathogenic *E. coli* (EPEC)	causative agent of diarrhea in humans, rabbits, dogs, cats and horses	Like ETEC, EPEC also causes diarrhea, but the molecular mechanisms of colonization .they use an adhesin known as intimin to bind host intestinal cells.
Enteroinvasive E. coli (EIEC)	found only in humans	EIEC infection causes a syndrome that is identical to *shigellosis***,** with profuse diarrhea and high fever.
Enterohemorrhagic E. coli (EHEC)	found in humans, cattle, and goats	EHEC can cause hemolytic-uremic syndrome and sudden kidney failure.

(Ecl-lab.com, 2017)

1.8.1.2 Source of contamination

- *E. Coli*-contaminated ground meat Also other meat items.
- *E. Coli* outbreaks connected with restaurant sustenance.
- Utilization from claiming crude milk and also unpasteurized cheeses stays a danger element to *E. coli*i infection.
- *E. coli* outbreaks followed should sprouts, lettuce, spinach, parsley, and other new handle.
- Water need been distinguished as that hotspot from claiming a few *E. coli* outbreaks.
- Animal-to-person transmission of *E. coli*. Ethnic minority transmission of *E. coli.*
- Feces of infected people (Mayoclinic.org, 2016).

1.8.2 *Salmonella Spp***.**

Salmonella is a gram-negative bacteria of the Enterobacteriaceae family. The two species of *Salmonella* are *Salmonella enterica* and *Salmonella bongori*. Many infections are due to ingestion of contaminated food. These types of bacteria are the causative agents of numerous diseases, such as bacteremia, enteric fever, and enterocolitis in a broad range of

organisms. Although some serovars of *S. enterica*, such as serovars Typhimurium and Enteritidis, can cause disease in a wide range of hosts, other strains have a greatly reduced range of host specificity (Darwin, K. H. & Miller, V. L.1999).

Figure 1.8.2: *Salmonella spp.*

1.8.2.1 Pathogenesis

Salmonella spoiling will be a standout amongst a significant number time permits reason for gastroenteritis (also known as 'gastro'). There are many distinctive sorts from claiming salmonella microscopic organisms and they happen in a number down home and wild animals, including birds, frequently creating ailment clinched alongside them. The class salmonella may be a part of the family enterobacteriaceae . It was found that *Salmonella Enteritidis* outbreaks were more common in the EU states and eggs were the most frequent vehicle of infection. Poultry products in the EU and dairy products in the United States were related to *Campylobacter* associated outbreaks. In Canada, *Escherichia coli* outbreaks were associated with beef. In Australia and New Zealand, *Salmonella typhiumurium* outbreaks were more common (Greig & Ravel, 2009).

Main types of Salmonella *entericas*erovars	**Description of the pathogen**
Salmonella entericaserovarTyphi.	Also known as *Salmonella typhi* .This bacterium will be those causative agenize from claiming typoid fever.
salmonella entericaserovar Typhimurium	Also called *salmonella typhimurium* .this causes diarrhea, abdominal cramps, spewing and nausea,
salmonella entericaserovar Enteritidis	Also called *salmonella enteritidis*. This bacterium increase in the rise of *S. Enteritidis* infections on the use of mass production chicken farms.

(Taylor & Francis, 2017)

1.8.2.2 Typical Symptoms of *Salmonella* infection

- Generalized systemic enteric fever
- Headache
- Malaise
- Anorexia
- Enlarged spleen and
- Constipation followed by more severe abdominal symptoms
- Rose spots on trunk in 25% of Caucasian patients
- Ulceration of Peyer's patches in ileum,
- Can produce hemorrhage or perforation.

1.8.2.3 Source of contamination

Salmonella infection usually results from ingestion of the bacteria from contaminated food, water or hands. Eggs, milk, meat or poultry are particularly high risk foods.Fruit and vegetables may also be contaminated, especially if manure has been used as fertilizer.

1.8.3 *Shigella spp.*

It consists of four species *Sh. dysenteriae, Sh. flexneri, Sh. boydii*and *Sh. sonnei,* all of which are regarded as human pathogens though they differ in the severity of the illness they cause. *Sh. sonnei* causes the mildest illness, while that caused by *Sh. boydii* and *Sh. flexneri* is of intermediate severity (Adams & Moss, 2008)

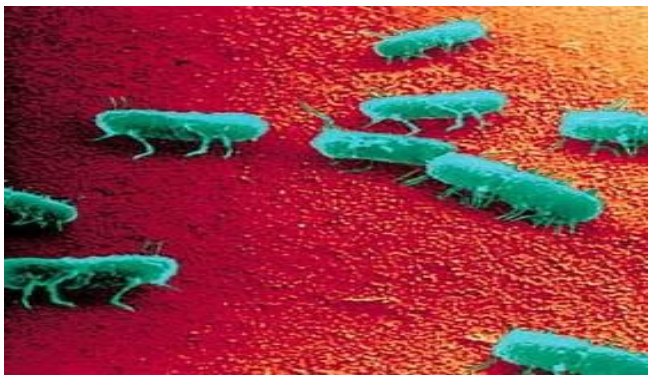


Figure 1.8.3: *Shigella Spp.*

1.8.3.1 Pathogenesis

Shigellosis is caused by a bacterial infection of the *Shigella* genus. *Shigella* bacteria are Gram-negative, rod-shaped, non spore-forming, facultative anaerobes. There are four *Shigella* species, only three of which are the major disease causing species. *S. flexneri* accounts for roughly 60% of shigellosis cases in the developing world and is the most frequently isolated species of *Shigella* worldwide .*S. sonnei* is the cause of 77% of cases in the developed world while only causing 15% of cases in the developing world, and it is also causes two-thirds of shigellosis cases in the United States. *S. dysenteriae* is the most common cause of dysentery epidemics, usually in confined populations with poor hygiene and little to no sanitation, for example environments such as refugee camps. *S. dysenteriae* is a major concern because it is the only species of *Shigella* that produces the Shiga toxin (Microbewiki.kenyon.edu, 2017)

1.8.3.2 Symptoms

Abdominal pain, vomiting and fever are accompanying a diarrhea which can range from a classic dysenteric syndrome of bloody stools containing mucus and pus. Illness lasts from 3 days up to 14 days in some cases and a carrier state may develop which can persist for several months.

1.8.3.3 Source of contamination

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

1.8.4 *Vibrio Spp.*

Historically, cholera has been one of the diseases most feared by mankind. *Vibrios* are Gram-negative pleomorphic (curved or straight), short rods which are motile with (normally) sheathed, polar flagella. Catalase and oxidase-positive cells are facultative anaerobic and capable of both fermentative and respiratory metabolism.

Figure 1.8.4: *Vibrio Spp.*

1.8.4.1 Pathogenesis

Vibrio cholerae may cause gastroenteritis through production of known toxins or unknown mechanism. *Vibrio parahaemolytitucs* strains capable of producing thermostable direct hemolysin (TDH) and/or TDH-related hemolysin are most important

cause of gastroenteritis associated with seafood consumption. *Vibrio vulnificus* is responsible for seafoodborne primary septicemia and its infectivity depends primarily on the risk factors of the host. *V. vulnificus* infection has the highest case fatality rate (50%) of any foodborne pathogen (Highveld.com, 2017).

1.8.4.2. Source of contamination

Cholera is regarded primarily as a waterborne infection, though food which has been in contact with contaminated water can often serve as the vehicle. Cholera is an acute intestinal infection causing profuse watery diarrhea, vomiting, circulatory collapse and shock. Many infections are associated with milder diarrhea or have no symptoms at all. If left untreated, 25-50% of severe cholera cases can be fatal. The disease is occasionally spread through eating raw or undercooked shellfish that are naturally contaminated (Cdc.gov, 2016).

1.9 Factors Affecting Growth of Microorganisms in Food

There are important factors which directly or indirectly affect the growth of microorganisms in food products. When microorganisms grow in food they cause varying degrees of change in the food's characteristics as a result of metabolic activity. Some of these changes, like those taking place during fermentation, are desirable, while others, like those resulting in food spoilage and food poisoning are undesirable (Hamad, S,2017)
The most important factors that affect microbial growth in foods can be summarized in the following categories:

Intrinsic factors:

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

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

Extrinsic Factors:

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

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

Implicit Factors:

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

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

1.10 Food Safety Class or Category

- **Environmental hygiene class / category:** Primarily food production and preparation should not be carried on in areas where the presence of potentially harmful substances would lead to an unacceptable level of such substances in food. This is to ensure all the safety, nutritional quality and acceptability of the delivered foods.
- **Personal Hygiene and cleanliness category:** Washing hands before handling food and often during food preparation Washing hands after going to the toilet Drying hands after hand washing Wearing clean protective clothing Wearing head covering to be a carrier of a disease or illness likely to be transmitted through food.

1.11 Economic Impact of Foodborne Illness

Every illness has an economic cost and same is the case with foodborne illness. However, the economic cost of health losses related to foodborne illnesses has not been extensively studied. There are few studies available which provide either incomplete cost estimates or their estimates are based on limiting assumptions (Buzby & Roberts, 2009). In the United States, data from Foodborne Diseases Active Surveillance Network (FoodNet) and other related studies contributed to estimates of the economic cost of foodborne illness (Greig, J. D., & Ravel, A,2009).

1.12 Frequency of Food Borne Illness in Different Countries

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

1.13 Transmission of Enteric Pathogens

Enteric pathogens that are believed to be capable of being transmitted by food workers include, but are not limited to, *E. coli*, *Salmonella* spp, *Shigella* spp. enteric pathogens are among the transient hand flora that can be easily removed by hand washing. In addition, pathogens such as Proteus,and chemical hazards which could originate from raw animal products, can contaminate hands from where they could be transferred to foods, equipment and other workers.Isolation of these organisms includes safeness-to-hand spread and indicating poor hygiene practices of the food handlers. In the present study' *Enterobacteriaceae* were identified from hands of 31.7% food handlers.This could be resulted due to difference in source population, and type of food establishment. Isolation of *Enterobacteriaceae* from hands reflects contamination with fecal matter, and inadequate and poor hand washing habit which may pose potential risk of food borne outbreaks.

1.14 Importance of Foodborne Disease Awareness

Food-borne disease outbreak (FBDO) is an important public health issue related to the food safety management in a country. Each year, unsafe food makes at least two billion people ill worldwide, or about one-third of the global population. The Centres for Disease Control and Prevention has identified more than 400 food-related illnesses. About two thirds of all outbreaks involve bacteria. The illnesses are caused either by the microorganisms themselves or by the toxins they release. The consumption of foods

contaminated by foodborne pathogenic microorganisms and toxins produced by them cause deaths, illnesses, hospitalization, and economic losses. Due to their widespread nature, foodborne diseases, in particular gastro-intestinal infections, represent a very large group of pathologies with a strong negative impact on public health. However, it is extremely important to note that most cases of foodborne disease in the region are not reported, so the true extent of the problem is unknown. In most countries of the region, the surveillance infrastructure for food-borne diseases of both microbiological and chemical etiology is weak or non-existent. This absence of reliable data on the burden of food-borne disease impedes understanding about its public health importance and prevents the development of risk based on foodborn illness (Chang, J. M., & Chen, T. H, 2003).

1.14.1 Improvement of Quality of Cafeteria's Food

Food handlers should receive training before starting work in any food establishment, with a periodic refreshing training. Food handlers must have got long course of training on food hygiene. Effective training of food handlers, may lead to an improvement in hygienic practices. Food handlers should cover hair and wear appropriate protective covering, cut their fingernails short and during handling they should remove jewellery from their hands. Persons working in food services have to go through periodic medical examination.Separating raw meat, poultry, fish and seafood, eggs, fruits and vegetable from other foods.Using separate equipment and utensils such as bowls, knives and cutting boards for handling raw foods.Storing food in separate containers to avoid contact between raw and prepared foods.Washing fruits and vegetables, especially if eaten raw.Removing outer leaves of leafy vegetables.Cooking food thoroughly; make sure that the temperature has reached 70°C.Reheating cooked food thoroughly.Avoiding leaving cooked food at room temperatures for more than 2 hours. Health agencies are increasingly conducting systematic reviews of foodborne disease outbreak investigations to develop strategies to prevent future outbreaks.

Literature Review

Food borne disease is an important health problem and is a leading cause of morbidity and mortality in developing countries. A cross sectional study conducted with the cafeteria foods in Jimma University evaluated the bacterial hand contamination and associated factors among food handlers working in the student cafeterias. Two thirty food handlers were tested to find out any bacterial contamination present in their hand rinse samples. About 50% food handlers samples were tested positive for one or more potential food borne bacterial contaminants. Enteric pathogens were identified in 32% samples. A total of 171 bacterial hand contaminants were isolated. The microorganism identified in the samples were *S. aureus, Klebsiella spp., E. coli, Enterobacter spp. , Citrobacter spp., Serratia marcescens, Pseudomonas aeruginosa, Proteus spp., Providencia rettegri, and salmonella spp* (Tsegaye Assefa1, 2015).

Another study reported cryptosporidiosis outbreak in a university campus in Washington, DC. *Cryptosporidium parvum* was detected in stool specimens of 16 (70%) of 23 ill students and 2 of 4 ill employees. The outbreak of foodborne diseases was due to *Cryptosporidium parvum* and it was indicated that ill food handler was the source for this outbreak (Quiroz ES, 2000).

Foodborne diseases are a public health problem in developed and developing countries like Ethiopia. It has been shown from a recent study in Addis Ababa University student's cafeteria,

Addis Ababa, Ethiopia that the health status of the food handlers, their personal hygiene, knowledge and practice of food hygiene are the risk factors of food contamination. A total of 172 food handlers were participated in the study. A high prevalence of intestinal parasite was found in food handlers. The infected food handlers are responsible for the contamination of foods and drinks and could serve as source of infection to consumers via food chain. Among the food handlers, 78 (45.3%) were found to be positive for different intestinal parasites.

About 71% *Entameoba histolytica/dispar* was found followed by *Giardia lamblia* 18 (18.8%), *Taenia* species 5 (5.2%), *Ascaris lumbricoides* 2 (2.1%), hookworm 2 (2.1%) and *Trichuris trichiura* 1 (1.1%). Stool cultures revealed 3.5% of *Salmonella* isolates which were sensitive to ciprofloxacin, amikacin and gentamicin. However, they were resistant to ampicillin, clindamycin, and erythromycin (Addis Aklilu, 2015).

There have been various reports of diarrhea with blood in Ethiopia Between 2006 and 2008. Shigelloses outbreaks were also reported in some parts of the country. Outbreak of diarrheal illness among students occurred in Addis Ababa University (AAU) Technology Campus. A total of 104 suspected cases were identified. *Shigella flexneri* (45%) was confirmed in stool culture. Fecal contamination was found in water stored in a tank in the cafeteria.The hygiene and sanitary conditions in the cafeteria, kitchen, and living area were not satisfactory
(Aragaw M, 2011).

Soriano et al. reported 19 (3.8%) yielded strains of enterotoxigenic *staphylococci*, and 10 (52.6%), 4 (21.1%), 3 (15.8%), and 2 (10.5%) of these strains produced different types of enterotoxins among 504 food samples. All these food samples were collected from cafeterias (Soriano JM, 2002).

Chapter 2

OBJECTIVE OF STUDY

Research Objective

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

To identify the presence of bacteria especially *E. coli*, *Salmonella*, *Shigella* and *Vibrio* species from different types of cafeteria foods in different private institutional premises situated in Dhaka city, Bangladesh.

Chapter 3

METHODOLOGY

METHODOLGY

3.1 Bacteriological Subculture

3.1.1 Sample Collection:

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

Sample Category:

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

Sample Processing:

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

3.1.3 Enrichment of the Organisms:

3.1.3.1 Enrichment of E. coli and Klebsiellaspp:

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

3.1.3.2 Enrichment of Salmonella spp and Shigellaspp:

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

3.1.3.3 Enrichment of Vibrio spp:

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

Fig.3.1: Enrichment of the Organisms

3.1.4 Selective Growth of the Organisms

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

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

3.1.4.2 Selective Growth of *Salmonella spp* and *Shigella spp:*

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

3.1.4.3 Selective Growth of *Vibrio spp*:

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

3.1.5 Sterilization Procedure:

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

Fig. 3.2: Autoclave and Hot air Oven

3.1.6 Preparation of Petri dishes

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

Fig. 3.4: Petri dishes preparation

3.1.7 Incubation

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

Fig. 3.5: Incubator

Table 3.1: Standard Colony Morphology of Suspected Organisms

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

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

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

- Laminar air flow cabinet (ESCO, Singapore)
- Petri dishes
- Autoclave (HIRAYAMA, Japan)
- Hot air oven (FN-500, Niive)

Agar media:

- MacConkey agar
- XLD agar
- TBX agar
- BGA agar
- TCBS agar

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

3.2 Biochemical Tests

3.2.1 Kliglar Iron Agar Test (KIA Test)

3.2.1.1 Test Tube Preparation for KIA Test

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

3.2.1.2 Inoculation for KIA Test

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

Fig. 3.6: Preparation of test tubes for KIA test

3.2.2 MIO Test

3.2.2.1 Test Tube Preparation for MIO Test

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

3.2.2.2 Inoculation for MIO Test

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

Fig. 3.7: Preparation of test tubes for MIO test

3.2.3 Citrate Test

3.2.3.1 Test Tube Preparation for Citrate Test

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

3.2.3.2 Inoculation for Citrate Test

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

Fig. 3.8: Preparation of test tubes for Citrate test

3.2.4 Urease Test

3.2.4.1 Test Tube Preparation for Urease Test

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

3.2.4.2 Inoculation for Urease Test

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

Fig. 3.9: Preparation of test tubes for Urease test

3.2.5 Oxidase test:

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

3.2.6 Apparatus & reagent used for Biochemical Tests

- Laminar air flow cabinet (ESCO, Singapore)
- Screw cap test tubes
- Autoclave (HIRAYAMA, Japan)
- Hot air oven (FN-500, Niive)
- Straight wire
- Spirit burner
- Incubator
- Distilled water
- Oxidase Reagents:
- Kovac's reagent
- Agar:
- Kliglar’s Iron Agar
- MIO agar
- Christensen’s Urea Agar
- Simmons citrate medium

Table 3.2: Standard Biochemical Test Results of Suspected Organisms

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

For KIA test, slant and butt portion of test tube is also observed to identify acid and alkali. K indicates acid and A indicates alkali. It can be K/A, A/K, K/K or even A/A for slant/butt.

3.3 Colony Counting Methodology

3.3.1. Cell counting and serial dilutions

3.3.1.2. Theory

In quantitative microbiology, we are concerned with determining the concentration of colony forming units (CFUs) in our sample – i.e., the number of CFUs per ml or per gram of the sample. More realistically, the concentration of CFUs in the sample could have been considerably greater. Counting the colonies on a plate inoculated with one ml of

sample may be impossible. We would like 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 succeedingly 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.

3.3.1.3. Materials Required

- Tubes
- Micropipette with tips
- Distilled water
- Bacteria sample
- Nutrient agar
- Petri dishes
- Water bath
- Alchohol
- Colony counter
- Conical Flask
- Labeling Tape

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.

3.3.1.5. Preparation of Serial Dilutions

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

3.3.1.6. 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

When the agar was set, the plate was incubated as appropriate.

3.3.1.5. Counting bacterial colonies

1. After an appropriate incubation period the plates were examined for colonial growth.

2. Colonies will form on the top of the agar as well as in the agar. Those on top of the agar will be larger but all colonies must be counted.

3. Plates were selected that appear to have between 30 - 300 colonies in and on the agar as this gives the best statistical representation of the number of bacteria in the undiluted sample.

4. Using a light box or colony counter (if one is available) and marker pen (put a dot above each colony as you count it), the number of colonies were counted in each

Chapter 4

Result

Result

4.1Bacterialcolonymorphology:

Table 4.1: Bacterial colony morphology isolated from different canteen foods

Name of University	Sample	Plates				
		MacConkey	**TBX**	**BGA**	**XLD**	**TCBS**
East West University	Sandwich1	Pink	Blue	No growth	No growth	Mo growth
	Jambu barger	colorless	No growth	No growth	No growth	yellow
	Egg curry	pink	No growth	No growth	No growth	yellow
	Burger	Colorless	No growth	No growth	No growth	No growth
	Tomato vorta	Pink &colorless	No growth	No growth	No growth	No growth
	Vegetable Roll 1	No growth	No growth	No growth	No growth	yellow
North South University	Sandwich2	pink	White	No growth	Red	yellow
	Chicken Patice	pink	No growth	No growth	No growth	Yellow
	Singara 2	pink	Colorless	No growth	No growth	yellow
	Dim chop	pink	No growth	No growth	No growth	Yellow
	Alur chop	pink	No growth	No growth	No growth	Green
University informarion and technology	Sandwich 3	colorless	No growth	No growth	No growth	No growth
	Chicken roll	No growth	No growth	No growth	No growth	Blackish green
	Vegetable Roll 2	No growth	blue	No growth	No growth	yellow

Table 4.1: (Bacterial colony morphology isolated from different canteen food samples). Around 14 food samples were collected from different university cafeterias in Dhaka city.

In total 12 samples were shown growth of different pathogenic or non pathogenic microorganisms. Of which, 10 samples were shown positive growth of our suspected organisms of *E.coli and Vibio spp*.

4.2 Bacterial colony morphology:

Table 4.2: Bacterial colony morphology isolated from different canteen food samples

Name of university	**sample**	**Plates**				
		MacConkey	**TBX**	**BGA**	**XLD**	**TCBS**
East West University	Somucha1	Pink, colorless	blue	No growth	No growth	Yellow
	Singara 1	pink	No growth	No growth	No growth	yellow
	Noodles 1	pink	blue	No growth	No growth	Yellow
	Dim vorta	No growth	No growth	No growth	No growth	Yellow
	Chicken masala	pink	No growth	No growth	No growth	Black&Yellow
	Jambu barger	Yellow& colorless	No growth	No growth	No growth	No growth
	Hot dog	no	blue	No growth	No growth	Yellow & Yellow with Black Dot
	Alu vorta	Mucoid pink	No growth	No growth	No growth	Yellow with black dot
	Mayonnaise	colorless	No growth	No growth	No growth	yellow
	Dim chop	No growth	No growth	No growth	No growth	Yellow
North South University	Chicken shashlik	No growth	No growth	No growth	No growth	yellow
	beguni	pink	green	No growth	No growth	Yellow&green
University of Liberal Arts Bangladeh	pizza	No growth	No growth	No growth	No growth	No growth
	salad	No growth	No growth	No growth	No growth	No growth

Around 21 food samples were collected from different university cafeterias in Dhaka city. In total, 13 samples were shown growth of different pathogenic or non pathogenic microorganisms. Of which, 4 samples were shown positive growth of our suspected organisms *E.coli* and *6* samples were shown positive growth of *Vibio spp.*

Table 4.3: Number of food samples with growth of suspected organisms determined by colony morphology (n=35)

Name of Universities	No. of of samples with +ve growth *E.coli*	No. of of samples with +ve growth *Vibrio spp*	No. of of samples with +ve growth *Salmonella*	No. of of samples with +ve growth *Shigella Spp*
East west University	9	8	1	0
North South University	6	4	0	0
University informarion and technology	3	5	0	0

From total 35 food samples that were collected from different university cafeterias, we found contamination in 25 (71%) samples (Table 4.1 and Table 4.2). Of which, 21 (60%) samples were suspected to be contaminated with our targeted organisms ((*E coli, Shigella, Salmonella* and *Vibrio* species).

Among 21 samples, 18 (85%) samples were suspected to be contaminated with *E coli*, 17 (80%) samples were suspected to be contaminated with *Vibrio spp*, 1 (4%) samples were suspected to be contaminated with *Salmonella.*

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

Samples	**Plates**	**Colony Morphology**	**M**	**I**	**O**	**Ci-trate**	**Urease**	**Oxidase**	**KIA**			organism
									Slunt/ butt	**gas**	**H_2S**	
Sand witch1	TCBS	Yellow	-	+	-	+	+	+	K/A	-	-	*Vibrio SPP*
Jambu Barger	TCBS	Yellow	+	+	-	-	-	-	K/A	-	-	
Egg curry	TCBS	Yellow	-	+	-	+	-	-	K/A	-	-	
Vegetable roll 1	TCBS	Yellow	+	+	-	+	-	-	K/A	-	-	
Dimchop	TCBS	Yellow	-	+	-	+	-	-	K/A	-	-	
pizza	TCBS	Yellow	+	+	+	+	-	-	K/A	-	-	
Chicken patices	TCBS	Yellow	+	+	-	+	-	-	K/A	-	-	
Dim vorta	TCBS	Yellow	-	+	-	+	-	-	K/A	-	-	
Dim chop	TCBS	Yellow	+	+	-	+	-	-	K/K	-	-	
Sandwitch 2	TCBS	Yellow	+	-	-	+	-	-	K/A	-	-	
Somucha 2	TCBS	Yellow	-	+	-	-	-	-	K/A	-	-	
Fucha	TCBS	Yellow	+	+	-	+	-	-	K/A	-	-	

Among 21 (60%) food samples were subjected for different biochemical test to identify our targeted organisms. Biochemical test results of about 19 (54%) food samples were

shown similarities with the standard biochemical test results of our targeted organisms (*E.coli, and Vibiospp.*) as compared.

Table 4.4 shows identification of the suspected organism (vibrio spp.) from different biochemical test.

Among 19 food samples were subjected for different biochemical test to identify our targeted organisms. Biochemical test results of about 12 (63%) food samples were shown similarities with the standard biochemical test results of our targeted organism *Vibrio spp.*

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

Samples	**Plates**	**Colony Morphology**	**M**	**I**	**O**	**Citrae**	**Urease**	**Oxidase**	**KIA**			**organism**
									Slunt /butt	**Gas**	**H_2S**	
Singara 1	TBX	Blue	-	+	-	+	-	-	A/A	-	+	
Somucha 1	MacConkey	Pink	+	+	-	+	-	-	A/A	-	+	*E.coli*
Veg roll2	TBX	Blue	+	+	-	-	-	-	K/A	-	+	
Alur chop	MacConkey	Pink	+	+	+	+	-	-	K/A	-	+	
Singara2	MacConkey	Pink	-	+	-	+	-	-	A/A	-	+	
Chicken pattice	MacConkey	Pink	+	+	-	+	-	-	A/A	-	+	
Burger	MacConkey	Pink	+	+	-	+	-	-	K/A	-	+	
Sandwitch 1	MacConkey	Pink	-	+	-	+	-	-	K/A	-	+	

Table 4.5 shows shows identification of the suspected organism (*E.coli*) from different Biochemical test

Biochemical test results of about 8(42%) food samples were shown similarities with the standard biochemical test results of our targeted organism *E.coli.*

Table 4.7: Incident of foodborne pathogen in various canteen food samples (n=35)

Pathogens	**Food catagory**				
	Salad items (N=1)	**Sauce items (N =1)**	**Fast food (N =24)**	**Lunch items (N=9)**	**Total food items N=35**
E .coli	Nd	Nd	8(33%)	Nd	8(22%)
Vibrio spp.	Nd	Nd	10(41%)	2(22%)	12(34%)

Among salad items, no suspected microorganism was found.

- ✓ In fast food sample were contained *E.coli*, 8(33%)
- ✓ Fast food sample were contained *Vibrio spp* 10(41%) and
- ✓ Lunch items were contained 2(22%) *Vibrio spp*

Table 4.8.1: Colony counting of various samples:

Name of University	Sample	Dilution 1	Dilution 2	Dilution 3	Dilution 4
East West University (EWU	Somucha1	Uncountable	Uncountable	76	17
	Singara 1	47	19	18	6
	Noodles 1	Uncountable	Uncountable	30	10
	Dim vorta	56	25	21	12
	Chicken masala	25	12	9	4
	Jambu barger	8	5	2	1
	Hot dog	Uncountable	Uncountable	51	13
	Alu vorta	Uncountable	23	20	14
	Mayonnaise	41	22	10	10
	Dim chop	Uncountable	Uncountable	Uncountable	30
	Sandwich1	Uncountable	Uncountable	60	13
	Jambu barger	Uncountable	Uncountable	60	22
	Egg curry	Uncountable	Uncountable	Uncountable	48
	Burger	5	6	0	0
	Tomato vorta	Uncountable	Uncountable	5	3
	Vegetable Roll 1	Uncountable	Uncountable	4	2

Table 4.8.2: Colony counting of various samples:

Name of University	Sample (Street food)	Dilution 1	Dilution 2	Dilution 3	Dilution 3
North South University (NSU)	Sandwich2	Uncountable	Uncountable	34	17
	Chicken patice	50	25	18	3
	Singara 2	Uncountable	32	11	10
	Dim chop	Uncountable	30	20	12
	Alur chop	25	12	9	4
	Chickenshask	8	5	2	1
	Beguni	Uncountable	Uncountable	30	13
University informarion and technology(UIT)	Sandwich 3	Uncountable	20	15	9
	Chicken roll	41	22	15	12
	Vegetable roll 2	Uncountable	Uncountable	Uncountable	30
Pacific Advantech university(PAU)	Dal vaji	5	4	0	0
University of Liberal Arts Bangladesh(ULAB)	Pizza	9	4	3	2
	Salad	5	2	0	0
	Biriani	5	6	0	0

Colony counting of various samples

For somucha 1plate 2 was selected as it has between 30-300 colonies. The other plates cannot be used because they are either too thick with colonies or too thin. The calculation of cell concentration in the original culture is:

76 colonies on plate 3X dilution factor of 1,000 = 76000 cells/ml.

For singara 1plate 1was 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:

47 colonies on plate 1X dilution factor of 10= 470cells/ml.

For Noodles 1 1plate 1was 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:

30colonies on plate 3X dilution factor of 1000= 30000cells/ml.

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

56 colonies on plate 1X dilution factor of 10= 560cells/ml.

For hot dog plate 3was 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:

51colonies on plate 3X dilution factor of 1000= 51000cells/ml.

For Mayonnaise plate 1was 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 cellconcentration in the original culture is:

41 on plate 1X dilution factor of 10= 410cells/ml.

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

30 on plate 4X dilution factor of 10000= 300000cells/ml.

For Sandwich1 plate 3was 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 cellconcentration in the original culture is:

60 on plate 3X dilution factor of 1000= 60000cells/ml.

For Jambu barger 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 cellconcentration in the original culture is:

60 on plate 3X dilution factor of 1000= 60000cells/ml.

For egg curry 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:

48 on plate 4X dilution factor of 10000= 480000cells/ml.

For Sandwich2 plate 3was selected as it has between 30-300 colonies. The other plates cannot be used because they are either too thick with colonies or too thin. The calculation of cell concentration in the original culture is:

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

For Chicken Patice plate 1was 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:

50 on plate 1X dilution factor of 10= 500cells/ml.

For Singara 2plate 2was 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:

32on plate 2X dilution factor of 100= 3200cells/ml.

For Dim chop plate 2was 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:

30on plate 2X dilution factor of 100= 3000cells/ml.

For beguni 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:

30on plate 3X dilution factor of 1000= 30000cells/ml.

Chicken roll 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:

30on plate 1X dilution factor of 10= 300cells/ml.

Vegetabl roll 2 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:

30on plate 4X dilution factor of 10000= 30000cells/ml.

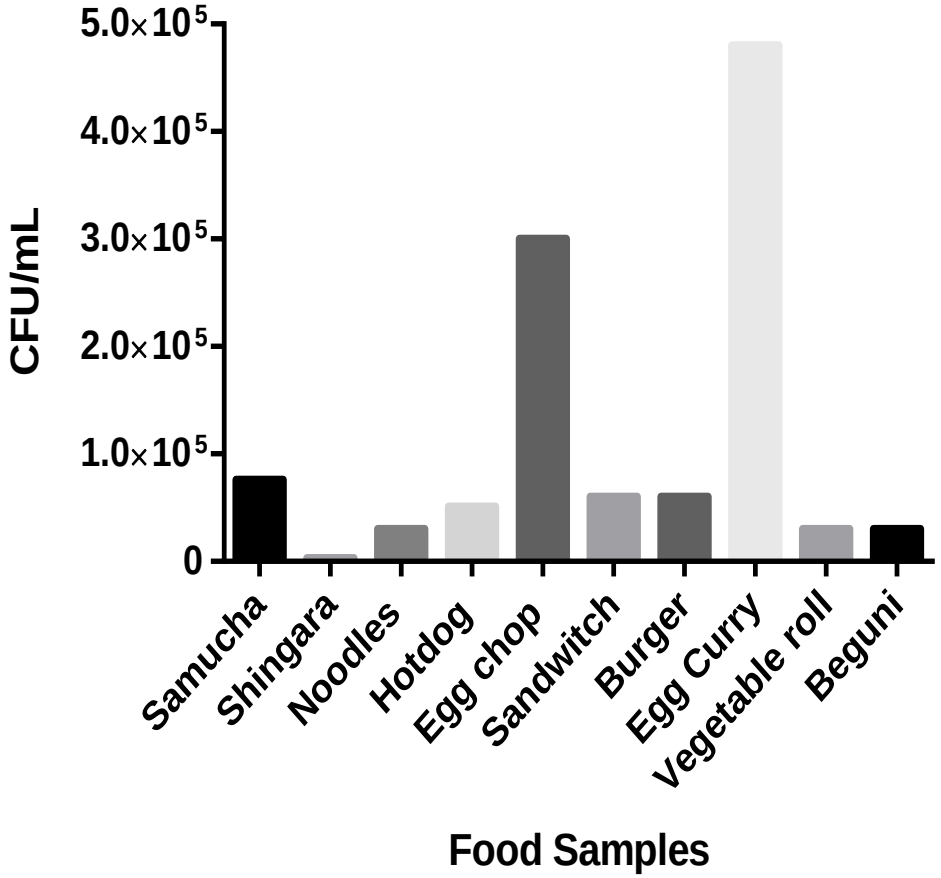
Rest of the colony was uncountable or more than 30-300 colonies, so the cell concentration in the culture cannot be calculated

Table 4.8.3: Number of colonies per ml of sample:

Sample Name	**Number of microorganism (cells/ml)**
Somucha1	76000 cells/ml.
singara 1	470cells/ml
Noodles 1	30000cells/ml
Dim vorta	560cells/ml
hot dog	51000cells/ml.
Mayonnaise	410cells/ml.
dim chop	300000cells/ml.
Sandwich1	60000cells/ml.
Jambu barger	60000cells/ml.
egg curry	480000cells/ml.
Sandwich2	34000cells/ml.
Chicken Patice	500cells/ml
Singara 2	3200cells/ml.
Dim chop	3000cells/ml.
beguni	30000cells/ml
Chicken roll	300cells/ml
Vegetabl roll 2	30000cells/ml

We have seen from this graph that highest colony formation in egg curry and egg chop sample.

Figure 1: Bacterial count in different food samples



Chapter 5

Discussion

Discussion and conclusion

At present time Food borne disease is an important health problem and is a leading cause of morbidity and mortality in developing countries like Bangladesh. The rate is rising because of insufficient supervision and monitoring by food safety officers. There is also lack of awareness, training in food safety and good hygiene practices among food handlers. Consumers should also be conscious about the food safety to avoid these diseases.

This study was designed to isolate and identify the enteric bacteria specially *E.coli, Vibio spp., Shigella spp. and Salmonella spp* from different types of Cafeteria foods from different universities. Seven cafeterias of different universities were chosen for collection of food samples. The universities were EWU, NSU, UIT, ULAB, DIU, UAP, SU etc
The main objective of this study was to isolate and identify enteric bacteria specially *E.coli, Vibio spp., Shigella spp. and Salmonella spp* from different types of cafeteria foods.

A total of 35 samples were collected from different cafeterias of universities in Dhaka city. Five different agar media were used for isolation and identification of our suspected organisms from the samples. The agar media were MacConkey, TBX (Tryptone Bile X-glucuronide Agar), BGA (Brilliant Green Agar), XLD (Xylose lysine deoxycholate) and TCBS (Thiosulfate citrate-bile salts sucrose). Microbial growth was observed in different media plates. Out of thirty five food samples 8 (42%) samples were suspected to be contaminated with *E.coli*, 12 (25%) samples were suspected to be contaminated with *Vibrio spp*.

A cross sectional study conducted with the cafeteria foods in Jimma University evaluated the bacterial hand contamination and associated factors among food handlers working in the student cafeterias. Two thirty food handlers were tested to find out any bacterial contamination present in their hand rinse samples. About 50% food handlers samples were tested positive for one or more potential food borne bacterial contaminants. Enteric pathogens were identified in 32% samples. A total of 171 bacterial hand contaminants were isolated. The microorganism identified in the samples were *S. aureus, Klebsiella*

spp., E. coli, Enterobacter spp. , Citrobacter spp., Serratia marcescens, Pseudomonas aeruginosa, Proteus spp., Providencia rettegri, and salmonella spp.

Another study reported cryptosporidiosis outbreak in a university campus in Washington, DC. *Cryptosporidium parvum* was detected in stool specimens of 16 (70%) of 23 ill students and 2 of 4 ill employees. The outbreak of foodborne diseases was due to *Cryptosporidium parvum* and it was indicated that ill food handler was the source for this outbreak (Quiroz ES, 2000).

A recent study in Addis Ababa University student's cafeteria, Addis Ababa, Ethiopia was conducted to evaluate that the health status of the food handlers, their personal hygiene, knowledge and practice of food hygiene are the risk factors of food contamination. A total of 172 food handlers were participated in the study. Among the food handlers, 78 (45.3%) were found to be positive for different intestinal parasites. About 71% *Entameoba histolytica/dispar* was found followed by *Giardia lamblia* 18 (18.8%), *Taenia* species 5 (5.2%), *Ascaris lumbricoides* 2 (2.1%), hookworm 2 (2.1%) and *Trichuris trichiura* 1 (1.1%). Stool cultures revealed 3.5% of *Salmonella* isolates (Addis Aklilu, 2015). In our study samples we have not evaluated the contamination related to health status of food handlers. We only tested the food samples from the cafeterias of different universities. Our study only estimated the bacteriological colonies.

There have been various reports of diarrhea with blood in Ethiopia. Shigelloses outbreaks were reported in some parts of the country. Outbreak of diarrheal illness among students occurred in Addis Ababa University (AAU) Technology Campus. A total of 104 suspected cases were identified. *Shigella flexneri* (45%) was confirmed in stool culture. Fecal contamination was found in water stored in a tank in the cafeteria. The hygiene and sanitary conditions in the cafeteria, kitchen, and living area were not satisfactory (Aragaw M, 2011). In our study we have found two bacteriological species and they were *E. coli* and *Vibrios*.

Soriano et al. reported 19 (3.8%) yielded strains of enterotoxigenic *staphylococci*, and 10 (52.6%), 4 (21.1%), 3 (15.8%), and 2 (10.5%) of these strains produced different types of enterotoxins among 504 food samples. All these food samples were collected from cafeterias (Soriano JM, 2002).

In our present study, 2 suspected organisms *E.coli* and *Vibio spp.* were found from 21 (40%) samples. From the biochemical test, 19 (54%) food samples were shown similarities with the standard biochemical test results of our targeted organisms (*E.coli, and Vibio spp.*). No *Salmonella spp*. was found from any food of our food samples. Seven biochemical tests were performed for characterizing the organisms but the serological test and PCR technology was not used to confirm the presence of pathogenic bacteria. Therefore, it cannot be said confidently that colonies of the media plates are the claimed ones. All 35 samples divided into 4 categories, eight fast food items 8(33%) sample was suspected to contain *E.coli* and 10 (48%) sample was suspected to contain *Vibrio* spp and in launch items, 2(22%) sample was suspected to contain *Vibrio* spp .

Foodborne illnesses in cafeteria foods have become major source of serious health problem due to microbial contamination. So, more focuses should be given in this sector and more research work should be carried out. In developing country like Bangladesh, it is also suggested that regular monitoring of the quality of cafeteria foods must be practiced to avoid food-borne infection in future. Due attention should be given by the government to improve knowledge about the food safety and quality standards of cafeteria foods in cafeterias of different universities. .

Chapter 6

Reference List

References

Adams, M & Moss, M 2007, 'Bacterial Agents of foodborne illness', Food microbiology, RSC Publishing, Cambridge, UK, pp. 249-260.

Assefa, T 2015, 'Assessment of bacterial hand contamination and associated factors among food handlers working in the student cafeterias of Jimma Main Campus, Jimma, South West Ethiopia', *Journal of Community Medicine & Health Education*, vol. 5, no. 2.doi: 10.4172/21610711.1000345.

Aklilu, A, Kahase, D, Dessalegn, M, Tarekegn, N, Gebremichael, S, Zenebe, S, Desta, K, Mulugeta, G, Mamuye, Y & Mama, M 2015, 'Prevalence of intestinal parasites, *salmonella* and *shigella* among apparently health food handlers of Addis Ababa University student's cafeteria, Addis Ababa, Ethiopia', *BMC Research Notes*, vol. 8, no. 1, p.17.

Aragaw, M, Tafese, T, Beyene, Z, Hailemariam, Z, Azaze, A, Luce, R &Addissie, A 2011, '*Shigellosis* outbreak at Addis Ababa University: March-April 2010', *Ethiopian Medical Journal, vol.* 49, no. 4, pp. 341-348.

Centre for Disease Control & Prevention 2016, 'Foodborne Germs and Illness', viewed 15 March 2017, http://www.cdc.gov/foodsafety/foodborne-germs.html

Centre for Disease Control & Prevention 2016, 'Sources of Infection & Risk Factors', viewed 10 June 2017, https://www.cdc.gov/cholera/infection-sources.html

Chang, J, M, Chen, T & H 2003, 'Bacterial foodborne outbreaks in Central Taiwan 1991-2010, *Journal of Food and Drug Analysis*, vol.11, no.1

Caister Academic Press, n.d., *'Vibrio spp*' viewed 20 June 2017, http://www.highveld.com/microbiology/vibrio.html

Darwin, K H, & Miller, VL 1999, 'Molecular basis of the interaction of *Salmonella* with the intestinal mucosa, *Clinical Microbiology Reviews*, vol. 12, no. 3, pp. 405–428.

Escherichia coli 2004, 'Pathogenic *E. coli'* viewed 20 June 2017,http://www.ecl-lab.com/en/*ecoli*/index.asp

Eng, S, Pusparajah, P, Ab Mutalib, N, Ser, H, Chan, K & Lee, L 2015, '*Salmonella:* a review on pathogenesis, epidemiology and antibiotic resistance', *Frontiers in Life Science*, vol. 8, no. 3, pp. 284-293.

Gormley, F, Little, C, Rawal, N, Gillespie, I, Lebaigue, S & Adak, G 2010, 'A 17-year review of foodborne outbreaks: describing the continuing decline in England and Wales (1992–2008)', *Epidemiology and Infection*, vol. 139, no. 5, pp.688-699.

Gould, D, Kraa, E, Dalton, C, Givney, R, Gregory, J, Stafford, R,& Kirk, M 2016, 'Food borne disease outbreaks in Australia, 1995 to 2000', *Communicable Diseases Intelligence Quarterly Report*, vol.28, no.2, p. 211.

Greig, JD & Ravel, A 2009, 'Analysis of foodborne outbreak data reported internationally for source attribution', *International Journal of Food Microbiology*, vol.130, no.2, pp.77-87.

Hamad, SH 2012, 'Factors affecting the growth of microorganisms in food', in R Bhat, AK Alias & GPaliyath (eds), *Progress in food preservation*, Wiley-Blackwell, Oxford, UK. doi: 10.1002/9781119962045.ch20

Jay, J, Loessner, M, & Golden, D 2005, *Modern food microbiology*, Springer, New York. doi: 10.1007/b100840

Käferstein, F 2003, 'Food borne diseases in developing countries: etiology, epidemiology and strategies for prevention*'International Journal of EnvironmentalHealth Research*, vol.13,no.1, pp. 161-168.

Kirk, M, Pires, S, Black, R, Caipo, M, Crump, J, Devleesschauwer, B, Döpfer, D, Fazil, A, Fischer-Walker, C, Hald, T, Hall, A, Keddy, K, Lake, R, Lanata, C, Torgerson, P,

Havelaar, A & Angulo, F 2015, 'World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis, *PLOS Medicine*, vol. 12, no. 12.

'*Shigellosis*' 2016, Microbe Wiki article, viewed 29 June 2017, https://microbewiki.kenyon.edu/index.php/Shigellosis

Mayoclinic 2016, '*E.coli* Causes- Mayo Clinic', viewed 19 June 2017, http://www.mayoclinic.org/diseases-conditions/e-coli/basics/causes/con-20032105

National Institute of Diabetes and Digestive and Kidney Diseases 2016, 'Foodborne Illnesses', viewed 9 June 2017, http://www.niddk.nih.gov/health-information/health-topics/digestive-diseases/foodborne-illnesses/Pages/facts.aspx.

Quiroz, E, Bern, C, MacArthur, J, Xiao, L, Fletcher, M, Arrowood, M, Shay, D, Levy, M, Glass, R &Lal, A 2000, 'An outbreak of cryptosporidiosis linked to a foodhandler, *The Journal of Infectious Diseases*, vol. 181, no. 2, pp.695-700.

Soriano, J, Font, G, Rico, H, Molto, J & Manes, J 2002, 'Incidence of enterotoxigenic staphylococci and their toxins in foods, *Journal of Food Protection*, vol. 65, no. 5, pp.857-860.