Microbiological Quality of Hospital Food Items Collected from Different Hospitals 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, Shaila Nasrin Shimul, hereby declare that the dissertation entitled "Microbiological Quality of Hospital Food Items Collected From Different Hospitals 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 2016-2017 of my research in the Department of Pharmacy, East West University, under the joint supervision and guidance of Professor Sufia Islam and 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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List of Abbreviations

Abbreviations	Full Name
EPEC	Enteropathogenic *E.coli*
EIEC	Enteroinvasive *E.coli*
ETEC	Enterotoxigenic *E.coli*
APW	Alkaline Peptone Water
BPW	Buffered Peptone Water
TSB	Trypticase Soy Broth
YE	Yeast Extract
TBX	Tryptone Bile X-glucoronide
TCBS	Thiosulfate Citrate Bile Salt-sucrose
BGA	Brilliant Green Agar
XLD	Xylose-Lysine Desoxycholate Agar
MIO	Motility Indole Ornithine
KIA	Kliglar's Iron Agar
HACCP	Hazard analysis and critical control points

Abstract

Food-borne illnesses generally cause disorders of the digestive tract; however, they can also lead to more serious consequences. Food-borne illness is the result of having microbial contaminated food, expired, or toxic food items. Hospital food has become a major health issue for the patients admitted in the hospital. The hospital food service is to provide in-patients with nutritious meals for their recovery and health. The meals are tailored to the specific health conditions of the patients. However, the contaminated foods served in the hospital may cause a potential health hazards for the patients. The objective of this study was to isolate and identify the presence of enteric bacteria (*E.coli, Salmonella, Shigella, Vibrio* species, *Aeromonas* species, *Plesiomonas shigelloides, Yersinia enterocolitica*) in different hospital food items collected from different Hospitals of Dhaka city, Bangladesh. Thirty one food items were collected from different hospitals in Dhaka city. The tested food samples were Hot Dog, Vaji, Egg fry, Banana vorta, Booter daal, Mixed vaji, Beef kabab, Bun, Pizza, Ruti, Roll, Porota, Daal, Chicken soup, Singara, Somucha, Naan, Soup, Daalvaji, Chicken roll, Jaw vaat, Dim chop, Mach vorta, Vegetable soup, Sauce, Salad, Coffee. Sterile polythene bags were used to collect these different samples. They were tested for the presence of microorganisms following conventional microbiological processes. Biochemical tests were performed for the confirmation of *E.coli, Salmonella, Shigella, Vibrio* species, *Aeromonas* species, *Plesiomonas shigelloides, Yersinia enterocolitica*. Out of thirty-one food samples, 4 (12.9%) were suspected to be contaminated with *E. coli*, 9 (29.03%) were suspected to be contaminated with *Vibrio* spp, 4 (12.9%) were suspected to be contaminated with *Aeromonas* spp. The patients can be more vulnerable to have these contaminated food items which are potential cause for food-borne illnesses. Food contamination in a hospital is often overlooked. Further study is needed to find out the contamination of different types of food items with large sample size.

Key Words: Hospital foods, *Escherichia coli*, *Aeromonas* spp, *Vibrio* spp, Food borne illness, Biochemical tests.

Chapter 1:
Introduction and literature review

1.1 Hospital Food Service

The goals of hospital food service are to provide in-patients with nutritious meals for their recovery and health and to present them with a nutritional model with meals tailored to their specific health conditions. When meals are carefully planned and served and when patients consume what they are served, the goals can be achieved (Kim 2010). Generally, the hospitals provide two kinds of treatments: the medical and the nutritional. The medical-treatment includes pharmaceuticals treatment, surgery and the nutritional-treatment includes carefully planned meals that provide all ingredients necessary for each patient's case. Both treatments are of equal importance and should be safe for patients. So meals or foods offered to patients inside the hospital environment are an essential part of their overall care for recovery. This is why the hospital food-service system is considered to be the most complicated and sophisticated production process within the hospitality sector (Kokkinakis 2011). The necessity of properly tailored food for patients in hospitals is a must alongside with medicines when it comes to recovery from illness. Studies have shown that malnourished patients stay 2-3 days longer in hospital than nourished patients, and malnourished patients are at higher risk of readmission within 30 days (Murphy 2017).

1.2 Hospital Food Items

Hospital food-menu is usually and meant to be tailored as well as processed according to the dietary needs of different patient populations based on different factors like as age, gender, culture, ethnicity, social and religious diversity, physical and mental-health needs (Scottish Government 2008).

Figure 1.1: Hospital Food.

In this study it is found that the common food items in different hospitals in Dhaka are like as Rice, Daal, Vegetable curry, Mixed-vegetable, Chicken curry, Khichuri, Beef curry, Egg curry, Sandwich, Ruti, Parata, Egg fry, Chicken soup, Thai soup, Vegetable soup, Salads and different snacks. This food items are served to in-patients according to their individual dietary needs and same items are also sold out in the hospital canteen for out-patients and for attendances, relatives of in-patients.

Figure 1.2: Different type of Hospital food served to patients in Bangladesh.

1.3 Hospital Food: Hygiene and Safety

1.3.1 Necessity of Maintaining Hygiene and Safety:

Food safety in hospitals is defined as an assurance that food will not cause harm to consumers when it is eaten by different patients and patients will be safe from different Microorganism based diseases from foods (Hanekom 2010). Hospital foods must be safe and hygienic to consume as it is an essential part of patient's recovery process. The process of hospital food preparing must also be monitored by a safe and hygienic cooking protocol, because there are many recorded cases of food-borne infections or diseases in hospitals. Sometimes the consequences of food borne diseases can bring more sufferings, even can be life-threatening for some patients who are in vulnerable groups like infants, old people (Mentziou 2014).

1.3.2 Challenges of Maintaining Hygiene and Safety

The maintenance of food safety becomes complex when the food is prepared and served to hospitalized patients, since they are more vulnerable for food borne diseases than general

population as most of them have low-immunity or many of them are immune-suppressed patients. Though food safety is an important concern but health institutions such as hospitals pay less attention. Nowadays, the concept of food safety is in increasing trend, because of the increasing outbreaks of food-borne diseases worldwide. Food safety is usually not prevailed due to lack of proper knowledge as well as international standard protocols. During food preparation, either from raw material or from cooked material, from any point, the contamination can be occurred (Adikari 2016). Food hygiene or safety in the hospital is mostly challenged by unexpected problems like as food-items that can be brought to the patients by the external visitors or relatives of them, which may create a high risk of both microbial and dietetic danger. For this reason, bringing outside-foods in hospitals should not be allowed by hospital's safety-personnel (Kokkinakis 2011).

1.3.3 Implementation of Hygiene and Safety

Concerning the safety and hygiene issue about foods in the hospitals, a number of measures can be taken by hospital authorities. Such as the HACCP system and implementing this system is crucial to maintain the optimum safety and hygienic environment in hospitals. HACCP is a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product (FDA 2017). The HACCP system consulters should strongly emphasize and encourage the utilization of Good Manufacturing Practices (GMPs) and Good Hygiene Practices (GHPs), focusing on personnel hygiene and personnel training. All kitchenware, food cutting boards and food contact surfaces of equipment, exclusive of cooling surfaces of equipment, used in the preparation of serving of food or drink, must be thoroughly cleaned after each use. Cooking surfaces of equipment must be cleaned every twenty-four hours. All utensils and food-contact surfaces of equipment used in the preparation, service, display, or storage must be thoroughly cleaned and sanitized prior to each use. Non-food contact surfaces of equipment must be cleaned at such intervals as to keep them in a clean and sanitary condition. Food items must be stored in accordance with standard dry food storage techniques (Kokkinakis 2011).

1.4 Patient Satisfaction about Hospital Food Service

Patient satisfaction has become a key criterion by which the quality of health care services is evaluated and foodservice is also now an integral part of evaluation to patients. In overall hospital patient satisfaction criterion, foodservice satisfaction sometimes go undervalued or unnoticed, as quality of nursing, physicians and technical medical care are more commonly identified. Several researchers have identified food quality as the most important determinant of patient foodservice satisfaction (Abdelhafez 2012). Patients, attendants are becoming more concerned about food quality and perceived value. There is an increased understanding that patients need nutritious, appealing food that tastes good in order for them to eat, regain their strength, and heal. There is also an increasing acceptance that healthy food can be tasty and patients have started value food, they now want to be healthier, and want fresher food, more choices, dietary preferences such as gluten-free, vegetarian, vegan, etc. Nutritious food that tastes good can also impact patient experience and patient satisfaction, and may contribute to a patient's overall sense of well-being and hopefulness on the road to recovery (Murphy 2017).

1.5 Food-borne Illness

World Health Organization defines Food-borne illnesses as diseases of infectious or toxic nature caused by consumption of contaminated foods or water (Addis 2015). Food-borne illnesses are not narrowed branch of disease rather covering a broad spectrum of diseases and are responsible for substantial morbidity and mortality worldwide. In both developing as well as developed countries it is a growing public health problem and is difficult to determine the exact mortality associated with food-borne illnesses. Generally food-borne illnesses are caused by the consumption of food containing pathogens such as bacteria, viruses, parasites or the food contaminated by poisonous chemicals or bio-toxins. Usually majority of the food-borne illness cases are less harmful and tolerable to healthy adult population but severe cases can occur in high risk groups that including infants, young children, the elderly and the immuno-compromised persons (Jahan 2012).

Most common symptoms of food-borne diseases are diarrhea, vomiting, abdominal cramps, headache and nausea. Before moving on to pathological test food-borne illnesses or diseases are usually diagnosed based on patient's history and the symptoms.

Though food-borne illness or diseases can cause health-hazard in different demographic populations around the world, but the prevention system of food-borne disease is not much difficult. Prevention can be done by implementing proper food safety control and precaution in places like hospitals, households, and restaurants through food production, processing and distribution keeping food clean, separation of raw and cooked, and cooking thoroughly, keeping food at safe temperature and using safe water and raw materials are some of the important points especially for safety of food of humans (Addis 2015).

1.6 Classification of Food-borne Illness

The term food-borne illness is used to describe illness resulting from the consumption of food products. This term is preferred to the term food poisoning because it encompasses a broader range of food source contaminants and is technically more appropriate. Food-borne illness needs to be distinguished from coincidental onset of symptoms while a person is eating, or no causal food-associated illness (MCKay 2015).

Food-borne infections: It is caused by consuming foods or liquids contaminated with bacteria, viruses, or parasites. These pathogens cause infection by:

- Invading and multiplying in the lining of the intestines and/or other tissues
- Invading and multiplying in the intestinal tract and releasing a toxin (bacteria only)

Food-borne intoxication: It is caused by consuming foods or beverages already contaminated with a toxin. Sources of toxins are as follows:

- Certain bacteria (pre-formed toxins)
- Poisonous chemicals
- Natural toxins found in animals, plants, and fungi (NCDC 2009).

1.7 Causes of Food-borne Illnesses

The majority of Food-borne illnesses are caused by harmful bacteria, viruses, parasites and chemicals. These pathogens are described as follows:

1.7.1 Bacteria

Bacteria are tiny organisms that can cause infections of the GI tract. Raw foods including meat, poultry, fish and shellfish, eggs, unpasteurized milk and dairy products, and fresh produce often contain bacteria that cause food-borne illnesses. Bacteria can contaminate food and making it harmful to eat. Foods may also be contaminated with bacteria during food preparation in a restaurant or home kitchen. Many types of bacteria cause Food-borne illnesses. Examples include-

- *Salmonella*, a bacterium found in many foods, including raw and undercooked meat, poultry, dairy products, and seafood. .
- *Shigella*, a bacterium spread from person to person. These bacteria are present in the stools of people who are infected. If people who are infected do not wash their hands thoroughly after using the bathroom, they can contaminate food that they handle or prepare.
- *Escherichia coli (E. coli),* which includes several different strains, only a few of which cause illness in humans. *E. coli O157:H7* is the strain that causes the most severe illness. Common sources of *E. coli* include raw or undercooked hamburger, unpasteurized fruit juices and milk, and fresh produce (NIDDK n.d.).

(a) (b)

Figure 1.3: Images of (a) *Salmonella* & (b) *Shigella,*

1.7.2 Viruses

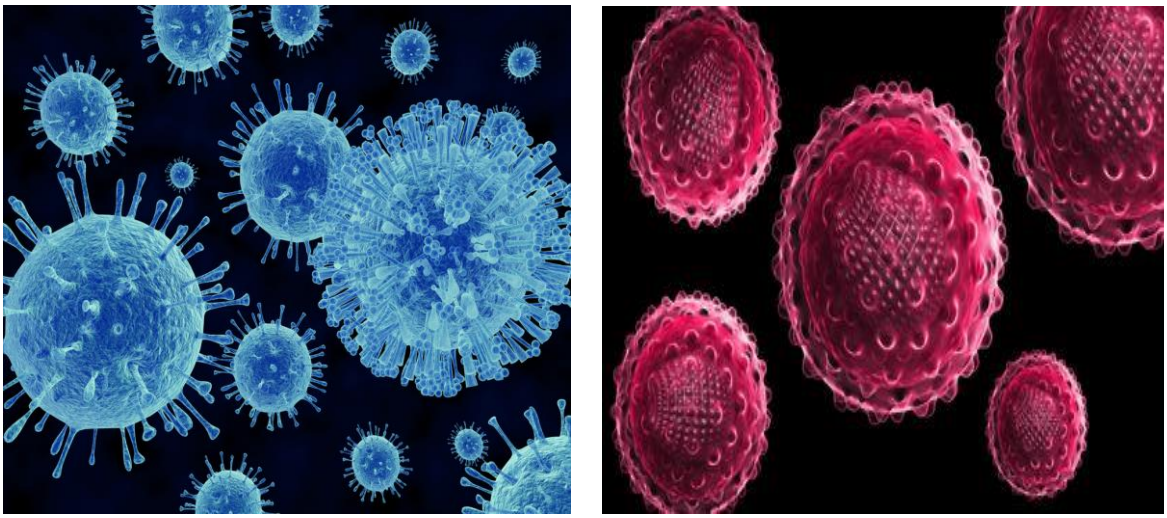
Viruses are tiny capsules, much smaller than bacteria that contain genetic material. Viruses cause infections that can lead to sickness. People can pass viruses to each other. Viruses are present in the stool or vomit of people who are infected. People who are infected with a virus may contaminate food and drinks, especially if they do not wash their hands thoroughly after using the bathroom.

Common sources of Food-borne viruses include-

- Food prepared by a person infected with a virus,
- Shellfish from contaminated water,
- Produce irrigated with contaminated water.

Common Food-borne viruses include-

- Norovirus, which causes inflammation of the stomach and intestines.
- Hepatitis A, which causes inflammation of the liver (NIDDK n.d.).

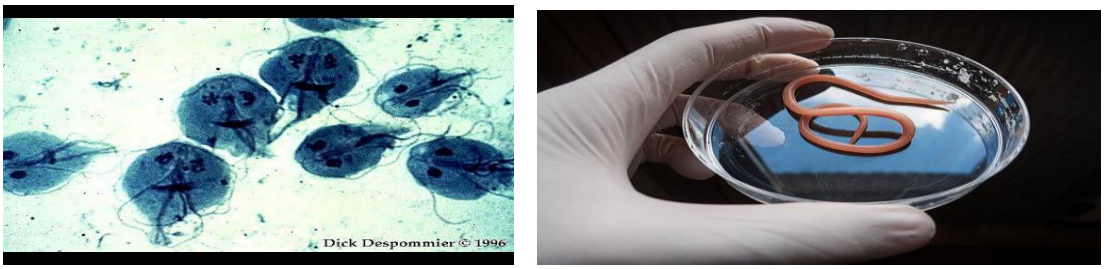


(a) (b)

Figure 1.4: Images of (a) Norovirus & (b) Hepatitis A.

1.7.3 Parasites

Parasites are tiny organisms that live inside another organism. *Cryptosporidium parvum* and *Giardia intestinalis* are parasites that are spread through water contaminated with the stools of people or animals that are infected. Foods that come into contact with contaminated water during growth or preparation can become contaminated with these parasites. *Trichinella spiralis* is a type of roundworm parasite. People may be infected with this parasite by consuming raw or undercooked pork or wild game (NIDDK n.d.).



(a) (b)

Figure 1.5: Images of (a) *Giardia intestinalis* & (b) *Trichinella spiralis*.

1.7.4 Chemicals

Harmful chemicals that cause illness may contaminate foods such as fish or shellfish, which may feed on algae that produce toxins, leading to high concentrations of toxins in their bodies, certain types of wild mushrooms and unwashed fruits & vegetables that contain high concentrations of pesticides (NIDDK n.d.).

Figure 1.6: Image of Different Types of Toxic Mushrooms.

1.8 Factors Influencing Microbial Growth in Food

The ability of microorganisms (except viruses) to grow or multiply in a food is determined by the food environment as well as the environment in which the food is stored, designated as the intrinsic and extrinsic environment of food. The most important factors that affect microbial growth in foods can be summarized in the following categories:

1.8.1 Intrinsic Factors

Intrinsic factors of a food include nutrients, growth factors, and inhibitors (or antimicrobials), water activity, pH, and oxidation–reduction potential.

- **Nutrients and Growth:**

Microbial growth is accomplished through the synthesis of cellular components and energy. The necessary nutrients for this process are derived from the immediate environment of a microbial cell and, if the cell is growing in a food, it supplies the nutrients. These nutrients include carbohydrates, proteins, lipids, minerals, and vitamins. Water is not considered a nutrient, but it is essential as a medium for the biochemical reactions necessary for the synthesis of cell mass and energy.

- **Growth Factors and Inhibitors in Food:**

Foods can also have some factors that either stimulate growth or adversely affect growth of microorganisms. The exact nature of growth factors is not known, but they are naturally present in some foods. An example is the growth factors in tomatoes that stimulate growth of some *Lactobacillus* species.

- **Water Activity and Growth:**

Water activity (*Aw*) is a measure of the availability of water for biological functions and relates to water present in a food in free form. In a food system, total water or moisture is present in free and bound forms. The free water in a food is necessary for microbial growth. It is necessary to transport nutrients and remove waste materials, carry out enzymatic reactions, synthesize cellular materials.

- **pH and Growth:**

The pH of a food has a profound effect on the growth and viability of microbial cells. Each species has an optimum and a range of pH for growth. In general, molds and yeasts are able to grow at lower pH than do bacteria, and Gram-negative bacteria are more sensitive to low pH than are Gram-positive bacteria.

- **Redox Potential, Oxygen, and Growth:**

The redox potential of a food is influenced by its chemical composition, specific processing treatment given, and its storage condition (in relation to air). On the basis of their growth in the presence and absence of free oxygen, microorganisms have been grouped as aerobes, anaerobes, facultative anaerobes, or microaerophiles. Like-Aerobes need free oxygen for energy generation, as the free oxygen acts as the final electron acceptor through aerobic respiration (Ray B 2005).

1.8.2 Extrinsic Factors

Extrinsic factors important in microbial growth in a food include the environmental conditions in which it is stored. These are temperature, relative humidity, and gaseous environment. (Ray B 2005)

- **Temperature:**

Microbial growth can occur over a temperature range from about 8° C up to 100 C at atmospheric pressure. The most important requirement is that water should be present in the liquid state and thus available to support growth (Adams & Moss 2007).

- **Relative Humidity:**

The storage of fresh fruit and vegetables requires very careful control of relative humidity. If it is too low then many vegetables will lose water and become flaccid. If it is too high then condensation may occur and microbial spoilage may be initiated (Adams & Moss 2007).

- **Gaseous Atmosphere:**

Oxygen is the most important gas in contact with food under normal circumstances. Its presence and its influence on redox potential are important determinants of the microbial associations that develop and their rate of growth (Adams & Moss 2007).

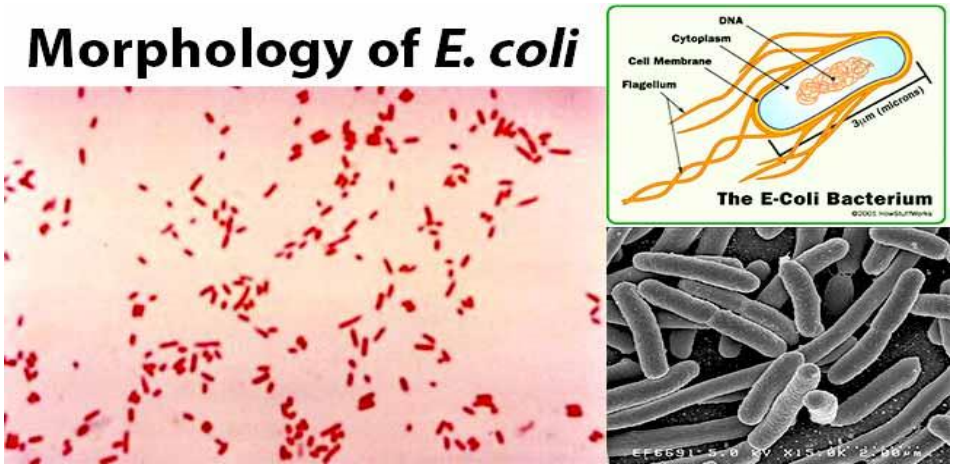
1.8.3 Implicit Factors

It is important in determining the nature of microbial associations found in foods. And also describes the properties of the organisms themselves, how they respond to their environment and interact with one another. Implicit factors include interactions between the microorganisms contaminating the food and between these microorganisms and the food (Adams & Moss 2007).

1.9 Description of Some Common Microorganism Responsible for Food borne Diseases:

1.9.1 *Escherichia coli*

E. coli is an almost universal inhabitant of the gut of humans and other warm-blooded animals where it is the predominant facultative anaerobe though only a minor component of the total micro flora. 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 immune suppressed patients, and meningitis in neonates.



Figue 1.7: *Escherichia coli*

1.9.1.1 The Organism and its Characteristics

Escherichia is the type genus of the Enterobacteriaceae family and *E. coli* is the type species of the genus. It is a catalase -positive, oxidase -negative, fermentative, short, Gram-negative, non-sporing rod. *E. coli* can be differentiated from other members of the Enterobacteriaceae on the basis of a number of sugar-fermentation and other biochemical tests. *E. coli* is a typical mesophile growing from 7‑10 ˚C up to 50 ˚C with an optimum around 37˚C.

1.9.1.2 Pathogenesis and Clinical Features

There are four major categories of diarrhoeagenic *E. coli* based on distinct, virulence properties.

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

Illness caused by ETEC usually occurs between 12 and 36 h after ingestion of the organism. Symptoms can range from mild a febrile diarrhea 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. 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˚C for 15 min and are acid resistant, and the heat-labile toxins (LT) which are inactivated at 60˚C after 30 min and at low pH.

- **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 a watery diarrhea which precedes the passage of stools containing blood, mucus, and fecal leukocytes.

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

When the properties of ETEC and EIEC were established it was noted that these strains were rarely of the same serotypes first associated with *E. coli* diarrhea in the 1950s. Subsequent investigation of some of these earlier strains in most cases failed to demonstrate the property of entero-invasiveness or the ability to produce ST or LT and yet they retained the ability to cause diarrhoea in volunteers. Symptoms of EPEC infection, malaise, vomiting and diarrhoea with stools containing mucus but rarely blood, appear 12‑36 h after ingestion of the organism. In infants, the illness is more severe than many other diarrhoeal infections and can persist for longer than two weeks in some cases.

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

EHEC, sometimes also known as Verotoxin-producing *E. coli* (VTEC), was first described in Canada where in some areas it rivals Campylobacter and Salmonella as the most frequent cause of diarrhea. EHEC has attracted attention not only because food-borne 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 hemolytic uremic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP).

1.9.1.3 Isolation and Identification:

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

1.9.1.4 Association with Foods:

Fecal contamination of water supplies and contaminated food handlers has been most frequently implicated in outbreaks caused by EPEC, EIEC and ETEC. A number of foods have been involved, including a coffee substitute in Romania in 1961, vegetables, potato salad, and sushi. In the United States, mould-ripened soft cheeses have been responsible for outbreaks in 1971, associated with EIEC in which more than 387 people were affected, and in 1983, caused by ETEC (ST). *E. coli* would not be expected to survive well in a fermented dairy product with a pH below 5 but, where contamination is associated with mould-ripening, the local increase in pH as a result of lactate utilization and amine production by the mould would allow the organism to grow (Adams & Moss 2007).

1.9.2 *Vibrio* species

Historically, cholera has been one of the diseases most feared by mankind. It is endemic to the Indian subcontinent where it is estimated to have killed more than 20 million people this century.

1.9.2.1 The Organisms and their Characteristics

Vibrios are Gram-negative pleomorphic (curved or straight), short rods which are motile with (normally) sheathed, polar flagella. Catalase and oxidase-positive cells are facultatively anaerobic and capable of both fermentative and respiratory metabolism. Sodium chloride stimulates the growth of all species and is an obligate requirement for some.

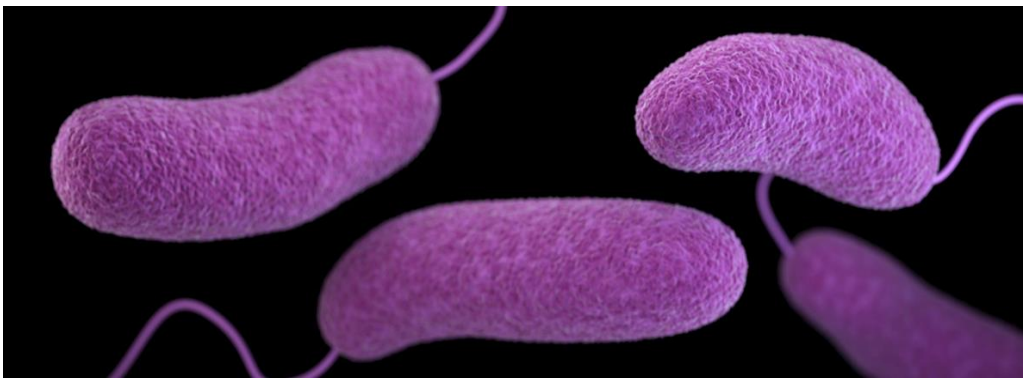


Figure 1.8: *Vibrio* species.

Growth of enteropathogenic *vibrios* occurs optimally at around 37˚C and has been demonstrated over the range 5 – 43˚C, although 10˚C is regarded as a more usual minimum in natural environments. When conditions are favorable, *vibrios* can grow extremely rapidly.

1.9.2.2 Pathogenesis and Clinical Features

Cholera usually has an incubation period of between one and three days and can vary from mild, self-limiting diarrhoea to a severe, life-threatening disorder. The infectious dose in normal healthy individuals is large when the organism is ingested without food. Individuals with low stomach acidity (hypochlorohydric) are more liable to catch cholera. Cholera is a non-invasive infection where the organism colonizes the intestinal lumen and produces a potent entero-toxin. In severe cases, the hyper-secretion of sodium, potassium, chloride, and bicarbonate induced by the entero-toxin results in a profuse, pale, watery diarrhoea containing flakes of mucus, described as rice water stools.

1.9.2.3 Isolation and Identification

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

1.9.2.4 Association with Foods

Cholera is regarded primarily as a waterborne infection, though food which has been in contact with contaminated water can often serve as the vehicle. Consequently a large number of different foods have been implicated in outbreaks, particularly products such as washed fruits and vegetables which are consumed without cooking (Adams & Moss 2007).

1.9.3 *Salmonella* species

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 food-borne illness worldwide.

1.9.3.1 The Organism and its Characteristics

Salmonellas are members of the Enterobacteriaceae. They are Gram negative, non-spore forming rods (typically 0.5 mm by 1–3 mm) which are facultatively anaerobic, catalase-positive, and oxidase-negative. 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. In frozen foods, numbers of viable *salmonella* decline slowly, the rate decreasing as the storage temperature decreases.

Salmonellas are primarily inhabitants of the gastrointestinal tract. They are carried by a wide range of food animals, wild animals, rodents, pets, birds, reptiles, and insects, usually without the display of any apparent illness. They can be disseminated via feces to soil, water, foods and feeds and thence to other animals (including humans).

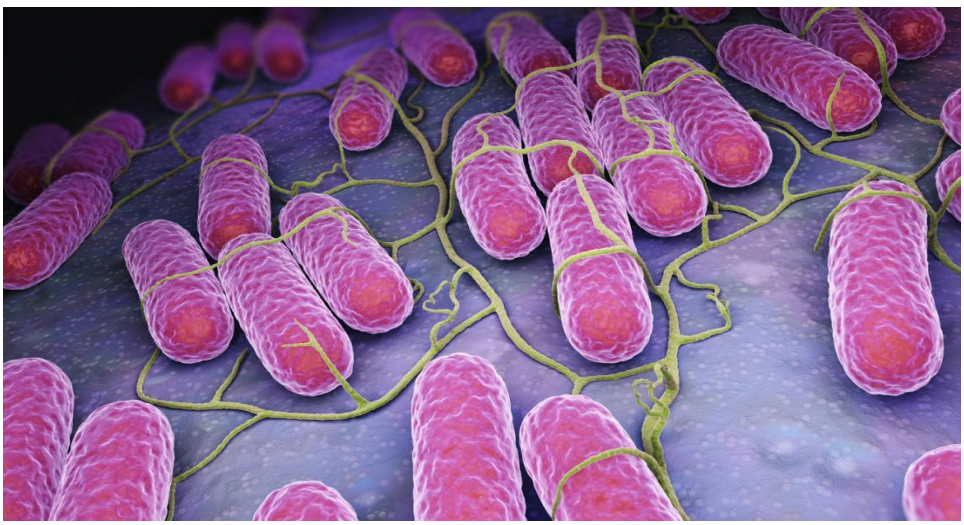


Figure 1.9: *Salmonella* species.

1.9.3.2 Pathogenesis and Clinical Features

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

Enteritis: Gastrointestinal infections are predominantly associated with those serotypes which occur widely in animals and humans. They can range in severity from asymptomatic carriage to severe diarrhea and are the most common type of salmonellosis. The incubation period for salmonella enteritis is typically between 6 and 48 h. The principal symptoms of mild fever, nausea and vomiting, abdominal pain and diarrhoea last for a few days but, in some cases, can persist for a week or more.

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 septicemic 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.

1.9.3.3 Association with Foods

Salmonellosis is described as a zoonotic infection since the major source of human illness is infected animals. Transmission is by the faecal–oral route whereby intestinal contents from an infected animal are ingested with food or water. A period of temperature abuse which allows the *salmonellae* to grow in the food and an inadequate or absent final heat treatment is common factors contributing to outbreaks. Meat, milk, poultry, and eggs are primary vehicles; they may be undercooked, allowing the salmonellas to survive, or they may cross contaminate other foods that are consumed without further cooking. Cross-contamination can occur through direct contact or indirectly via contaminated kitchen equipment and utensils. Human transmission can occur if the faecally contaminated hands of an infected food handler touch a food which is then consumed without adequate cooking, often after an intervening period in which microbial growth occurs (Adams & Moss 2007).

1.9.4 *Shigella* species

The genus *Shigella* was discovered as the cause of bacillary dysentery by the Japanese microbiologist Kiyoshi Shiga in 1898. It consists of four species *Sh. dysenteriae*, *Sh. flexneri, Sh. boydii* and *Sh. sonnei,* all of which are regarded as human pathogens though they differ in the severity of the illness they cause.

1.9.4.1 The Organism and its Characteristics

Shigellas are members of the family Enterobacteriaceae. They are non-motile, non-spore forming, Gram-negative rods which are catalase positive, 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 food-borne enteric pathogens have, but such evidence as is available suggests that their survival characteristics are in fact similar to other members of the Enterobacteriaceae. They are typical mesophiles with a growth temperature range between 10–45˚C and heat sensitivity comparable to other members of the family. They grow best in the pH range 6–8 and do not survive well below pH 4.5.

Figure 1.10: *Shigella* species.

1.9.4.2 Pathogenesis and Clinical Features

Shigellas cause bacillary dysentery in humans and other higher primates. The incubation period can vary between 7 h and 7 days although food-borne outbreaks are commonly characterized by shorter incubation periods of up to 36 h. Symptoms are of abdominal pain, vomiting and fever accompanying a diarrhoea which can range from a classic dysenteric syndrome of bloody stools containing mucus and pus, in the cases of *Sh. dysenteriae, Sh. flexneri* and *Sh. boydii,* to a watery diarrhoea with *Sh. sonnei*. Illness lasts from 3 days up to 14 days in some cases. 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.

1.9.4.3 Isolation and Identification

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

1.9.4.4 Association with Foods

Food-borne cases of shigellosis are regarded as uncommon though some consider the problem to be greatly underestimated. The limited range of hosts for the organism certainly suggests that it is relatively insignificant as a food-borne problem when compared with say Salmonella. In food-borne cases, the source of the organism is normally a human carrier involved in preparation of the food. In areas where sewage disposal is inadequate the organism could be transferred from human feces by flies (Adams & Moss 2007).

1.9.5 *Aeromonas* species

Currently, *Aeromonas* (principally *A. hydrophila*, but also *A. caviae* and *A. sobria*) has the status of a food-borne pathogen of emerging importance. Like *Listeria monocytogenes*, *Plesiomonas*, and *Yersinia enterocolitica*, it has attracted attention primarily because of its ability to grow at chill temperatures, prompting the concern that any threat it might pose will increase with the increasing use of chilled foods.

1.9.5.1 The Organism and its Characteristics

Aeromonas are Gram-negative, catalase-positive, oxidase-positive rods which ferment glucose. They are generally motile by a single polar flagellum. Its principal reservoir is the aquatic environment such as freshwater lakes and streams and wastewater systems. The numbers present will depend on factors such as the nutrient level and temperature. Although it is not resistant to chlorine, it is found in potable water, where it can multiply on the low level of nutrients available in piped water systems. It has also been isolated from a wide range of fresh foods and is a transient component of the gut flora of humans and other animals.

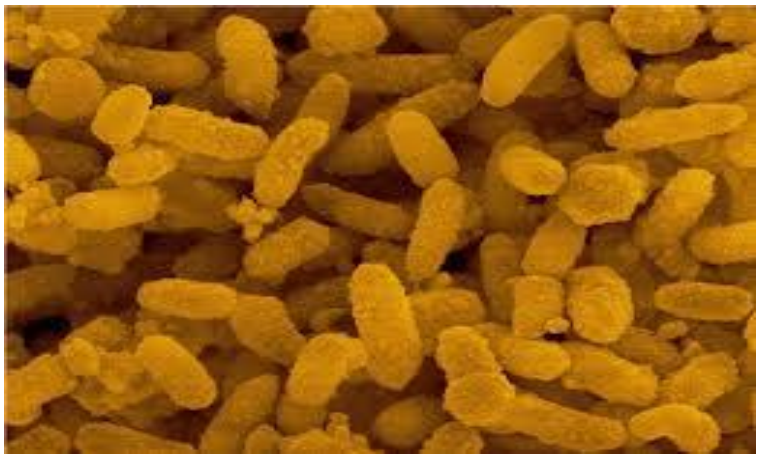


Figure 1.11: *Aeromonas* species.

1.9.5.2 Pathogenesis and Clinical Features

Gastroenteritis associated with *Aeromonas* occurs most commonly in children under five years old. It is normally mild and self-limiting mostly characterized by profuse watery diarrhoea, although dysenteric stools may sometimes be a feature. Vomiting is not usually reported. *Aeromonas* spp., particularly *A. hydrophila* and *A. sobria*, produce a range of potential virulence factors including a number of distinct cytotoxic and cytotonic

enterotoxins. Three cytotonic enterotoxins have also been described which act like cholera toxin, stimulating accumulation of high levels of cAMP within epithelial cells.

1.9.5.3 Isolation and Identification

In some instances enrichment media such as alkaline peptone water are used, but where high numbers are present direct plating is usually sufficient. Species of the Hydrophila group grow on a wide range of enteric media but may often be misidentified as 'coliforms' since many strains can ferment lactose. Most cannot ferment xylose and this is a useful distinguishing feature used in several media. As well as bile salts, ampicillin is used as a selective agent in media such as starch ampicillin agar, blood ampicillin agar and some commercial formulations. Colonies which give the characteristic appearance of *Aeromonas* on the medium concerned and are oxidase-positive are then confirmed with biochemical tests.

1.9.5.4 Association with Foods

Apart from their possible role in gastroenteritis, food and water are also probably the source of the severe extra-intestinal *Aeromonas* infections associated with immune-compromised individuals. *Aeromonas* of the Hydrophila group have been isolated from a wide range of fresh foods including fish, meat, poultry, raw milk, and salad vegetables as well as water. The ability of some strains to grow at very low temperatures can lead to the development of high numbers under chill conditions and they can be an important part of the spoilage flora of chilled meats. They are unlikely to survive even mild cooking procedures but may be introduced as post-process contaminants from uncooked produce or contaminated water (Adams & Moss 2007).

1.9.6 *Plesiomonas shigelloides*

Plesiomonas shigelloides is the only species of the genus whose name is derived from the Greek word for neighbour; an allusion to its similarity to *Aeromonas*. Its position as a causative agent of food-borne illness also bears some similarity to *Aeromonas*.

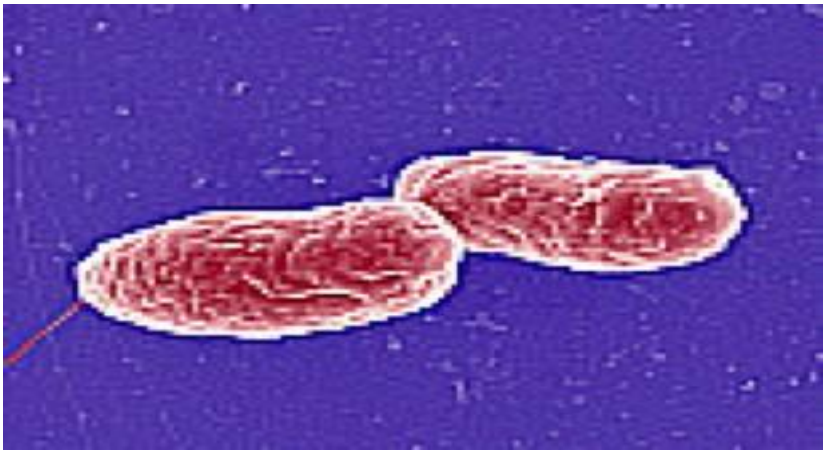


Figure 1.12: *Plesiomonas shigelloides.*

1.9.6.1 The Organism and its Characteristics

A member of the family Enterobacteriaceae (previously classified in the Vibrionceae), *P. shigelloides* is a short, catalase-positive, oxidase-positive, Gram-negative rod. It is motile by polar, generally lophotrichous flagella in contrast to *Aeromonas* and *Vibrio* which are monotrichous. It grows over a temperature range from 8-10°C to 40-45°C with an optimum at around 37°C. It is not markedly heat resistant and is readily eliminated by pasteurization treatments. The organism is ubiquitous in surface waters and soil, more commonly in samples from warmer climates. Carriage in cold-blooded animals such as frogs, snakes, turtles, and fish is common and it has Bacterial Agents of Food-borne Illness been isolated from cattle, sheep, pigs, poultry, cats and dogs. It is not normally part of the human gut flora.

1.9.6.2 Pathogenesis and Clinical Features

Cases of *P. shigelloides* infection are more common in warmer climates and in travellers returning from warmer climates. The usual symptoms are mild watery diarrhoea free from blood or mucus. Symptoms appear within 48 h and persist for several days. Motility appears to be an important factor and evidence has been presented for an entero-toxin causing fluid secretion in rabbit ligated ileal loops.

1.9.6.3 Isolation and Identification

The relatively recent growth of interest in *P. shigelloides* is reflected in the use of 'second-hand' media in its isolation. Alkaline peptone water and tetra thionate broth

have both been used for enrichment culture of *P. shigelloides* at 35‑40˚C and *salmonella*‑*shigella* and MacConkey agars have been used as selective plating media. Selective plating media have been developed such as inositol/brilliant green/bile salts, Plesiomonas agar. Isolates can be readily confirmed on the basis of biochemical tests.

1.9.6.4 Association with Foods

Fish and shellfish are a natural reservoir of the organism and, with the exception of one incident where chicken was implicated, they are the foods invariably associated with *Plesiomonas* infections. Examples have included crab, shrimp, cuttle fish and oysters (Adams & Moss 2007).

1.9.7 *Yersinia enterocolitica*

Yersinia enterocolitica is one of three species of the genus *Yersinia* recognized as human pathogens; *Y. enterocolitica* causes predominantly Bacterial Agents of Food-borne Illness gastroenteritis, while *Y. pseudotuberculosis* is associated mainly with mesenteric adenitis.

Figure 1.13: *Yersinia enterocolitica.*

1.9.7.1 The Organism and its Characteristics

Yersinia enterocolitica is a member of the Enterobacteriaceae; an asporogenous, short (0.5‑1.0 by 1‑2 mm) Gram-negative rod which is facultative anaerobic, catalase-positive and oxidase-negative. It can grow over a wide range of temperature, from -1˚C to +40˚C, with an optimum around 29˚C and has a number of temperature-dependent phenotypic characteristics.

Y. enterocolitica can be isolated from a range of environmental sources including soil, fresh water and the intestinal tract of many animals.

1.9.7.2 Pathogenesis and Clinical Features

Illness caused by *Y. enterocolitica* occurs most commonly in children under seven years old. It is a self-limiting enterocolitis with an incubation period of 1‑11 days and lasting for between 5 and 14 days, although in some cases it may persist for considerably longer. Symptoms are predominantly abdominal pain and diarrhoea accompanied by a mild fever; vomiting is rare. A problem of post-infection complications such as arthritis and erythema nodosum (araised, red skin lesion) can occur in adults, the latter particularly in women.

1.9.7.3 Isolation and Identification

A large number of procedures for the isolation and detection of *Y. enterocolitica* have been developed. Enrichment procedures usually exploit the psychrotrophic character of the organism by incubating at low temperature, but this has the disadvantage of being slow with the attendant possibility of overgrowth by other psychrotrophs present. The most commonly used enrichment media are phosphate buffered saline (PBS) or tryptone soya broth (TSB) most usually incubated at 4˚C for 21 days. *Y. enterocolitica* and related species are more alkali resistant than many other bacteria so the pH of enrichment media is sometimes adjusted to 8.0‑8.3 or cultures subjected to a short post-enrichment alkali treatment. The best results for the selective isolation of *Y. enterocolitica* from foods and enrichment broths have been obtained with cefsulodin/ irgasan/ novobiocin (CIN) agar.

1.9.7.4 Association with Foods

Pigs are recognized as chronic carriers of those *Y. enterocolitica* serotypes most commonly involved in human infections. The organism can be isolated most frequently from the tongue, tonsils and, in the gut, the caecum of otherwise apparently healthy animals (Adams & Moss 2007).

Chapter: 2

Research Objective

2.1 Research Objectives

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

To isolate and identify the presence of enteric bacteria especially *E. coli, Salmonella, Shigella, Vibrio* species, *Aeromonas* species, *Plesiomonas shigelloides, Yersinia enterocolitica* from different type of Hospital food items from different Hospitals of Dhaka city, Bangladesh.

Chapter: 3

Methodology

3.1 Bacteriological Subculture

3.1.1 Sample Collection

About 31 food samples were randomly chosen and collected from different Hospitals of Dhaka city. These samples were collected in different sealed poly bags to prevent their contact with any other source that can contaminate the samples. All 31 samples were tested for identifying the presence of enteric bacteria especially *E. coli, Salmonella, Shigella, Vibrio* species, *Aeromonas* species, *Plesiomonas shigelloides, Yersinia enterocolitica*.

3.1.2 Sample Processing

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

3.1.3 Enrichment of the Organisms

3.1.3.1 Enrichment of *E.coli* spp

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* and *Shigella* spp

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.

Figure 3.1: Enrichment of the Organisms

3.1.4 Selective Growth of the Organisms

3.1.4.1 Selective Growth *E.coli* 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* 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.

Figure 3.2: Autoclave and Hot air Oven

Figure 3.3: Laminar Air Flow Cabinet

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.

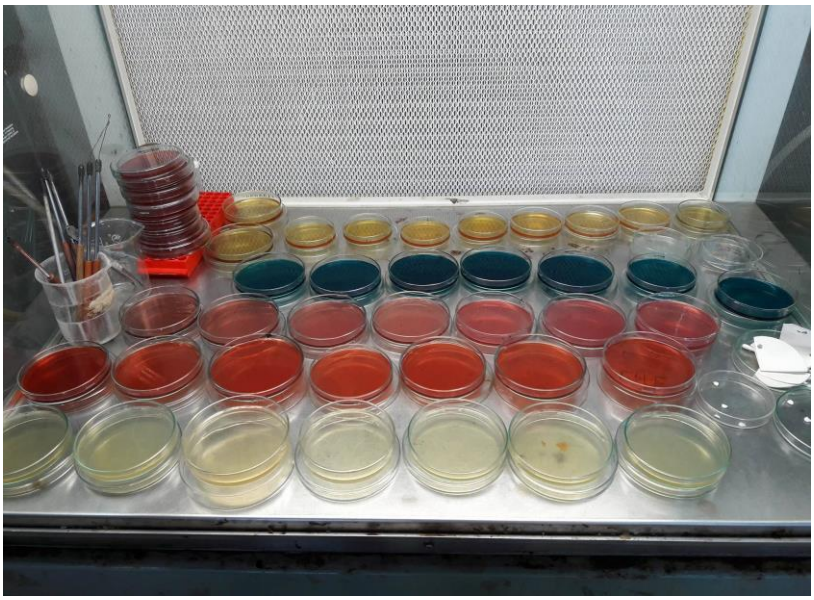


Figure 3.4: Petri dishes preparation

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.

Figure 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 listed below on the table 3.1:

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

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

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

Agar:

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

Figure 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.

Figure 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.

Figure 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.

Figure 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
- 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
- Media preparation bottle

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 inoculums to 9 parts of diluents) 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

- Alcohol

- Colony counter

- Conical Flask

- Labeling Tape.

3.3.1.4 Procedure

There are four major steps in the procedure:

1. Preparation of serial dilutions

2. Mixing the serial dilutions into agar

3. Counting the resulting bacterial colonies

4. Calculation of total numbers of viable bacteria from these counts.

3.3.1.5 Preparation of Serial Dilutions

- A sample was taken containing the bacteria to be counted.

- Four test tubes were taken and labeled them 10^{-1} to $10^{-4.}$

- Nine mL of distilled water was pipette into each of the tubes.

- 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.

- Therefore the following dilutions of the original sample were obtained.

Table 3.3: Calculation of Dilution Factors

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.

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

3.3.1.7 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 Bacterial colony morphology

Table 4.1: Bacterial colony morphology isolated from different hospital food samples.

Name of the hospital	**Sample name**	**Plates**				
		MacConkey	**TBX**	**TCBS**	**XLD**	**BGA**
Bangabandhu Sheikh Mujib Medical University (BSMMU)	Hot Dog	Pink	No growth	No growth	No growth	No growth
	Vaji	Pink	Blue	No growth	No growth	No growth
	Egg fry	No growth	Blue	No growth	No growth	No growth
	Banana vorta	No growth	No growth	Yellow	No growth	No growth
	Booter Daal	Colorless	No growth	No growth	No growth	No growth
	Mixed vaji	No growth	No growth	No growth	No growth	No growth
Dhaka Medical College (DMC)	Beef kabab	Pink	No growth	Yellow	No growth	No growth
	Hot Dog	No growth	No growth	Yellow	No growth	No growth
	Bun	No growth	No growth	Yellow	No growth	No growth
	Pizza	No growth	No growth	Yellow	No growth	No growth
	Ruti	No growth	No growth	Yellow	No growth	No growth
	Egg fry	No growth	No growth	Yellow	No growth	No growth
	Roll	No growth	No growth	Yellow	No growth	No growth
Monowara Hospital (MH)	Porota	No growth	No growth	No growth	No growth	No growth
	Daal	No growth	No growth	No growth	No growth	No growth
	Chicken soup	No growth	No growth	No growth	No growth	No growth
Salauddin Hospital (SH)	Singara	No growth	No growth	No growth	No growth	No growth
	somucha	No growth	No growth	No growth	No growth	No growth

Table 4.2: Bacterial colony morphology isolated from different hospital food samples.

Name of the hospital	**Sample name**	**Plates**				
		MacConkey	**TBX**	**TCBS**	**XLD**	**BGA**
Islami Bank Hospital (IBH)	Naan	No growth	No growth	Green	No growth	No growth
	Soup	No growth	No growth	No growth	No growth	No growth
	Daal vaji	No growth	No growth	No growth	No growth	No growth
	Chicken roll	No growth	No growth	No growth	No growth	No growth
	Jaw vaat	No growth	No growth	No growth	No growth	No growth
	Singara	No growth	No growth	No growth	No growth	No growth
Khidma Hospital (KH)	Dim vaji	Colorless	No growth	No growth	No growth	No growth
	Dim chop	Colorless	No growth	No growth	No growth	No growth
	Mach vorta	Colorless	No growth	No growth	No growth	No growth
Uttara Cresent Hospital (UCH)	Vegetable soup	No growth	No growth	No growth	No growth	No growth
	Sauce	No growth	No growth	No growth	No growth	No growth
	Salad	No growth	No growth	No growth	No growth	No growth
	Coffee	No growth	No growth	No growth	No growth	No growth

Table 4.1 & Table 4.2 Show bacterial colony morphology isolated from different hospital food samples. Thirty-one food samples were collected from seven different hospital canteens in Dhaka city. Of which, 16 samples show positive growth of our suspected organisms (*E.coli, Vibrio* spp., *Aeromonous* spp.) and 15 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=18).

Name of hospital	**No. of samples with +ve growth by *E.coli***	**No. of samples with +ve growth by *Vibrios spp.***	**No. of samples with +ve growth by *Aeromonus spp.***	**No. of samples with +ve growth by *Plesiomonus spp.***	**No. of samples with +ve growth by *Yersinia spp.***
BSMMU	4	1	1	0	0
DMC	1	7	0	0	0
Islami Bank Hospital	0	1	0	0	0
Khidma Hospital	0	0	3	0	0

Table 4.3 Shows number of food samples with growth of suspected organisms determined by colony morphology. From total 31 food samples, 16 (51.61%) samples were suspected to be contaminated with our targeted organisms (*E coli, Vibrio* species, and *Aeromonus* species). In total 16 samples, 5 (31.25%) samples were suspected to be contaminated with *E coli*, 9 (56.25%) with *Vibrio,* 4 (25%) with *Aeromonous* species.

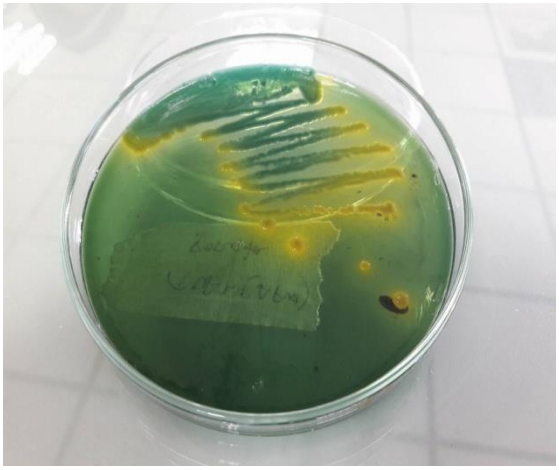


Figure 4.1: Bacterial colony (yellow) On TCBS agar plate.

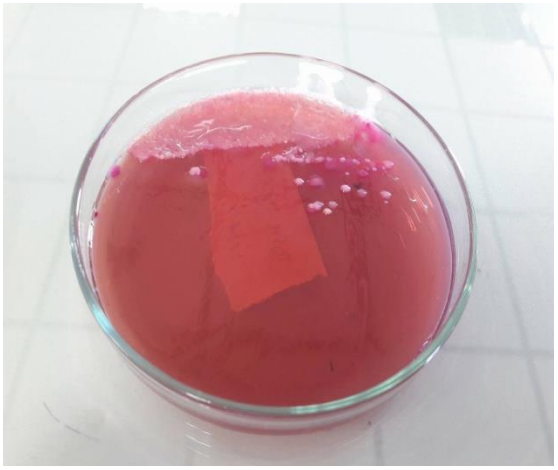


Figure 4.2: Bacterial colony (Pink) On MacConkey agar plate.

4.2 Suspected organism from different biochemical test:

Table 4.4: Identification of the suspected organism (*E.coli* species) from different biochemical tests.

Sample name	**Plate**	**Colony morphology**	**KIA**			**Citrate**	**MIO**			**Urease**	**Organism**
			Slant/ Butt	**H_2S**	**Gas**		**Mortality**	**Indole**	**Ornithine**		
Hot Dog	MAC	Pink	A/A	-	+	-	+	-	-	-	*E.Coli*
Vaji	TBX	Blue	A/A	-	+	-	+	-	-	-	
Vaji	MAC	Pink	A/A	-	+	-	+	-	-	-	
Egg fry	TBX	Blue	A/A	-	+	-	-	-	-	-	
Beef kabab	MAC	Pink	A/A	-	+	+	+	-	-	-	

Table 4.4 Shows identification of *E.coli* species from different biochemical test. Biochemical test results for the samples Hot Dog, Vaji, Egg fry, Beef kabab matched with the standard results for *E.coli* species. So, we can say that the samples may contain the *E.coli* species.

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

Sample name	**Plates**	**Colony morphology**	**KIA**			**Citrate**	**MIO**			**Urease**	**Organism**
			Slant/ Butt	**H_2S**	**Gas**		**Mortality**	**Indole**	**Ornithine**		
Naan	TCBS	Green	K/A	-	+	+	+	-	-	-	*Vibrio* sp.
Beef kabab	TCBS	Yellow	K/A	-	-	+	+	-	-	-	
Hot Dog	TCBS	Yellow	K/A	-	-	+	+	-	-	-	
Bun	TCBS	Yellow	K/A	-	-	+	-	-	-	-	
Pizza	TCBS	Yellow	K/A	-	-	+	+	-	-	-	
Ruti	TCBS	Yellow	K/A	-	-	+	+	-	-	-	
Egg fry	TCBS	Yellow	K/A	-	+	+	+	-	-	-	
Roll	TCBS	Yellow	K/A	-	+	+	+	-	-	-	
Banana vorta	TCBS	Yellow	K/A	-	+	-	+	-	+	-	

Table 4.5 Shows identification of *Vibrio* species from different biochemical test. Biochemical test results for the samples Naan, Beef kabab, Hot Dog, Bun, Pizza, Ruti, Egg fry, Roll and banana vorta matched with the standard results for *Vibrio* species. So, we can say that the samples may contain the *Vibrio* species.

Table 4.6: Identification of the suspected organism *Aeromonus* species from different biochemical tests.

Sample name	**Plates**	**Colony morphology**	**KIA**			**Citrate**	**MIO**			**Urease**	**Organism**
			Slant/ Butt	**H_2S**	**Gas**		**Mortality**	**Indole**	**Ornithine**		
Dim vaji	MAC	Colorless	K/A	+	+	+	+	+	-	-	*Aeromonas* spp.
Dim chop	MAC	Colorless	A/A	-	+	+	+	+	-	-	
Mach vorta	MAC	Colorless	K/K	+	+	+	+	+	-	-	
Booter Daal	MAC	Colorless	K/K	-	-	+	+	-	-	-	

Table 4.6 Shows identification of *Aeromonous* species from different biochemical test. Biochemical test results for the samples Dim vaji, Dim chop, Mach vorta and booter daal matched with the standard results for *Aeromonous.* So, we can say that the samples may contain the Aeromonous species.

Table 4.7: Presence of suspected organisms in selected food samples after biochemical test (n=18)

Hospital name	***E.coli***	***Vibrio* spp.**	***Aeromonus* spp.**
BSMMU	4	1	1
DMC	1	7	0
Islami Bank Hospital	0	1	0
Khidma Hospital	0	0	3

Table 4.7 shows presence of suspected organisms in selected food samples after biochemical test. From the results of biochemical test we found 18 of our suspected bacteria. Among them, from Bangabandhu Sheikh Mujib Medical University (BSMMU)

we got 4 *E.coli*, 1 *Vibrio*, 1 *Aeromonus* spp. ; from Dhaka Medical College (DMC) we got 1 *E.coli*, 7 *Vibrio*; from Islami Bank Hospital we got 1 *Vibrio*; from Khidma Hospital we got 3 *Aeromonus* spp. In total we got 5 (27.77%) *E.coli*, 9 (50%) *Vibrio*, 4 (22.22%) *Aeromonus* spp.

Table 4.8: Presence of food borne pathogens in hospital food samples (n=13)

Pathogen	**Food categories**								Total food items (n=31)
	Fried items (n=12)	Curry items (n=7)	Baked items (n=4)	Soup (n=3)	Rice (n=1)	Sauce (n=1)	Salad (n=1)	Beverage (n=1)	
E.coli	3 (25%)	2 (28.5%)	Nd	Nd	Nd	Nd	Nd	Nd	5 (16.12%)
Vibrio spp.	4 (33.3%)	1 (14.2%)	4 (100%)	Nd	Nd	Nd	Nd	Nd	9 (29.03%)
Aeromonus spp.	2 (16.6%)	2 (28.5%)	Nd	Nd	Nd	Nd	Nd	Nd	4 (12.90%)

Table 4.8 shows the incidence of food borne pathogens in various food samples. Among 12 fried items, 3 (25%) samples were suspected to contain *E.coli*, 4 (33.3%) samples were suspected to contain *Vibrio* and 2 (16.6%) samples were suspected to contain *Aeromonus* spp. Among 7 curry items, 2 (28.5%) samples were suspected to contain *E.coli*, 1 (14.2%) sample was suspected to contain *Vibrio* and 2 (28.5%) samples were suspected to contain *Aeromonus* spp. Among 4 baked items, 4 (100%) samples were suspected to contain *Vibrio*.

4.3 Bacterial colony counting:

After an appropriate incubation period the plates were examined for colonial growth. 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. 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. Using a light box or colony counter and marker pen, the numbers of colonies were counted.

Table 4.9: Colony counting of various hospitals food samples.

Hospital name	Sample name	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Bangabandhu Sheikh Mujib Medical University	Booter Daal	Uncountable	Uncountable	Uncountable	Uncountable
	Vaji	Uncountable	Uncountable	70	40
	Hot Dog	Uncountable	Uncountable	Uncountable	Uncountable
	Banana vorta	Uncountable	Uncountable	Uncountable	Uncountable
	Egg Fry	Uncountable	Uncountable	Uncountable	60
	Mixed vaji	Uncountable	Uncountable	Uncountable	Uncountable
Dhaka Medical College	Bun	Uncountable	Uncountable	Uncountable	Uncountable
	Roll	Uncountable	Uncountable	Uncountable	Uncountable
	Hot Dogg	Uncountable	Uncountable	Uncountable	Uncountable
	Pizza	Uncountable	Uncountable	Uncountable	Uncountable
	Ruti	Uncountable	Uncountable	Uncountable	Uncountable
	Egg Fry	Uncountable	Uncountable	Uncountable	Uncountable
	Beef Kabab	Uncountable	Uncountable	Uncountable	Uncountable
Islami Bank Hospital	Naan	Uncountable	Uncountable	Uncountable	Uncountable
	Soup	Uncountable	Uncountable	Uncountable	Uncountable
	Daal vaji	85	62	22	9
	Chicken roll	55	40	15	7
	Jaw vaat	88	65	7	2
	Singara	Uncountable	Uncountable	Uncountable	Uncountable
Khidma Hospital	Dim vaji	Uncountable	Uncountable	Uncountable	Uncountable
	Dim chop	Uncountable	Uncountable	19	2
	Mach vorta	Uncountable	Uncountable	Uncountable	Uncountable
Monowara Hospital	Porota	Uncountable	Uncountable	Uncountable	Uncountable
	Daal	Uncountable	Uncountable	Uncountable	Uncountable
	Chicken soup	Uncountable	Uncountable	Uncountable	Uncountable
Salauddin Hospital	Singara	Uncountable	Uncountable	Uncountable	Uncountable
	somucha	Uncountable	Uncountable	Uncountable	Uncountable
Uttara Cresent Hospital	Vegetable soup	Uncountable	Uncountable	Uncountable	Uncountable
	Sauce	Uncountable	Uncountable	Uncountable	Uncountable
	Salad	Uncountable	Uncountable	Uncountable	Uncountable
	Coffee	Uncountable	Uncountable	Uncountable	Uncountable

Table 4.9 shows Colony counting of various street food samples. For Vaji, 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:

70 colonies on plate 3 x dilution factor of 1000 = 70,000 cells/ml.

Dilution factor for plate 1: 10

Dilution factor for plate 2: 100

Dilution factor for plate 3: 1000

Dilution factor for plate 4: 10000

Using above mentioned formula and dilution factors we got the number of microorganism per ml of sample of hospital food items in table 4.10.

Table 4.10: Number of colony forming unit (CFU) per ml of hospital food sample.

Sample name	Vaji	Egg Fry	Daal vaji	Chicken roll	Jaw vaat
Number of CFU (cells/ml)	$7.0x10^4$	$6.0x10^5$	$8.5x10^2$	$5.5x10^2$	$8.8x10^2$

Table 4.10 shows that the number of microorganism per ml for Vaji is $7.0x10^4$; for Egg Fry is $6.0x10^5$; for Daal vaji is $8.5x10^2$; for Chicken roll is $5.5x10^2$; for Jaw vaat is $8.8x10^2$.

Chapter: 5

Discussion & Conclusion

5.1 Discussion and Conclusion

Hospital food has become a major community health issue and matter of concern for all of us in present situation. Because that food is served to hospitalized patient to maintain their needed nutritional value and to help them to recover from the disease. A lot of food-borne disease outbreaks are occurring every year worldwide by hospital food because of contamination occurred in the food by various type of pathogens. The reasons behind of contamination of food include lack of appropriate knowledge of hygiene and lack of supervision while preparing the food under unhygienic environment.

The present research work was therefore carried out to find out the presence of enteric bacteria specially *E.coli, Salmonella, Shigella, Vibrio* species, *Aeromonas* species, *Plesiomonas shigelloides*, *Yersinia enterocolitica* from different types of hospital foods items collected from different Hospital 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 Hospital food items. MacConkey and TBX agar were used for the identification and isolation of *E.coli*. TCBS Agar is highly selective for *Vibrio* species isolation. XLD and BGA were used for isolation of *Salmonella* and *Shigella* species. MacConkey agar was used for the identification and isolation of *Aeromonas* species, *Plesiomonas shigelloides*, *Yersinia enterocolitica* from food samples. Sometimes we didn't find any growth in agar media.

The reason of no growth may include the following:

a) Sometimes fresh foods were collected early in the morning so no contamination occurred yet,

b) Sometimes food were hot which prevented growth of bacteria,

c) The quality of the hospital food was good & well maintained.

In this study, 31 different hospital food samples were tested from 7 different hospitals of Dhaka city, Bangladesh. Among all 31 samples, we found contamination in 16 (51.61%) samples with our targeted organisms (*E coli, Shigella, Salmonella, Vibrio* species, *Aeromonas* species, *Plesiomonas shigelloides*, *Yersinia enterocolitica)* and 15 samples

shows no growth in those agar media. Among 16 contaminated food samples, we observed 5 (27.77%) samples were suspected to be contaminated with *E.coli*, 9 (50%) samples were suspected to be contaminated with *Vibrio spp,* 4 (22.22%) samples were suspected to be contaminated with *Aeromonas spp*. But we didn't find any sample which was contaminated with *Salmonella, Shigella.*

A study was conducted to assess microbiological safety of street vended foods from May to November, 2014 in Jigjiga City. One hundred thirty-two samples of street foods were aseptically collected from four 'kebeles' of Jigjiga City. The study revealed that 95(72%) of the food samples had pathogenic bacteria contaminations. Three different bacterial species were isolated: *E. coli* 68(51.5%), *S. aureus* 85(64.4%) and 26 (19.7%) *Salmonella* species. The highest incidence of *S. aureus* 23/33(69%) was seen in 'Sambusa'; the highest incidence of *E. coli* 24/33(73.5%) was observed in 'Pasta', while the highest Salmonella incidence was observed in 'Ades' (Bereda et al. 2016).

A study was performed to determine the prevalence of bacterial infestation among the food handlers attending the public health center laboratory in Sari, northern Iran from September 2013 to August 2014. Stool samples, fingernail specimens of both hands and nasal swabs were collected from 220 male and female food handlers of different jobs, aged between 17-65 years. The samples were cultured on bacteriological culture media and bacterial species were identified following standard procedures. Of the total 220 subjects examined, 62.2% showed positive culture for different bacterial species from their fingernail contents, 65.4% were found to be harboring *Staphylococcus aureus* in their nostrils and 0.9% tested positive for *Shigella boydii*from stool samples. *Staphylococcus aureus* was the predominant bacteria isolated from fingernail specimens (46%), followed by *Escherichia coli* (29.2%), *Coliforms* (18.2%) and *Pseudomonas aeruginosa* (6.6%) (Nasrolahei 2016).

Another study was carried out to find out the presence of common enteric bacteria, such as, *Shigella*, *Salmonella*, pathogenic *E. coli* and *Vibrio* spp. in the street vended foods around the student campus of East West University (EWU) in Dhaka, Bangladesh. The study focused on the socio-economic, behavioral characteristics and practices with street foods among the students of EWU and the street food vendors around the campus. A total of 50 street food samples were randomly collected from fixed and mobile vendors around the EWU area and tested for the presence of microorganisms following conventional

microbiological processes. In order to describe the characteristics and practices of students of EWU, 225 students were randomly selected and interviewed by using a structured questionnaire. Based on the availability, 150 vendors were also interviewed by using a structured questionnaire. Among 50 food samples, 46 (92%) had bacterial contamination of which, 6 samples (12%) were confirmed to contain different species of *E coli* and *Shigella* (Islam S et al. 2015).

Another study was performed in order to determine the level of bacterial contamination on the hands of food handlers (n=30) who work in the kitchen of a military training hospital. A total of 180 samples were collected from bare and gloved hands before and during food preparation. A total of 16 different bacteria were isolated, of which the most common was *Staphylococcus aureus* (126/180; 70%), diphtheroid bacilli (39/180; 21.7%), *Bacillus* spp. (19/180; 10.5%), and *Escherichia coli* (14/180; 7.8%). Fifty-one of 60 (85%) gloved hand samples were collected during work, 57 (95%) of the bare hand samples were collected before work, and all of the bare hand samples collected during work were positive. Poor hand hygiene was indicated by high levels of *S. aureus* and *E. coli* on samples taken from bare and gloved hands. Although bacterial loads on gloved hand samples were found to be significantly lower (p<0.05) than ungloved hand samples, these loads were not within acceptable limits (Ayçiçek et al. 2004).

Our study also shows the presence of food-borne pathogens in various hospital food samples. Total 31 samples divided into 8 categories, among them 12 fried items were available and from that 3 (25%) samples was suspected to contain *E.coli,* 2 (16.6%) sample was suspected to contain *Aeromonus spp.* and 4 (33.3%) samples were suspected to contain *Vibrio.* Among 7 curry items, 2 (28.5%) samples were suspected to contain *E.coli,* 1 (14.2%) samples were suspected to contain *Vibrio* and 2 (28.5%) samples were suspected to contain *Aeromonus spp.* Among 4 baked items, 4 (100%) sample was suspected to contain *Vibrio.*

If we compare our study with above mentioned four studies, then we can observe that *E.coli* was found in different categories of food samples in all the studies. Study on street-vended foods around the EWU campus was contaminated with *E.coli* and *Shigella*. Though we had objective to identify *Shigella,* but we did not find any *Shigella* spp in our food sample. We had also objective to identify *Salmonella* species. However, no *Salmonella* was identified in our food samples. Other studies identified the *Salomonella*

species in 26 street food samples in Jigjiga City. We have identified *Vibrio* species and *Aeromonas* species in our present study.

Hospital foods have become major source of serious health problem due to different type of microbial contamination. So, more focuses should be given in this sector and more research work should be carried out in developing country like Bangladesh to search out the quality, hygiene, safety of hospital food. It is also suggested that regular monitoring should be done for observing the quality, hygiene, well-maintained environment for preparing food for hospitalized patients to avoid food-borne infection in future. There is a huge lack of knowledge on food safety and hygiene among the people who prepare the hospital food, handle all the system, equipment, utensils & also who deliver the food to individual patient. Enough attention should be given by the government to improve knowledge about food safety, quality and hygiene standards of hospital food.

Chapter: 6

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