An assessment of 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, Md. Yeasin Sheakh, hereby declare that the dissertation entitled "An assessment of 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 2017 of my research in the Department of Pharmacy, East West University, under the supervision and guidance of 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

This is to certify that the thesis entitled "An assessment of microbiological quality of hospital food items collected from different hospitals 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 Md. Yeasin Sheakh, ID: 2014-1-70-015, 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
EPEC	Enteropathogenic *E.coli*
EPIC	Enteroinvasive *E.coli*
ETEC	Enterotoxigenic *E.coli*
APW	Alkaline Peptone Water
BPW	Buffered Peptone Water
TSB	Trypticase Soya Broth
YE	Yeast Extract
TBX	Triptone Bile X- glucuronide
TCBS	Thiosulfate Citrate Bile Salt sucrose
BGA	Brilliant Green Agar
XLD	Xylose Lysine Desoxycholate Agar
MIO	Mortality Indole Ornithine
KIA	Kligler's Iron Agar
HACCP	Hazard analysis and critical control points

ABSTRACT

In healthcare settings a nutritious diet is essential for the patients in hospital. Food must be safe and of good quality. Meals or foods offered to the patients inside the hospital environment are an essential part of their overall care for recovery. Bacterial contamination of hospital food items can be hazardous for the health and safety of the patients. Therefore, the present research work was undertaken to find out the presence of enteric bacteria specially *E. coli*, *Salmonella*, *Shigella* and *Vibrio* species from different hospital food of Dhaka city, Bangladesh. Five agar media MacConkey, Tryptone Bile X-glucoronide (TBX) agar, Thiosulfate Citrate Bile Salt-sucrose (TCBS) agar, Brilliant Green Agar (BGA) and Xylose-Lysine Desoxycholate agar (XLD) were used to observe the presence of our targeted microorganisms in food items. Seven biochemical tests were performed to identify the targeted organisms. The tests are KIA, citrate, motility, indole, ornithine, urease, and oxidase test. Out of thirty-one food samples 7 (36.85%) food samples were suspected to be contaminated with *E. coli*, 9 (37.37%) food samples were suspected to be contaminated with *Vibrio* species, 3 (15.79%) food samples were suspected to be contaminated with *Aeromonas* species. All these enteric pathogens could be the potential cause for foodborne illnesses and may further deteriorate the patients' health. So, the hospital canteen authority should take necessary steps to maintain good quality of food to ensure patients health safety.

Keywords: Hospital food, Foodborne illness, Public health risk, Enteric bacteria, Biochemical test, *E. coli*, *Vibrio*, *shigella*, *Salmonella*, *Aeromonas*.

Chapter1
Introduction & Literatura Review

1.1 Hospital food

In healthcare settings a nutritious diet is essential for patient treatment and recovery, so food must be safe, of good quality, wholesome, and served at times that are convenient and appropriate not only at conventional mealtimes. Many hospitals have canteens serving visitors, but inpatients, outpatients and staff can often also purchase food. Food from these outlets must also be safe for patients and staff (Lund and O'Brien 2009). 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 (Kokkinakis et al. 2011).

Most of the food served in hospitals affects not only the health of the patients, their visitors and employees but also the broader community, society and the environment. If hospitals can't even serve healthy food to critically ill patients, there is no hope that left for us (Kirk et al. 2015). Poor food quality has become the standard complaint of hospital patients. In the past, such complaints were rare. In ancient time, many Islamic hospitals served their food by maintaining a good quality especially for noblemen and for kings but few historians have doubted the benefits that food and rest offered to hospital patients. In recent years' hospital food related different surveys have been conducted where cost-cutting and the difficulties of catering to everyone's tastes make it impossible to please everyone. The only relief for most people comes in the progressive shortening of patients' hospital stays. While patients' periods of hospitalization in the early Victorian period averaged more than a month, most patients now spend days, rather than weeks, in hospital (Science Museum's History of Medicine n.d.).

1.2 Hospital Food Items

There are so many hospitals in Dhaka city. Include the cafeterias served with so many variation of foods.Different types of foods are prepared in the hospital for patients according to their disease condition so that they can meet their nutritional needs. 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.

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: Different types of hospital food items

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 Drawbacks 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 isusually 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, fromany 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 surfacesof 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 storagetechniques (Kokkinakis, 2011).

1.4 Food borne illness

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 bio toxins. 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 Centers 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 (Assefa, et al. 2015). The Environmental Health Specialists Network (EHSNet) was established to conduct research and surveillance that would contribute to a greater understanding of the risk of foodborne illness associated with restaurants and to translate knowledge of how and why outbreaks occur into improved prevention practices. In a recent report conducted by the World Health Organization (WHO), it was revealed that 1 in 10 people fall ill globally due to foodborne diseases while more than 91 million people are affected in developing countries despite various research and intervention measures toward food safety. Similarly, 2.2 million children die of diarrhea annually in developing countries. Occurrence of foodborne diseases however, is more prevalent in developing countries due to poor hygiene, lack of potable drinking water, contaminated inappropriate food storage facilities and lack of food safety education (Stratev, et al. 2017). Preliminary data from Food Net, a collaborative program comprised of 10 state health departments, CDC, the Food and Drug Administration, and the U.S. Department of Agriculture's Food Safety and Inspection Service, listed the most common pathogens for food-borne illnesses for 2009, from highest to lowest incidence, as *Salmonella*, *Campylobacter*,*Shigella*,*Cryptosporidium*,*STECO157*, *STEC* non-*O157*, *Vibrio*, *Listeria*,*Yersina*, andCyclospora.Compared to previous years, there has been an increased incidence of *Vibrio*, while there has been a decrease in *Shigella*and *STECO157*. These dataalso show that children less than 4 years of age have the highest reported incidence of food-borne illnesses; however, adults 50 years of age have the highest hospitalization and fatality rates. The organisms that were most responsible for hospitalization of adults, in descending order, are *STECO157*, *Salmonella*,*Yersinia*,*Vibrio*, *STEC*non-*O157*, *Shigella*, *Cyclospora*, *Cryptosporidium*, and *Campylobacter*, *Listeria*had the highest fatality rate inadults over 50, followed by *Vibrio* (Linscott 2011).

1.5 Types of Microbial Food-borne Diseases

On the basis of mode of illnesses, food borne disease (FBD) can be arbitrarily divided into three groups.

1.5.1 Intoxication

Illness in this case occurs as a consequence of ingestion of a pre formed bacterial or a mold toxin due to its growth in a food. A toxin has to be present in the contaminated food. Once the microorganism has grown and produced toxin in a food, there is no need of viable cells during the consumption of the food for illness to occur. e.g. Staphylococcal food poisoning (Roy 2005).

1.5.2 Infection

Illness occurs as a result of the consumption of food and water contaminated with enter pathogenic bacteria. It is necessary for the cells of enter pathogenic bacteria to remain alive in the food or water during consumption. The viable cells even if present in small numbers have the potential too establish and multiply in the digestive tract to cause the illness. e.g., Salmonellosis (Roy 2005).

1.5.3 Toxicoinfection

Illness occurs from the ingestion of a large number of viable cells of some pathogenic bacteria through contaminated food and water. Generally, the bacterial cells either sporulate or die and release toxin to produce the symptoms. e.g. Bacillus cereus Gastroenteritis (Roy 2005).

1.6 Common Pathogens Responsible for Food-borne Diseases

Pathogens	Examples	Sign & symptoms
Bacteria	*Salmonnelaspecies* *Camphylobactorjejuni* *Shigellaspecies* *Listeriamonocytogenes* *Vibriospecies* *Yersiniaspecies*	Abdominal pain, diarrhea, chills, fever, vomiting, nausea, jaundice, Anorexia,
Parasites	*Cyclospora* species *Entamoebahistolytica* *Toxoplasmacaris*	Severe intestinal distress, abdominal pain, cramps.
Toxins	*Staphylococcusaureus* *Clostridiumperfringens*	Abdominal pain, diarrhea, vomiting,
Miscellaneous	Monosodium glutamate	Visual hallucination and ataxia in children

1.7 Factors Affecting 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:

1.7.1 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: 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 (Dilbaghi and Sharma 2007).

Water activity: 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: 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 (Dilbaghi and Sharma 2007).

1.7.2 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. (Dilbaghi and Sharma 2007).

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(Dilbaghi and Sharma 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 (Dilbaghi and Sharma 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(Dilbaghi and Sharma 2007).

1.7.3 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 (Dilbaghi and Sharma 2007).

1.8 Description of Some Common Microorganism Responsible for Food borne Diseases

1.8.1 *Escherichia coli*

Escherichiacoli(commonly abbreviated*E. coli*) is a Gram-negative, rod-shapedbacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in

humans. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2, and by preventing the establishment of pathogenic bacteria within the intestine. *E. coli* and related bacteria constitute about 0.1% of gut flora, and fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause diseases (Adams and Moss 2008).

1.8.2 Pathogenesis

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

1.8.3 *Shigatoxin-producing E. coli* (*STEC*)

STEC may also be referred to as Verocytotoxin-producing *E. coli* (*VTEC*) or enterohemorrhagic *E. coli* (*EHEC*). This pathotype is the one most commonly known bacteria associated with food-borne outbreaks. First recognized as a cause of human disease in 1982, *EHEC* causes bloody diarrhea (hemorrhagic colitis), non-bloody diarrhea and hemolytic uremic syndrome (HUS).

Figure 1.2: ***Escherichia coli***

The key virulence factor for *EHEC* is Stx, which is also known as verocytotoxin (VT). Stx consists of five identical B subunits that are responsible for binding the holotoxin to the glycolipid globotriaosylceramide (Gb3) on the target cell surface, and a single A subunit that cleaves ribosomal RNA, causing protein synthesis to cease12. The Stx family contains two subgroups Stx1 and Stx2 that share approximately 55% amino acid homology. Stx is produced in the colon and travels by the bloodstream to the kidney, where it damages renal endothelial cells and occludes the microvasculature through a combination of direct toxicity and induction of local cytokine and chemokine production,

resulting in renal inflammation. This damage can lead to HUS, which is characterized by hemolytic-anaemia, thrombocytopoenia and potentially fatal acute renal failure. Stx also induces apoptosis in intestinal epithelial cells, a process that is regulated by the Bcl-2 family 44 (Adams and Moss 2008).

1.8.4 *Enterotoxigenic E. coli (ETEC)*

ETEC causes watery diarrhea, which can range from mild, self-limiting disease to severe purging disease. The organism is an important cause of childhood diarrhea in the developing world and is the main cause of diarrhea in travelers to developing countries. *ETEC* colonizes the surface of the small bowel mucosa and elaborates enterotoxins, which give rise to intestinal secretion. Colonization is mediated by one or more proteinaceous fibrillar colonization factors (CFs), which are designated by CFA (colonization factor antigen), CS (coli surface antigen) or PCF (putative colonization factor) followed by a number. More than 20 antigenically diverse CFs have been characterized, yet epidemiological studies indicate that approximately 75% of human *ETEC* express either by CFA/I, CFA/II or CFA/IV) (Adams and Moss 2008).

1.8.5 *Enteropathogenic E. coli (EPEC)*

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

A characteristic intestinal histopathology is associated with *EPEC* infections; known as **'**attaching and effacing**'** (A/E), the bacteria intimately attach to intestinal epithelial cellsand cause striking cytoskeletal changes, including the accumulation of polymerized actin directly beneath the adherent bacteria. The microvilli of the intestine are effaced and pedestal-like structures on which the bacteria perch frequently rise up from the epithelial cell. The ability to induce this A/E histopathology is encoded by genes on a 35-kb pathogenicity island (Adams and Moss 2008).

1.8.6 *Enteroinvasive E. coli (EIEC)*

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

indistinguishable from that due to infection by other *E. coli* pathogens (Adams and Moss 2008).

1.9 *Salmonella* species

Salmonella is an important bacterial genus which causes one of the most common formsof food poisoning worldwide. It is one of the most extensively studied bacterial species in terms of its physiology, genetics, cell structure, and development. It is also one of the most extensively characterized bacterial pathogens and is a leading cause of bacterial gastroenteritis. *Salmonella* is capable of causing a variety of disease syndromes: enteric fever, bacteremia, enterocolitis, and focal infections (NCBI 1996).

1.9.1 Characteristics

The growth and survival of *Salmonella species* in foods is influenced by a number of factors such as temperature, pH, salt content and the presence of preservatives. For example,survival of *Salmonella flexneri* has been shown to increase with: decreasing temperature, increasing pH, and decreasing NaCl concentration Once ingested, *Salmonella species* must survive the acidic environment of the stomach and invade the epithelial cells of the colon to enable infection. *Salmonella species* multiply inside the colonic epithelial cells and spread to adjacent cells, leading to the death of the infected cells. The colon becomes inflamed and ulcerated and the dead mucoid cells are shed, resulting in the bloody mucoid diarrhoea often characteristic of *Salmonella* infection (NCBI 1996).

Figure 1.3: *Salmonella* species

1.9.2 Pathogenesis

Salmonellosis includes several syndromes (gastroenteritis, enteric fevers, septicemia, focal infections, and an asymptomatic carrier state). Particular serovars show a strong propensity to produce a particular syndrome (*Salmonella typhi*, *Salmonell paratyphi-A*, and *Salmonella schottmuelleri* produce enteric fever; *Salmonella choleraesuis* produces

septicemia or focal infections; *Salmonellatyphimurium*and*Senteritidis*produce gastroenteritis); however, on occasion, anyserotype can produce any of the syndromes. In general, more serious infections occur in infants, in adults over the age of 50, and in subjects with debilitating illnesses. Most non-typhoidal s*almonellae* enter the body when contaminated food is ingested. Person-to-person spread of *salmonellae* also occurs. To be fully pathogenic, *salmonellae* must possess a variety of attributes called virulence factors. These include: the ability to invade cells, a complete lipopolysaccharide coat, the ability to replicate intracellularly, and possibly the elaboration of toxin. After ingestion, the organisms colonize the ileum and colon, invade the intestinal epithelium, and proliferate within the epithelium and lymphoid follicles. The mechanism by which *salmonellae* invade the epithelium is partially understood and involves an initial binding to specific receptors on the epithelial cell surface followed by invasion. Invasion occurs by the organism inducing the enterocyte membrane to undergo **"**ruffling**"** and thereby to stimulate pinocytosis of the organism. Invasion is dependent on rearrangement of the cell cytoskeleton and probably involves increases in cellular inositol phosphate and calcium. Attachment and invasion are under distinct genetic control and involve multiple genes in both chromosomes and plasmids. After invading the epithelium, the organisms multiply intracellularly and then spread to mesenteric lymph nodes and throughout the body via the systemic circulation; they are taken up by the reticuloendothelial cells. The reticuloendothelial system confines and controls spread of the organism. However, depending on the serotype and the effectiveness of the host defenses against that serotype, some organisms may infect the liver, spleen, gallbladder, bones, meninges, and other organs. Fortunately, most serovars are killed promptly in extra intestinal sites, and the most common human Salmonella infection, gastroenteritis remains confined to the intestine (NCBI 1996).

1.10 *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 *Shigellady senteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*, all of which are regarded as human pathogens though they differ in the severity of the illness they cause. *Shigella. dysenteriae* has been responsible for epidemics of severe bacillary dysentery in tropical countries but is now rarely encountered in Europe and North America where *Shigella*

sonnei is more common. *Shigellasonnei* causes the mildest illness, while that caused by *Shigellaboydii* and *Shigellaflexneri* is of intermediate severity (Adams and Moss 2008).

1.10.1 Characteristics

Shigella species of the *Enterobacteriaceae* family, is gram-negative rod-shaped pathogenicbacteria. They are non-motile, non-encapsulated, and facultative anaerobes that do not ferment lactose, or do so slowly. Different serogroups, considered as species, can be differentiated by their biochemical properties, phage or colicin susceptibility, and polyvanlent antisera can detect specific polysaccharide antigens. *Shigelladysenteriae* is considered the most virulent, and can produce a potent cytotoxin known as Shigatoxin.

1.10.2 Pathogenesis

*Shigella*causes bacillary dysentery in humans and other higher primates. Studies withhuman volunteers have indicated that the infectious dose is low; of the order of 10–100 organisms. The incubation period can vary between 7 hour and 7 days although food-borne outbreaks are commonly characterized by shorter incubation periods of up to 36 hours.

Figure 1.4: *Shigella* species

Symptoms are of abdominal pain, vomiting and fever accompanying a diarrhea which can range from a classic dysenteric syndrome of bloody stools containing mucus and pus, in the cases of *Shigelladysenteriae*, *Shigellaflexneri* and *Shigellaboydii*, to a watery diarrhea with Shigellasonnei. Illness lasts from 3 days up to 14 days in some cases and a carrier state may 250Bacterial Agents of Food borne Illness develop which can persist for several months. Milder forms of the illness are self-limiting and require no treatment but *Shigella* dysenteries infections often require fluid and electrolyte replacement and antibiotic therapy. Shigellosis is an invasive infection where the organism's invasiveproperty is encoded on a large plasmid (Adams and Moss 2008).

1.11 *Vibrio* species

*Vibrio*is a family of Gram-negative bacteria that can cause a variety of illnesses inhumans. The most famous form of *Vibrio* is *Vibriocholerae*, the bacterium that causes cholera. *Vibriocholerae* has been the cause of seven worldwide pandemics and countless deaths over the last couple of centuries. Except for *Vibriocholerae* and *Vibriomimicus*, all require saltwater for growth. Therefore, seawater and raw or undercooked shellfish are common infection routes for *Vibrio* (Adams and Moss 2008).

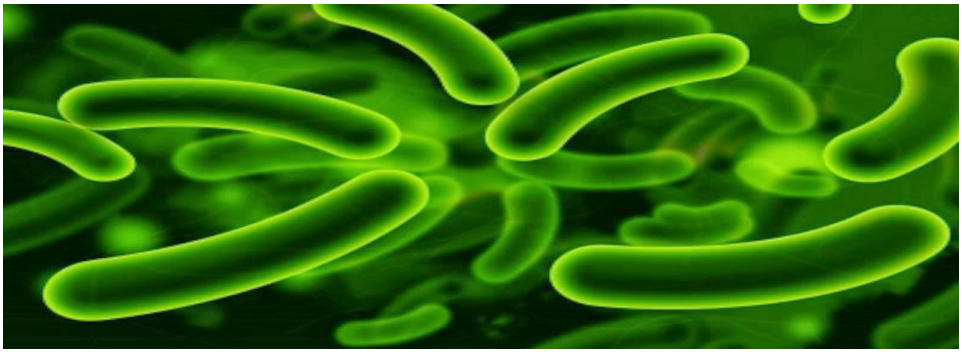

Figure 1.5: *Vibrio* species

1.11.1 Characteristics

*Vibrios*are 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 metabolisms.

1.11.2 Pathogenesis

Cholera usually has an incubation period of between one and three days and can vary from mild, self-limiting diarrhea to a severe, life threatening disorder. The infectious dose in normal healthy individuals is large when the organism is ingested without food or buffer, of the order of 1010 cells, but is considerably reduced if consumed with food which protects the bacteria from stomach acidity. Studies conducted in Bangladesh indicate that 103 –104 cells may be a more typical infectious dose. Individuals with low stomach acidity (hypochlorohydric) are more liable to catch cholera. Cholera is a non-invasive infection where the organism colonizes the intestinal lumen and produces a potent enterotoxin. In severe cases, the hyper secretion of sodium, potassium, chloride, and bicarbonate induced by the enterotoxin results in a profuse, pale, watery diarrhea containing flakes of mucus, described as rice water stools (Adams and Moss 2008).

1.12 *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.

1.12.1 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. Itsprincipal reservoir is the aquatic environment such as freshwater lakesand streams and wastewater systems. The numbers present will dependon 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 pipedwater systems. It has also been isolated from a wide range of fresh foodsand is a transient component of the gut flora of humans and other animals (Adams and Moss 2008).

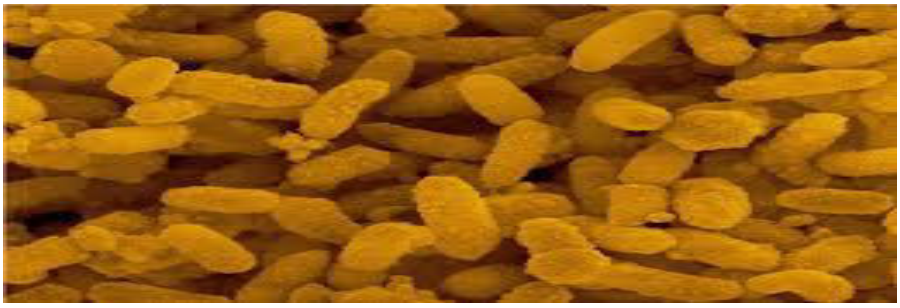

Figure 1.6: *Aeromonas* species

1.12.2 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 species* particularly *Aromona shydrophila* and *Aeromonas 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.

1.12.3 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 (Adams and Moss 2008).

1.13 *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*.

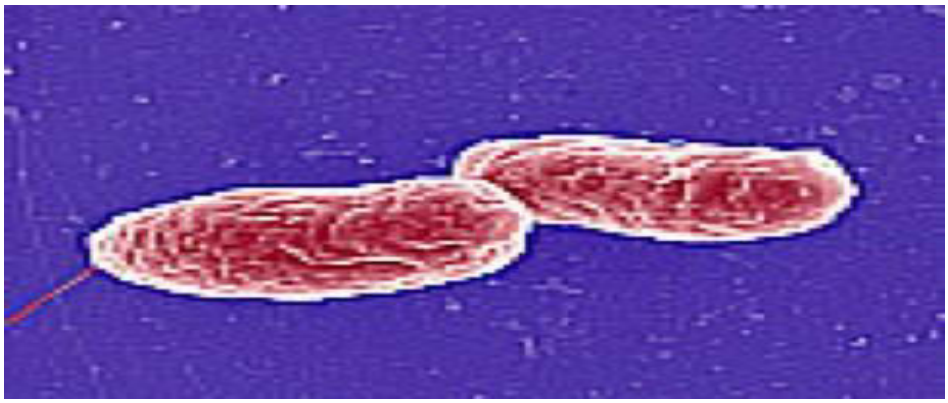

Figure 1.7: *Plesiomonas shigelloides*

1.13.1 The Organism and its Characteristics

A member of the family Enterobacteriaceae (previously classified in the *Vibrionceae*), *Plesiomonasshigelloides* 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 asfrogs, 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 (Adams and Moss 2008).

1.13.2 Pathogenesis and Clinical Features

Cases of *Plesiomonasshigelloides* 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.

1.13.3 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 and Moss 2008).

1.14 Prevalence of Food-borne Illness Around the world

According to WHO, a disease outbreak is the occurrence of cases of disease in excess of what would normally be expected in a defined community, geographical area or season. Expression of the similar symptoms or sickness by two or more of the individuals after consumption of the same contaminated food is labeled as an outbreak of food-borne illness. The description of outbreak includes time, place, and person distribution (Jahan 2012).

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

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

Zealand, *Salmonella typhiumurium* outbreaks were more common (Greig and Ravel 2009). A study was conducted in the United States, to describe the epidemiology of food-borne illness outbreaks in schools, colleges and universities. The data from January 1, 1973, to December 31, 1997 was reviewed. In majority (60%) of the outbreaks the etiology was unknown. Among the outbreaks with a known etiology, in 36% of outbreak reports *Salmonella* was the most commonly identified pathogen. However, the highest mortality was caused by *Listeria monocytogenes*. Viral pathogens were responsible for 33% of the outbreaks. Among the viral pathogens, norovirus was the most common causative agent (Lynch, et al. 2006).

In 2002, a salmonellosis outbreak occurred in five states of U.S. It occurred after consuming ground beef. During this outbreak, forty-seven cases were reported; out of which 17 people were hospitalized and one death was reported (Lynch et al., 2006).

In England and Wales, 2429 food-borne outbreaks were reported from 1992 to 2008. Approximately half of the outbreaks were caused by *Salmonella* species. Poultry and red meat was the most commonly implicated foods in the causation of outbreaks. The associated factors in most outbreaks were cross-contamination, lack of adequate heat treatment and improper food storage (Gormley, et al. 2011).

In central Taiwan, 274 outbreaks of food-borne illness including 12,845 cases and 3 deaths were reported during 1991 to 2000. Majority (62.4%) of the outbreaks were caused by bacterial pathogens. The main etiologic agents were *Bacillus cereus*, *Staphylococcusaureus*, and *Vibrio parahaemolyticus*. The important contributing factor was improperhandling of food. The implicated foods included seafood, meat products and cereal products (Chang and Chen 2003).

Chapter 2
Objective

2.1 Research Objective

To isolate and identify the presence of enteric bacteria especially *E. coli*, *Salmonella*, *Shigella*, *Vibrio* and *Aeromonas* species from different hospital food canteen in Dhaka city.

Chapter 3
Methodology

3.1 Bacteriological Subculture

3.1.1 Sample Collection About 31food samples were randomly chosen and collected from different hospital canteen 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.

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*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* species5 gm solid sample were mixed well with 45 ml of BPW (Buffered Peptone Water) broth and incubated at 37 ºC for 18-24h.

3.1.3.3 Enrichment of *Vibrio* species5 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 Organism

3.1.4 Selective Growth of the Organisms

3.1.4.1 Selective Growth *E. coli* species: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* species: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* species: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

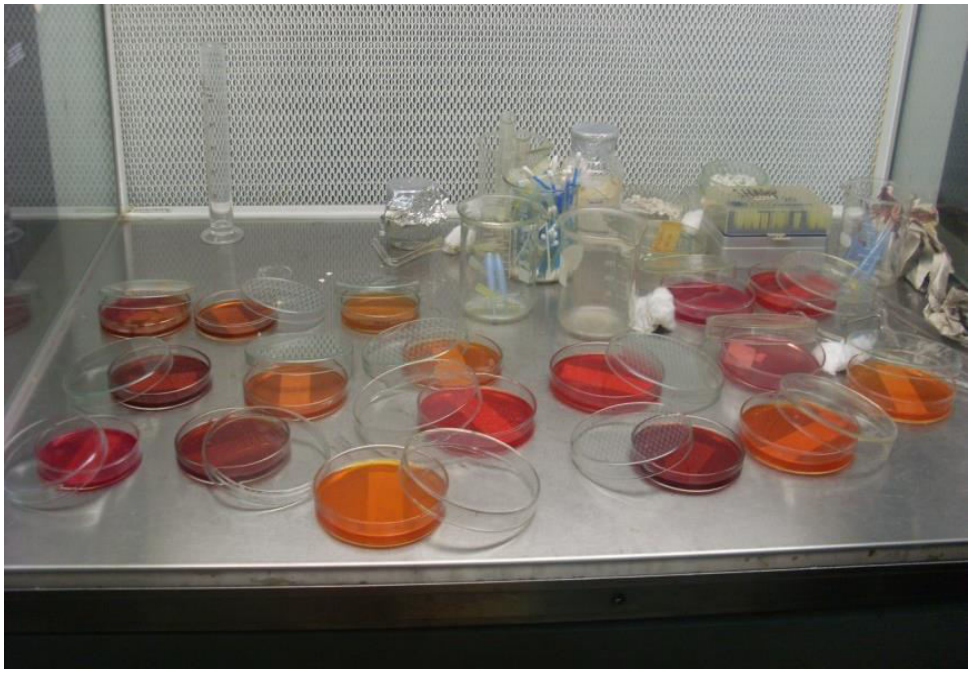

Figure 3.4: Petri dishes preparation

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:

Organism	Media	Appearance
E.coli	MacConkey	Lactose fermenting pink colonies, Non Lactose fermenting colorless colonies
	TBX	Blue colonies
Vibrio	TCBS	Large yellow colonies, Green colonies
Aeromonous	MacConkey	White/colorless colonies
Plesiomonas	MacConkey	White/colorless colonies
Yersinia	MacConkey	White/colorless colonies

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

- Laminer air flow cabinet (ESCO, Singapore)
- Petridish
- Autoclave (HIRAYAMA, Japan)
- Hot air oven (FN-500,Niive)

Agar

- Mackonkey agar
- XLD agar
- BGA agar
- TBX agar
- TCBS agar

Enrichment Broth:

- Trypticase Soya Broth (TSB)
- 0.3% Yeast extract (YE)
- Buffered Peptone Water (BPW)
- Alkaline Peptone Water (APW)
- Inoculating loop
- Spirit burner
- Hand gloves
- Mortar and pestle
- Incubator
- Measuring cylinder
- 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 (1inch) 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 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.

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 3.8: 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 tube
- Autoclave (HIRA YAMA, Japan)
- Hot Air Oven (FN-500, Niive)
- Straight wire
- Sprit burner

- Hand gloves
- Incubator
- Measuring cylinder

Oxidase Reagent

- Kovac's reagent

Agar

- Kligler's Iron agar
- MIO agar
- Christensen's urea agar
- Simmons citrate medium
- Analytical balance
- Media bottle

Figure 3.9: Bacterial colony on different media

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 (Simmions Citrate agar) test		Blue color	No change in color of media (Green color)
Urease test		Pink purple color	No change in color
Oxidase test		Blue color of colony (avoid blue color after 10 seconds)	No color change of colony
KIA	H2S	Black color	No black color
	Gas production	Bubble production	No bubble production

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 successively 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
- Distill water
- Burner
- Bacteria sample
- Nutrient agar
- Petri dish
- Water bath
- Alcohol
- Colony counter
- Conical Flask
- Labelling tape

3.3.1.4 Procedure

There are four major steps in the procedure:

1.Preparation of serial dilution.

2.Mixing the serial dilution into agar.

3.Counting the bacterial colonies.

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

3.3.1.5 Preparation of Serial Dilutions

1.A sample was taken counting the bacteria to be counted.

2.Four test tube were taken and labelled them.

3.Nine ml of distilled water was pipette into each of the tubes.

4.One gm of the undiluted sample was given into the tubes. The contents were mixed and using a new pipette.

5.This was continued until transfers had been completed for four times.

6.There for 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.

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 4.1.1: Bacterial colony morphology isolated from different hospital food samples.

Name of the hospital	Sample	Plates				
		Mac Conkey	TBX	XLD	BGA	TCBS
Bangabandhu Sheikh Mujib Medical Hospital	MosurDaal	No growth	Blue	No growth	No growth	No growth
	Puisakh	Dark pink	No growth	No growth	No growth	No growth
	Sandwich	Pink	Blue	No growth	No growth	No growth
Salauddin Hospital	Kabab	Pink	No growth	No growth	No growth	No growth
	Mayonase	Large pink	No growth	No growth	No growth	No growth
	Halwa	No growth	No growth	No growth	No growth	Yellow
Monowara Hospital	Sandwich	No growth	No growth	No growth	No growth	Yellow
	Bun	No growth	No growth	No growth	No growth	Yellow
Panpacific Hospital	Pizza	No growth	Blue	No growth	No growth	No growth
	Singara	No growth	No growth	No growth	No growth	Yellow
	Chicken patis	No growth	No growth	No growth	No growth	Yellow

Table 4.1.1: shows bacterial colony morphology isolated from different hospital food samples. Thirty-one food samples were collected from six different hospital canteens in Dhaka city. Of which, 18 samples show positive growth of our suspected organisms (*E. coli, Vibrio* species,*Aeromonous* species) and 13samples show no growth of organism.

Table 4.1.2: Bacterial colony morphology isolated from different hospital food samples.

Name of the hospital	Sample	Plates				
		Mac Conkey	**TBX**	**XLD**	**BGA**	**TCBS**
Kidhma Hospital	Begun vorta	No growth	No growth	No growth	No growth	Yellow
	Morichvorta	Colorless	No growth	No growth	No growth	Green
	Aloovorta	Colorless	No growth	No growth	No growth	No growth
	Singara	Colorless	No growth	No growth	No growth	No growth
Uttara cresent Hospital	Thai soup	No growth	No growth	No growth	No growth	Yellow
	Singara	No growth	No growth	No growth	No growth	Yellow
	Halwa	No growth	No growth	No growth	No growth	Yellow

Table 4.1.2: shows bacterial colony morphology isolated from different hospital food samples. Thirty-one food samples were collected from six different hospital canteens in Dhaka city. Of which, 18 samples show positive growth of our suspected organisms (*E. coli, Vibrio* species,*Aeromonous* species) and 13samples show no growth of organism.

Table 4.2: Number of food samples with growth of suspected organisms determined bycolony morphology (n=19)

Name of hospital	**No.of samples with +ve growth by *E.coli***	**No. of samples with +ve growth by *Vibrio* species.**	**No. of samples with +ve growth by *Aeromonas* species.**	**No. of samples with +ve growth by *Plesiomonus*species.**	**No. of samples with +ve growth by *Yersinia* species.**
Bangabandhu Sheikh Mujib Medical University	4	0	0	0	0
Salauddin Hospital	2	1	0	0	0
Monowara Hospital	1	2	0	0	0
Khidma Hospital	0	2	3	0	0
Panpacific Hospital	0	2	0	0	0
Uttara cresent Hospital	0	2	0	0	0

Table 4.2: shows number of food samples with growth of suspected organisms determined by colony morphology. From total 31 food samples, 19 (61.3%) samples were suspected to be contaminated with our targeted organisms (*E coli,Vibrio* species,and *Aeromonas* species). In total 19 samples, 7 (36.85%) samples weresuspected to be

contaminated with *E coli*, 9(47.37%) samples with *Vibrio,* 3(15.79%) samples with *Aeromonous* species.

4.2 Suspected organism from different biochemical test

Table 4.3: Identification of the suspected organism (*E. coli* species) from different biochemical tests.

Sample Name	**Plate**	**Colony Morphology**	**KIA**			**Citrate**	**MIO**			**Urease**	**Organism**
			Slunt/ butt	**gas**	**H_2S**		**Mortality**	**Indole**	**Ornithin**		
MosurDaal	TBX	Blue	A/A	+	-	-	-	Not done	-	-	
PuiShakh	MAC	Dark Pink	A/A	+	-	-	-	Not done	-	-	
Sandwich	TBX	Blue	A/A	+	-	-	+	Not done	-	-	
Sandwich	MAC	Pink	A/A	+	-	-	+	+	-	-	*E.coli*
Kabab	MAC	Pink	A/A	+	-	-	+	+	-	-	
Mayonase	MAC	Large Pink	A/A	+	-	-	+	+	-	-	
Pizza	TBX	Blue	A/A	+	-	-	-	+	-	-	

Table 4.3: shows identification of *E. coli* species from different biochemical test. Biochemical test results for the samples Mosur Daal, PuiShakh, Sandwich, Kabab, Mayonase, Pizzamatched with the standard results for *E. coli* species. So, we can say that the samples may contain the *E. coli* species.

4.3 Suspected organism from different biochemical test

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

Sample Name	**Plate**	**Colony Morphology**	**KIA**			**Citrate**	**MIO**			**Urease**	**Organism**
			Slunt/butt	**gas**	**H_2S**		**Mortality**	**Indole**	**Ornithin**		
Begun vorta	TCBS	Yellow	K/A	-	-	+	-	+	-	-	
Morichvorta	TCBS	Green	K/A	+	-	+	+	+	+	-	
Sandwich	TCBS	Yellow	K/A	-	-	+	-	+	-	-	
Bun	TCBS	Yellow	K/A	+	+	+	+	+	-	-	
Singara	TCBS	Yellow	K/A	+	-	+	+	+	-	-	*Vibrio* species
Chicken petis	TCBS	Yellow	K/A	+	+	+	+	+	-	-	
Thai soup	TCBS	Yellow	K/A	+	-	+	+	+	-	-	
Singara	TCBS	Yellow	K/A	+		+	+	+	-	-	
Halwa	TCBS	Yellow	K/A	+		+	-	-	-	-	

Table 4.4: shows identification of *Vibrio* species from different biochemical test Biochemical test results for the samples Begun vorta, Morichvorta, Sandwich, Bun, Singara, Halwa matched with the standard results for *Vibrio* species. So, we can say that the samples may contain the *Vibrio* species.

Table 4.5: Identification of the suspected organism *Aeromonas* speciesfrom different biochemical test.

Sample Name	**Plate**	**Colony Morphology**	**KIA**			**Citrate**	**MIO**			**Urease**	**Organism**
			Slunt /butt	**gas**	**H_2S**		**Mortality**	**Indole**	**Ornithin**		
Morich vorta	MAC	Colorless	K/A	-	+	+	+	+	-	-	*Aeromonas* species.
Singara	MAC	Colorless	K/A	+	+	+	+	+	-	-	
Aluvorta	MAC	Colorless	K/A	+	+	+	-	+	-	-	

Table 4.5: shows identification of *Aeromonasspecies* from different biochemical test. Biochemical test results for the samples Morichvorta, Singara, Aluvorta matched with the standard results for *Aeromonas* species. So, we can say that the samples may contain the *Aeromonas* species.

4.4 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 4.6.1 Colony counting of various hospitals food samples

Hospital name	Sample name	Dilution 1	Dilution 2	Dilution 3	Dilution 4
;Bangabandhu Sheikh Mujib Medical University	MosurDaal	Uncountable	Uncountable	70	50
	Sandwich	Uncountable	Uncountable	63	30
	Puishakh	Uncountable	Uncountable	Uncountable	70
	Porota	Uncountable	Uncountable	Uncountable	Uncountable
	Chicken curry	Uncountable	Uncountable	30	25
	Vaji	Uncountable	Uncountable	Uncountable	Uncountable
Salauddin	Kabab	Uncountable	Uncountable	Uncountable	200
	Halwa	Uncountable	Uncountable	Uncountable	Uncountable
	Mayonase	60	40	20	4
Panpacific Hospital	Chicken petis	Uncountable	Uncountable	Uncountable	Uncountable
	Singara	Uncountable	Uncountable	Uncountable	Uncountable
	Pizza	Uncountable	Uncountable	Uncountable	Uncountable
	Sandwich	Uncountable	Uncountable	Uncountable	84
	Chicken fry	Uncountable	Uncountable	38	2
	Bun	Uncountable	Uncountable	Uncountable	Uncountable

Table 4.6.1: shows Colony counting of various hospital canteen food samples. For MosurDaal, 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.

Table 4.6.2 Colony counting of various hospitals food samples

Hospital name	Sample name	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Khidma Hospital	Aluvorta	Uncountable	Uncountable	Uncountable	280
	Morichvorta	Uncountable	Uncountable	Uncountable	Uncountable
	Begun vorta	Uncountable	Uncountable	Uncountable	Uncountable
	Dim vorta	Uncountable	Uncountable	Uncountable	Uncountable
Monowara Hospital	Sandwich	Uncountable	Uncountable	Uncountable	Uncountable
	Bun	Uncountable	Uncountable	Uncountable	Uncountable
	Cake	Uncountable	Uncountable	Uncountable	Uncountable
Peoples Hospital	Singara	Uncountable	Uncountable	Uncountable	Uncountable
	Somucha	Uncountable	Uncountable	Uncountable	Uncountable
Uttara Cresent Hospital	Thai soup	Uncountable	Uncountable	Uncountable	Uncountable
	Singara	Uncountable	Uncountable	Uncountable	Uncountable
	Sauce	Uncountable	Uncountable	Uncountable	Uncountable
	Soup	Uncountable	Uncountable	Uncountable	Uncountable
	Daal	Uncountable	Uncountable	Uncountable	Uncountable

Table 4.6.2: shows Colony counting of various hospital canteen food samples. For Aluvorta, 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 street food items.

Table 4.7: Number of Colony Forming Unit (CFU) per ml of hospital food sample

Samples	No. of CFU per ml
MosurDaal	7.0×10^4
Sandwich	6.3×10^4
Puisakh	7.0×10^5
Chicken curry	3.0×10^4
Kabab	2.0×10^6
Mayonase	6.0×10^2
Chicken fry	3.8×10^4
Aluvorta	2.8×10^5

Chapter 5
Discussion & Conclusion

5.1 Discussion & conclusion

Prevention of foodborne infection in healthcare settings is essential. Most of the foodborne out-breaks in healthcare settings could have been prevented if good hygienic practice and HACCP principles had been followed. Food safety policy in a hospital should involve persons such as a consultant in communicable disease control, control of infection officer and EHOs as well as the catering management. The policy should include commitment to good hygienic practice, an HACCP system, and procedures to ensure that suppliers of food and water have satisfactory food safety policies (Lund and O'Brien 2009).

The present research work was therefore carried out to find out the presence of enteric bacteria specially *E. coli, Salmonella, Shigella*, *Vibrio*speciesand*Aeromonas*species from different types of hospital canteen foods collected from different private and public 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 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 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 was hot which prevented growth of bacteria.

In this study, 31 different food samples were tested. Among them, 19 (61.3%) samples were suspected to be contaminated with our targeted organisms (*E coli,Vibrio* species,and *Aeromonas*species). In total 19 samples, 7 (36.85%) samples weresuspected to be contaminated with *E coli*, 9(47.37%) samples with *Vibrio,* 3(15.79%) samples with *Aeromonas*species.

A study was conducted to assess bacterial hand contamination and associated factors among food handlers working in the student cafeterias of Jimma university at south west Ethopia from May 2012 to April 2013.The study revealed that 114(49.6%) carriage of potential food borne bacterial hand contaminants and 73(31.7%) were tested positive for

enteric bacterial hand contaminants. The following food borne bacterial hand contaminants were isolated with the corresponding prevalence rate: *S*. aureus 54(23.5%), *Klebsiella*spp. 37(16.1%), *E. coli* 25(10.9%), *Enterobacter*spp. 21(9.1%), *Citrobacter*spp. 10(4.3%), *Serratiamarcescens* 6(2.6%), *Pseudomonasaeruginosa* 8(3.5%), *Proteus*spp. 5(2.2%), *Providenciarettegri* 3(1.3), and *salmonella*spp.2(0.9%) (Assefa, et al. 2015). Another study was conducted in Amravati, India. Forty water sample of panipuri were aseptically collected from eleven locations of Amravati City. Analysis of the food samples revealed that 93% of panipuri water samples had high loads of bacterial pathogens such as *Escherichia coli* (41%), *Staphylococcus aureus* (31%), *Klebsiella*spp. (20%), *Pseudomonas*spp. (5%) and yeast (3%). It is suggested that regular monitoring of the quality of street foods must be practiced to avoid any food-borne infection in future (Tambeker, et al. 2011).

In developing country like Bangladesh, hospital foods are causing serious health hazards due to microbial contamination. This health hazards are also due to the lack of knowledge on food safety and hygiene among the people who prepare the hospital food, handle all the system including equipment, utensils and also the person who is serving the food to individual patients. To get rid of these hazards it is suggested that regular monitoring should be done for observing the quality, hygiene, and also maintaining suitable environment for preparing foods for hospitalized patients to avoid food-borne infection. The government can play a vital role by providing appropriate knowledge about food safety, quality and hygiene standards of hospital food. Before these hazards become an epidemic, more focuses should be given in this sector and more research work should be carried out in developing countries like Bangladesh to search out the quality, hygiene and safety of hospital food.

Chapter 6
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6.1 References

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