Study on Bacteriological Quality of FoodsCollected 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

Jeba Ahmed
ID: 2013-1-73-001

Department of Pharmacy

East West University

Dhaka, Bangladesh

Declaration by the Candidate

I, Jeba Ahmed hereby declare that the dissertation entitled "Study on Bacteriological Quality of Foods Collected from Different Hospitals in Dhaka City" submitted by me to the Department of Pharmacy, East West University and in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, work carried out by me during the period 2013-1-73-001 of my research in the Department of Pharmacy, East West University, under the supervision and guidance of Dr. Sufia Islam, Professor, 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.

....

Jeba Ahmed

ID: 2013-1-73-001

Department of Pharmacy

East West University, Dhaka, Bangladesh

Certificate by the Supervisor

This is to certify that the thesis entitled "Study on Bacteriological Quality of Foods Collected from different Hospitals in Dhaka City " 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 Jeba Ahmed 2013-1-73-001, during the period 2016-2017 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.

....

Dr. Sufia Islam
Professor
Department of Pharmacy
East West University, Dhaka, Bangladesh

Certificate by the Co-Supervisor

This is to certify that the thesis entitled "Study on Bacteriological Quality of Foods Collected from Different hospitals in Dhaka City " 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 Jeba Ahmed 2013-1-73-001, during the period 2016-2017 of her research in the Department of Pharmacy, East West University, under the supervision and guidance of Nafisa Tanjia, Senior Lecture, Department of Pharmacy, East West University. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

____

Nafisa Tanjia

Senior Lecture
Department of Pharmacy
East West University, Dhaka, Bangladesh

Certificate by the Chairperson

This is to certify that the thesis entitled "Study on Bacteriological Quality of Foods Collected from Different Hospitals in Dhaka City " 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 Jeba Ahmed, ID: 2013-1-73-001, during the period 2016-2017 of his research in the Department of Pharmacy, East West University.

____

Dr. Chowdhury Faiz Hossain

Professor and Chairperson

Department of Pharmacy

East West University, Dhaka, Bangladesh

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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

Hospital should provide a nutritious diet for the treatment and recovery of patients Therefore, the food must be safe, good quality, wholesome, and should be served at times that are convenient and appropriate for the patients. Food may be a source of contamination in a hospital which is often overlooked. The patient can be more vulnerable if the foods are not free of contamination. Prevention of foodborne infection in healthcare settings is essential. The present research work was therefore untaken to find out the presence of enteric bacteria specially E. coli,*Aeromonas Species*and*Vibrio* species from different types of fooditems collected from different hospitals 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 total 30 food samples we have found the presence of enteric bacteria E.coli, Vibrio, and *Aeromonas Species*in 17 (56.6%) food samples. All these enteric pathogens could be the potential cause for further illness of the patients in hospitals. Therefore, hospital authority should take necessary steps to prevent the unhygienic hospital food items. Along with the management of the hospital, other health professionals such as doctors, pharmacists, nurses should take the initiative for preventing contamination of the hospital food items to ensure health safety.

Keywords: Hospital foods, Public health risk, Enteric bacteria,Biochemical test, E.coli, Vibrio,*Aeromonas Species.*

Chapter -01

Introduction and Literature Review

1.Hospital Foods :

Patients need a nutritious diet for the treatment and recovery. Therefore, the food must be safe, good quality, wholesome, and should be served at times that are convenient and appropriate for the patients. Conventional mealtimes are not always best for the patients for consumption of foods. 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 hospital meals or foods are an essential part of their overall care for recovery. In many hospitals meals are prepared and cooked in the hospital kitchen and distributed directly to the wards. Most of the food served in hospitals affects not only the heath of the patients, their visitors and employees but also broader, community, society, and the environment. 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, i.e. not only at conventional mealtimes. As Hippocrates said "lets food be thy medicine and medicine be thy food ". Hospital saves lives in time of emergencies. but they're also meant to be healing institutions that teach patients how to take better care of themselves once they check out. Unfortunately, just like it can be confusing how to eat out, hospital staff don't seem to understand how to feed you or your loved ones when you check in.So, doctors and patients also should be careful and cautious in purchasing foods to ensure their own health safety.

Figure 1.1: Hospital Foods

1.2.Types of hospital foods:

There are so many hospitals in Dhaka city .Include the cafeterias are surved 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.

The following diets are used in hospital:-

- Normal diet
- clear liquid diet
- full liquid diet
- low salt diet
- low-fat diet
- in anemia
- renal diet
- GI soft diet
- NPO (nil per oral)

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

1.2.2.Meals are served at the following times:

Breakfast	6:30am – 9:30am
Lunch	11:30am – 2:30pm
Dinner	4:30pm – 8:00pm

1.3.Maintaining Hygiene and Safety:

Hospital foods must be safe and hygienic to consume as it is an essential part of patient's recovery process. 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). 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).

1.4.How to contaminate the hospital foods:

1.4.1.Handling of Foods:

Unhygienic management of hospital foods by various number of seller has been normally identified to be the source of pollution. The sellers can be transporters of pathogens such as *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter* as well as *S. aureus*who ultimately handover these food borne dangers to the clients. The hands of the food sellers are the most vital vehicle for the transmission of organisms from different body parts such as faces, nose, and skin to the food. The findings from investigation is that Salmonella, non-typhi salmonellae, *Campylobacter* and *E. coli* can persist on fingertip.

1.4.2.Bacterial contamination on hands of hospital food handlers:

When using the toilet, employee might contaminate his hands or bacteria might be spread from raw. Microorganisms on human skin can be divided into two groups, permanent and transitory.There are some Coagulase-negative staphylococci (an CNS), diphtheroid bacilli (Corynebacterium, Propionibacterium strains), some types of Acinetobacter, and some members of Enterobacteriaceae are in the permanent bacteria group (Garner &Favero, 1986; Lowbury, Lilly, & Bull, 1964; Miller, 1994). The only pathogen microorganism in the permanent bacteria group of the human skin is Staphylococcus aureus(Lowbury et al., 1964). Nearly all the causative microorganisms of infectious diseases belong to transient group (Fuerst, 1983; Snyder, 1994). The transient microorganisms found on hands vary significantly according to the surfaces contacted, and that there are microorganisms characteristic for skin, respiratory system, stool, and peri-anal region (Fuerst, 1983). The hands of food service employees can be vectors in the spread of foodborne disease because of poor personal hygiene or cross-contamination. For example, meat to salad greens by food handlers hands.

1.5.Food-borne Illness

Food-borne illness, also called "food-borne disease," "food-borne infection," or "food poisoning, is a common, costly but preventable public health problem. Each year, 1 in 6 Americans gets sick by consuming contaminated foods or beverages. Many different disease-causing microbes, or pathogens, can contaminate foods, so there are many different food-borne infections. In addition, poisonous chemicals, or other harmful substances can cause food-borne diseases if they are present in food.

The Centers for Disease Control and Prevention (CDC) estimates that 48 million food borne illness cases occur in the United States every year. At least 128,000 Americans are hospitalized, and 3,000 die after eating contaminated food each year. Food-borne illness costs Americans billions of dollars each year, and serves as a constant challenge for consumers, researchers, government and industry (Centers for Disease Control and Prevention [CDC] 2015).

1.6.TYPES OF FOODBORNE ILLNESS

The term foodborne 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. Foodborne illness needs to be distinguished from coincidental onset of symptoms while a person is eating, or noncausal food-associated illness. Foodborne illness may be the result of bacterial, viral, or parasitic contamination, or noninfectious toxins such as ciguatera. Illness can also be the result of toxins produced by bacterial contamination (eg, botulism). Although foodborne illness from infectious contamination is usually the result of improper food preparation or handling practices, bacterial contamination has also been commonly observed. Foreign body contamination or other non–foreign body contaminants (eg, chemicals) can be introduced inadvertently or deliberately, based on a desire to substitute a less expensive compound, circumvent a regulatory restriction or standard, or to cause harm. State public health laboratories have increased capabilities and capacities to address these issues through the Food Emergency Response Network (FERN).

1.7.Major Source of Foodborne Illness:

A. Bacteria

Bacteria are minute organisms that can cause infections of the GI tract. All bacteria are not destructive to humans. Some destructive bacteria may already be present in foods when they are bought. Raw foods including meat, poultry, fish and shellfish, eggs, unpasteurized milk and dairy products, and fresh produce often contain bacteria causing foodborne illnesses. Bacteria can contaminate food, making it destructive to eat during growth, harvesting or slaughter, processing, storage, and delivery. Foods may also be contaminated with bacteria during food preparation in a restaurant or home kitchen. If food preparers do not thoroughly wash their hands, kitchen utensils, cutting boards, and other kitchen surfaces that come into contact with raw foods, cross-contamination may occur. If hot food is not kept hot enough or cold food is not kept cold enough, bacteria may multiply. Bacteria multiply quickly when the temperature of food is between 40 and 140 degrees. Cold food should be kept below 40 degrees and hot food should be kept above 140 degrees. Bacteria multiply more slowly when food is refrigerated, and freezing food can further slow or even stop the spread of bacteria.

However, bacteria in refrigerated or frozen foods become active again when food is brought to room temperature. Systematically cooking food kills bacteria.

Many types of bacteria cause foodborne illnesses. Examples include

- *Salmonella*, a bacterium found in many foods, including raw and undercooked meat, poultry, dairy products, and seafood. *Salmonella* may also be present on egg shells and inside eggs.

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

- *Shigella,* a bacterium spread from person to person. These bacteria are present in the stools of people who are infected. If people who are infected do not wash their hands thoroughly after using the bathroom, they can contaminate food that they handle or prepare. Water contaminated with infected stools can also contaminate produce in the field.
- *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.

- *Listeria monocytogenes (L. monocytogenes),* which has been found in raw and undercooked meats, unpasteurized milk, soft cheeses, and ready-to-eat deli meats and hot dogs .

- *Vibrio,* a bacterium that may contaminate fish or shellfish.

- *Clostridium botulinum (C. botulinum),* a bacterium that may contaminate improperly canned foods and smoked and salted fish.

B. Viruses

Viruses are minute organism, much slighter than bacteria, contain genetic material. Viruses cause infections, leading to sickness. People can pass viruses to each other. Viruses are remain 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 foodborne viruses include food

 prepared by a person infected with a virus shellfish from contaminated water produce

 irrigated with contaminated water

Common foodborne viruses include noroviru

 which causes inflammation of the stomach and intestines hepatitis

 A, which causes inflammation of the liver

C. Parasites

Parasites are minute organisms, living inside another organism. *Cryptosporidium parvum*and *Giardia intestinalis*are parasites which spread through water contaminated with the stools of people or animals who are infected. Foods that come into contact with contaminated water during growth or preparation can become contaminated with these parasites. Food preparers who are infected with these parasites can also contaminate foods if they do not thoroughly wash their hands after using the bathroom and before handling food. Example: *Trichinellaspiralis*is a type of roundworm parasite.

D. Chemicals

Harmful chemicals that cause illness may contaminate foods such as Fish orshellfish, which may feed on algae that produce toxins, leading to high concentrations of toxins in their bodies. Some types of fish, including tuna and mahimahi, may be contaminated with bacteria that produce toxins if the fish are not properly refrigerated before they are cooked or served. Certain types of wild mushrooms.Unwashedfruits and vegetables that contain high concentrations of pesticides .

1.8.Factors Affecting Growth of Microorganisms

1.8.1. Intrinsic Parameters

These parameters are as follows:

Moisture

 content

 Oxidation-reduction potential (Eh) Nutrient

 content (water, source of energy, source of nitrogen, vitamins and related growth factors, minerals)
 Antimicrobial constituents.

1.8.2. Extrinsic Parameters

The extrinsic parameters of foods are those belongings of the storage environment that affect both the foods and their microorganisms. Those of greatest importance to the welfare of food-borne organisms are as follows:
temperature of storage
relative
 humidity of environment Presence
 and concentration of gases Presence
 and activities of other microorganisms

1.8.3. Implicit Factors

A third set of factors that are vital in determining the nature of microbial associations found in foods are designated as implicit factors, belongings of the organisms themselves, how they react to their environment and interrelate with one another. An organism's specific growth rate can determine its importance in a food's microflora; those with the highest specific growth rate are probable to rule over time. This will depend upon the situations prevailing; many moulds can grow properly well on fresh foods such as meat, but they grow more slowly than bacteria and are out-competed. (Adams & Moss, 2008).
The food processor decreases potential problems from microorganisms in several ways:

1.9.Bacterial Agents of Food-borne Illness :

1.9.1)*Salmonella* species :

Salmonella is a significant bacterial genus, originating one of the most common forms of food poisoning worldwide. It is one of the most broadly 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 chief cause of bacterial gastroenteritis. *Salmonella*

is capable of causing a variety of disease syndromes: enteric fever, bacteremia, enterocolitis, and focal infections (Darwin, 1999).

Figure 1.3: ***Salmonella*** ***s*pp**

i. Microbiological Characteristics:

Salmonella is a rod-shaped, motile, aerobic and facultative anaerobe, non-spore forming and gram-negative organism. It can grow from 5°C up to 47°C, with an optimum temperature of 37°C. *Salmonella* is heat sensitive and can be readily destroyed at pasteurization temperature. *Salmonella* is a general name used for a group of more than 2,000 closely related bacteria that cause illness by reproducing in the digestive tract. Each *Salmonella* serotype shares common antigens and has its own name; *Salmonella enteritidis*was the commonest serotype isolated from human clinical specimens (Bayu et al., 2013)

ii. Pathogenesis and Clinical Features :

Generalized systemic enteric fever, headache, malaise, anorexia, enlarged spleen, and constipation followed by more severe abdominal symptoms; rose spots on trunk in 25% of Caucasian patients; complications include ulceration of Peyer's patches in ileum, can produce hemorrhage or perforation; Common enterocolitis may result without enteric fever; characterized by headache, abdominal pain, nausea, vomiting, diarrhea, dehydration may result; case fatality of 16% reduced to 1% with antibiotic therapy (Adams & Moss, 2008).

iii. 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. 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 carriers are generally less important than animals in the transmission of salmonellosis. 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, 2008)

Typical Symptoms of *Salmonella* Infection:

- Generalized systemic enteric fever
- Headache
- Malaise
- Anorexia
- Enlarged spleen and
- Constipation followed by more severe abdominal symptoms

1.9.2.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 microflora. Strains of *E. coli* were first recognized as a cause of gastroenteritis by workers in England investigating summer diarrhoea in infants in the early 1940s. Until 1982, strains producing diarrhoea were classified into three types based on their virulence properties: enteropathogenic*E. coli* (EPEC), enteroinvasive*E. coli* (EIEC), and enterotoxigenic*E. coli* (ETEC). They are not very common causes of food-borne illness in developed countries, but and important cause of childhood diarrhoea in less developed countries (Adams & Moss, 2008)

Fig1.4: ***Escherichia spp***

i. 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. Genetically, E. coli is very closely related to the genus Shigella, although characteristically it ferments the sugar lactose and is otherwise far more active biochemically than Shigella spp. Late lactose fermenting, non-motile, biochemically inert strains of E. coli can however be difficult to distinguish from Shigella. E. coli can be differentiated from other members of the Enterobacteriaceae on the basis of a number of sugar-fermentation and other biochemical tests (Adams & Moss, 2008).

ii. Pathogenesis 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 a mild afebrile diarrhoea to a severe choleralike syndrome of watery stools without blood or mucus, stomach pains and vomiting. The illness is usually self-limiting, persisting for 2–3 days, although in developing countries it is a common cause of infantile diarrhoea where it can cause serious dehydration.

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 diarrhoea which precedes the passage of stools containingblood, mucus, and faecal leukocytes. The infective dose of EIEC appearhigher than for *Shigella*and this is thought to be a reflection of the organism's greater sensitivity to gastric acidity.

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* diarrhoea in the 1950s. 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 diarrhoea. *E. coli* O157:H7 is the most common EHEC serotype reported, although others do occur. EHEC has attracted attention not only because foodborne transmission is more common than with other diarrhoeagenic*E. coli,* but because the illness it causes can range from a non-bloody diarrhoea, through haemorrhagic colitis, to the life threatening conditions haemolyticuraemic syndrome (HUS) and thrombotic thrombocytopaenicpurpura (TTP) (Adams & Moss, 2008).

iii. Isolation and Identification

Selective techniques for *E. coli* mostly exploit the organism's tolerance of bile and other surfactive 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 it is 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 pink colonies; non-lactose fermenters such as *Salmonella, Proteus,* and *Edwardsiella,* with rare exceptions produce colourless colonies (Adams & Moss, 2008

iv. Association with Foods

Faecal contamination of water supplies and contaminated food handlers have 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. Outbreaks caused by EHEC serotype O157:H7 have mostly involved undercooked ground meat products and occasionally raw milk. Cattle seem to be an important reservoir of infection and O157:H7 has been isolated from 0.9–8.2% of healthy cattle in the UK (Adams & Moss, 2008).

Symptoms of *Escherichia coli* Infection:

- Nausea.
- Vomiting.
- Stomach cramps.
- Diarrhea that often is bloody.
- Fever of about 100 F to 101 F (37.7 C to 38.3 C)
- Malaise.
- Loss of appetite.
- Mild dehydration.

1.9.3) *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 in 19th century. It was Robert Koch who firmly established the causal link between *Vibrio cholerae*and cholera when working in Egypt in 1886.

Figure1.4: ***Vibrio*****Spaecies**

i. 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. The optimum level for the growth of clinically important species is 1–3%.

ii. 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 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. In severe cases, the hyper-secretion of sodium, potassium, chloride, and bicarbonate induced by the enterotoxin results in a profuse, pale, watery diarrhoea containing flakes of mucus, described as rice water stools. Unless the massive losses of fluid and electrolyte are replaced, there is a fall in blood volume and pressure, an increase in blood viscosity, renal failure, and circulatory collapse. In fatal cases death occurs within a few days. In untreated outbreaks the death rate is about 30–50% but can be reduced to less than 1% with prompt treatment by intravenous or oral rehydration using an electrolyte/glucose solution (Adams & Moss, 2008).

iii. 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. Tellurite/bile salt broth (pH 9.0 –9.2) is a more selective enrichment medium and can be incubated overnight. 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. cholerae*produces yellow colonies. Individual species can then be differentiated on the basis of further biochemical tests (Adams & Moss, 2008).

iv. 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. Foods coming from a contaminated environment may also carry the organism, for example sea foods and frog's legs. In the current pandemic in South and Central America, an uncooked fish marinade, in lime or lemon juice, ceviche has been associated with some cases (Adams & Moss, 2008)

Typical Symptoms *Vibrio* of Infection

- Watery diarrhea.
- Abdominal cramps.
- Nausea.
- Vomiting.
- Fever.

1.9.4.*Aeromonas Species*:

Aeromonas (principally *A. hydrophila*, but also *A. Caviae* and *A. sobria*) has the status of a foodborne 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. It was first isolated from drinking water by Zimmerman in 1890 and the following year from frog's blood by Sanarelli. They called their isolates *Bacillus punctata* and *Bacillus hydrophilus* respectively and it was not until the 1930s that the genus *Aeromonas* was first described. Although the taxonomy is still not settled, more recent studies have led to the recognition of two major groups within the genus: the Salmonicida group, which contains the non-motile *Aeromonassalmonicida* and several sub-species, and the Hydrophila–Punctata group containing a number of motile species, including *A. hydrophila A. sobria, and A. caviae.*(Adams & Moss, 2008)**.**

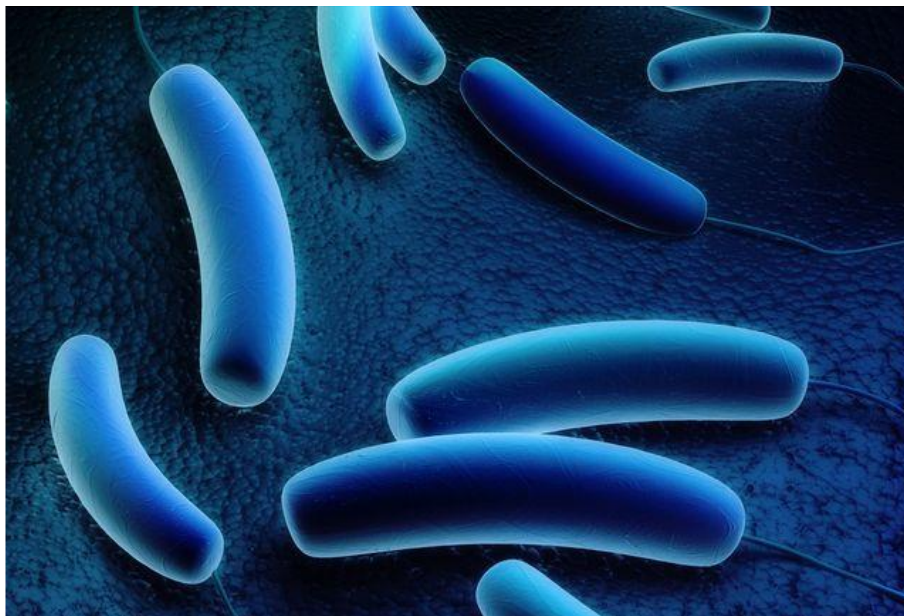

Figure1.5:Aeromonas Species

Pathogenesis:

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. Most clinical strains of A. hydrophila and A. sobria produce aerolysin, a heat-labile,b-haemolytic, cytotoxic enterotoxin with a molecular mass of 52 kDa. Three cytotonicenterotoxins have also been described which act like cholera toxin, stimulating accumulation of high levels of cAMP within epithelial cells. Only one of these shows any marked structural similarity to cholera toxi*n as* measured by cross reactivity with cholera toxin antibodies. (Adams & Moss, 2008)

Typical Symptoms Aeromonas Infection*:*

- *traveler's diarrhea,*
- *watery diarrhea,*

1.9.5.*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.

1.9.5.1 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.

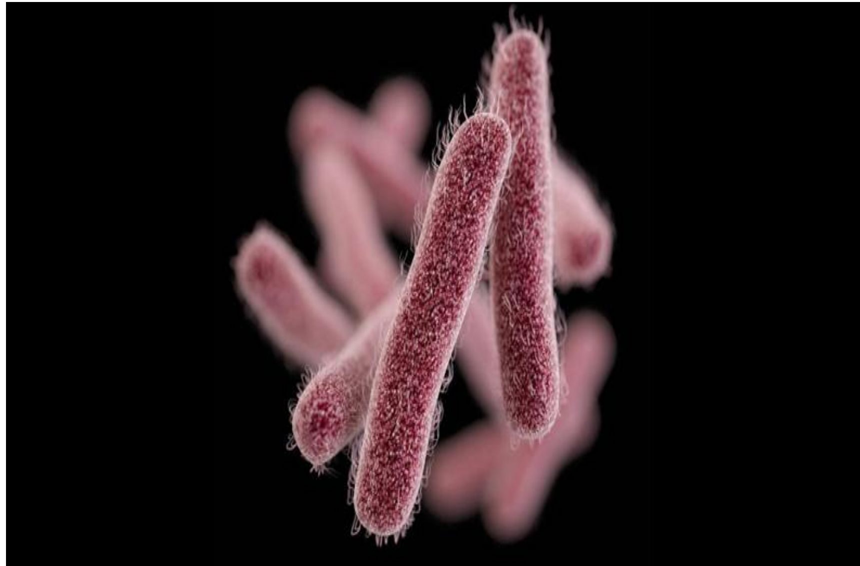

Figure*1.6:Shigella*species

1.9.5.2.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.

1.9.5.3 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.

1.9.5.4 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, 2008).

Chapter 2

Objective of the Study

2.1 Research objective

The objective of this research work was to isolate and identify the presence of enteric bacteria especially *E.coli, Aeromonas Species* and *Vibrio* species from different types of hospital fooditems collected from different hospitals of Dhaka city, Bangladesh.

Chapter-3

Methodology

3.1.1 Sample Collection

About 30 food samples were randomly chosen and collected from different hospital canteens 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 30 samples are tested for the identification of enteric bacteria especially *E col*i, *Vibrio* species, *Aeromonas* species.

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 Organism

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 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

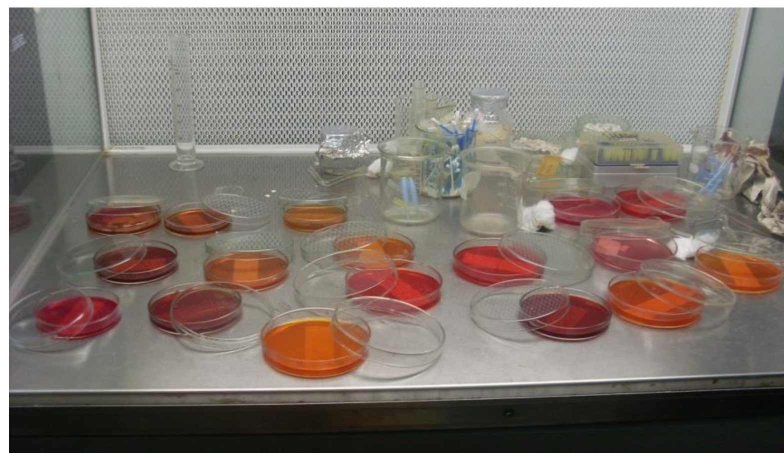

Figure 3.4: Petri dishes preparation

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:

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

Enrichment Broth:

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

3.2 Biochemical Tests

3.2.1 Kliglar Iron Agar Test (KIA Test)

3.2.1.1 Test Tube Preparation for KIA Test

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

3.2.1.2 Inoculation for KIA Test

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

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.

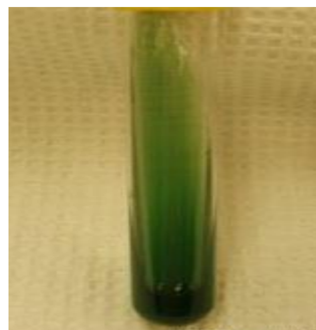

Figure 3.8: Preparation of test tubes for Citrate test

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
- Analytical balance
- 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

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 colonyforming units (CFUs) in our sample – i.e., the number of CFUs per ml or per gram of the sample. More realistically, the concentration of CFUs in the sample could have been considerably greater. Counting the colonies on a plate inoculated with one ml of sample may be impossible. We would like to have "countable" plates – containing between 30 and 300 colonies. If fewer than 30, we run into greater statistical inaccuracy. If greater than 300, the colonies would be tedious to count and also would tend to run together. So we now get into "dilution theory" to accomplish the equivalent of plating out succeedingly smaller amounts of sample. Making serial decimal dilutions (i.e., successive 1/10 dilutions, each made by adding one part of inoculum to 9 parts of diluent) and inoculating one ml into each of the plates, we can construct a plating procedure that is equivalent to the above.

3.3.1.3 Materials Required

- Tubes
- Micropipette with tips
- Distilled water
- Bacteria sample
- Nutrient agar
- Petri dishes
- Water bath
- 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.

Tubes	Dilution	Dilution	Dilution factor
	10^{-1}	1/10	10^{1}
	10^{-2}	1/100	10^{2}
	10^{-3}	1/1,000	10^{3}
	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
Results

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**
Panpacific hospital	Somucha	No growth	Isolated blue	No growth	No growth	No growth
	Porota	No growth	Isolated blue	No growth	No growth	No growth
	Daal	No growth	Isolated blue	No growth	No growth	No growth
	Mustard Sauce	No growth	Blue	No growth	No growth	No growth
	Vaji	No growth	No growth	Yellow	No growth	No growth
Salahuddin hospital	Porota	No growth	No growth	Yellow	No growth	No growth
	Daal	Colorless	No growth	Yellow	No growth	No growth
	Vaji	Colorless	No growth	Yellow	No growth	No growth
	Burger	Colorless	No growth	Yellow	No growth	No growth
	Sauce	No growth	No growth	No growth	No growth	No growth
Uttara crescent hospital	Clear soup	No growth	Blue	yellow	No growth	No growth
	Dim vaji	No growth	No growth	Yellow	No growth	No growth
	Porota	Colorless	No growth	Yellow	No growth	No growth

Peoples hospitals	vaji	No growth	No growth	No growth	No growth	No growth
	daal	No growth	No growth	No growth	No growth	No growth
	Sauce	No growth	No growth	No growth	No growth	No growth
	kabab	No growth	No growth	No growth	No growth	No growth
	bun	No growth	No growth	No growth	No growth	No growth
	vaji	No growth	No growth	No growth	No growth	No growth
BSMMU	Singara	No growth	No growth	No growth	No growth	No growth
	halwa	No growth	No growth	No growth	No growth	No growth
	somucha	No growth	No growth	No growth	No growth	No growth
	sabji	No growth	No growth	No growth	No growth	No growth
	daal	No growth	No growth	No growth	No growth	No growth
Khidma hospital	daaal	No growth	No growth	No growth	No growth	No growth
	Sauce	No growth	No growth	No growth	No growth	No growth
	vaji	No growth	No growth	No growth	No growth	No growth
	porota	No growth	No growth	No growth	No growth	No growth
Monowara hospital	naan	No growth	No growth	No growth	No growth	No growth
	Kabab	No growth	No growth	No growth	No growth	No growth
Peoples hospitals	vaji	No growth	No growth	No growth	No growth	No growth
	daal	No growth	No growth	No growth	No growth	No growth
	Sauce	No growth	No growth	No growth	No growth	No growth

Table4.1 Shows bacterial colony morphology isolated from different food samples. Thirty food samples were collected from seven different hospital canteens in Dhaka city. Of which, 17 samples show positive growth of our suspected organisms (*E.coli, Vibrio* spp., *Aeromonous* spp.) and 13samples show no growth in these agar media.

Table4.2: Number of food samples with growth of suspected organisms determined bycolony morphology (n=17).

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.***
Salahudin hospital	0	4	4	0	0
Uttaracrescent hospital	1	3	0	0	0
Panpacific hospital	4	1	0	0	0

Table4.2: Shows number of food samples with growth of suspected organisms determined by colony morphology. From total 30food samples, 17 (56.677%) samples were suspected to be contaminated with our targeted organisms (*E coli,Vibrio* species,and *Aeromonus*species). In total 17samples, 5(29.05%) samples weresuspected to be contaminated with *E coli*, 8(47.25%) with *Vibrio,*4 (23.52%) with *Aeromonous*species.

Suspected organism from different biochemical test:

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

Sample name	Plates	Colony-morphology	KIA			Citrate	MIO			Urease	Organism
			Slunt/Butt	H_2S	Gas		Mortality	Indole	Ornithine		
samucha	TBX	Isolated blue	A/A	-	+	+	+	+	-	-	*E.Coli*
porota	TBX	Isolated Blue	A/A	-	+	+	-	+	-	-	
daal	TBX	Isolated blue	A/A	-	+	+	-	+	-	-	
sauce	TBX	Blue	A/A	-	+	+	-	+	-	-	
Clear soup	TBX	blue	A/A	-	+	+	+	+	-	-	

Table 4.3: Shows identification of *E.coli* species from different biochemical test. Biochemical test results for the samplesSamucha ,daal , sauce, porota, clear soup are matched with the standard results for *E.coli*species. Therefore, the samples may contain the *E.coli* species.

Table4.4: Identification of the suspected organism (*Vibrio*species) from differentbiochemical tests.

Sample name	Plates	Colony morphology	KIA			Citrate	MIO			Urease	Organism
			Slunt/ Butt	H_2S	Gas		Mortality	Indole	Ornithine		
vaji	TCBS	Green	K/A	-	+	+	+	+	-	-	*Vibrio* sp.
porota	TCBS	Yellow	K/A	-	+	+	+	+	-	-	
daal	TCBS	Yellow	K/A	+	-	+	+	+	-	-	
Burgur	TCBS	Yellow	K/A	-	+	+	-	+	-	-	
Clear soup	TCBS	Yellow	K/A	-	+	+	+	+	-	-	
Dim vaji	TCBS	Yellow	K/A	-	+	+	+	-	-	-	
porota	TCBS	Yellow	K/A	-	+	+	+	+	-	-	
vaji	TCBS	Yellow	K/A	-	+	+	+	-	-	-	

Table 4.4: Shows identification of *Vibrio* species from different biochemical test. Biochemical test results for the samplesporota, daal, burger, clear soup, dim vaji, porota, vajimatched with the standard results for *Vibrio* species. Therefore, the samples may contain the *Vibrio* species.

Table4.5: Identification of the suspected organism *Aeromonus* species from different biochemical tests.

Sample name	Plates	Colony morphology	KIA			Citrate	MIO			Urease	Organism
			Slunt/ Butt	H_2S	Gas		Mortality	Indole	Ornithine		
Daal	MAC	Colorless	K/A	+	+	+	+	+	-	-	*Aeromonus* spp.
vaji	MAC	Colorless	A/A	-	+	+	+	-	-	-	
burger	MAC	Colorless	K/K	-	+	+	+	-	-	-	
porota	MAC	colorless	K/A	-	+	+	-	+	-	-	

Table 4.5: Shows identification of *Aeromonous*species from different biochemical test. Biochemical test results for the samples daal ,vaji, burger ,porotamatched with the standard results for *Aeromonous.*Therefore,the samples may becontained the Aeromonous species.

Table 4.6: Presence of suspected organisms in selected food samples after biochemicaltest (n=17)

Name of the hospitals	*E.coli*	*Vibrio spp.*	*Aeromonus spp.*
Panpacific hospital	4	1	0
Uttara crescent hospital	1	3	0
Salahuddin hospital	0	4	4

Table 4.6: shows presence of suspected organisms in selected food samples after biochemical test. From the results of biochemical test we found 17 of our suspected bacteria.we got1*E.coli*, 3*Vibrio*; fromUttaracrescent hospital; 4*E.coli*, 1*Vibrio from panpacific hospital;*4*Vibrio* , *4aermonus* from salahuddin hospital*; In* total we got 5 (29.677%) *E.coli*, 8(47.05%) *Vibrio*, 4 (23.50%) *Aeromonus spp*.

Table 4.7: Presence of food borne pathogens in various street-vended and expired foodsamples (n=30)

Pathogen	**Food categories**								Total food items (n=30)
	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	2 (16%)	1 (14.5%)	Nd	1(33.5%)	Nd	1(100%)	Nd	Nd	5 (29.67%)
Vibrio spp.	3(25%)	3 (42.2%)	1(100%)	1(33.2%)	Nd	Nd	Nd	Nd	8 (47.05%)
Aeromonus spp.	1(8.6%)	2 (28.5%)	1 (25%)	Nd	Nd	Nd	Nd	Nd	4 (23.50%)

Table 4.7:shows the incidence of food borne pathogens in various food samples. Among 12fried items, 2(16%) sample was suspected to contain *E.coli,* 1 (8.6%) sample was suspected to contain *Aeromonus spp.* and 3(25.0%) samples were suspected to contain *Vibrio.* Among 7 curry items, 1 (14.5%) samples were suspected to contain *E.coli,*3 (42.2%) samples were suspected to contain *Vibrio*and 2 (28.5%) samples were suspected to contain *Aeromonus spp.* Among 4 baked items, 1 (25%) sample was suspected to contain *Vibrio and* 1 (25.%) samples were suspected to contain *Aeromonus spp.*Among soup 3 items 1 (33.5%) samples were suspected to contain *E.coli,*1 (33.2%) samples were suspected to contain *Vibrio.* Among sauce 1 items,1 (100%) samples were suspected to contain *E.coli.*

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 4.8: Colony counting of various hospitals food samples.

Hospital name	Sample name	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Panpacific hospital	porota	Uncountable	Uncountable	Uncountable	Uncountable
	Vaji	Uncountable	Uncountable	uncountable	105
	daal	Uncountable	Uncountable	Uncountable	Uncountable
	soumucha	Uncountable	uncountable	uncountable	uncountable
	Mustard sauce	Uncountable	Uncountable	Uncountable	uncountable
Uttara crescent hospital	Clear soup	Uncountable	Uncountable	Uncountable	Uncountable
	Egg fry	Uncountable	Uncountable	Uncountable	Uncountable
	porota	Uncountable	Uncountable	Uncountable	Uncountable
Salahuddin hospital	burgur	Uncountable	uncountable	uncountable	uncountable
	porota	Uncountable	uncountable	uncountable	uncountable
	Daal	uncountable	uncountable	173	100
	vaji	uncountable	uncountable	uncountable	150
	sauce	uncountable	uncountable	uncountable	uncountable
Peoples hospitals	vaji	60	50	25	20
	daal	uncountable	uncountable	uncountable	uncountable
	Sauce	uncountable	uncountable	uncountable	uncountable
	kabab	uncountable	uncountable	uncountable	uncountable

	bun	uncountable	uncountable	uncountable	uncountable
	vaji	uncountable	uncountable	uncountable	uncountable
BSMMU	Singara	uncountable	uncountable	uncountable	uncountable
	halwa	60	50	25	20
	somucha	uncountable	uncountable	uncountable	uncountable
	sabji	uncountable	uncountable	uncountable	uncountable
	daal	uncountable	uncountable	uncountable	uncountable
Khidma hospital	daaal	uncountable	uncountable	uncountable	uncountable
	Sauce	uncountable	uncountable	uncountable	uncountable
	vaji	uncountable	uncountable	uncountable	uncountable
	porota	uncountable	uncountable	uncountable	uncountable
Monowara hospital	naan	uncountable	uncountable	uncountable	uncountable
	Kabab	uncountable	uncountable	uncountable	uncountable

Table 4.8 shows Colony counting of various hospitals' food samples. ForVaji, plate 4 was selected as it has between 30-300 colonies. The other plates cannot be used because they are either too thick with colonies or too thin. The calculation of cell concentration in the original culture is:

150 colonies on plate 4 x dilution factor of 1000 = 1500,000 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 .

Table 4.9: Number of microorganism per ml of street food sample

Sample name	Vaji	halwa	daal	vaji	vaji
Number of microorganism (cells/ml)	$15x10^5$	$6x10^3$	$173x10^3$	$6x10^3$	$15x10^5$

Table 4.9 shows that the number of microorganism per ml for Vaji is$15x10^5$; for halwa is $6x10^3$; for Daalvaji is $173x10^3$; for vajiis from another two hospitals are $6x10^3$ and $15x10^5$respectively.

Chapter -5

Discussion and Conclusion

Discussion and Conclusion

Food safety is an important issue for the patients, caregivers and employees in the hospital. Hospital is a source of contamination. Infection can easily spread to the individuals if it is not properly controlled. Food is another source of contamination in a hospital which is often overlooked. The patient can be more vulnerable if the foods are not free of contamination.At present time, hospital foods are needed to 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. A lot of food-borne disease outbreaks are occurring every year worldwide. The reasons behind this includes lack of appropriate knowledge and supervision on hospital food , preparation of food under insanitary conditions and displaying food openly which also lead to further contamination by dust, insects, rodents and hands of intending consumers.

The present research work was therefore carried out to find out the presence of enteric bacteria specially *E.coli, Salmonella, Shigellaand Vibrio*species from different types of food items collected from different hospitals 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. MAC were used for isolation of *Aeromonous*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, 30 different food samples were tested. Among them, 30 samples were collected from 4 hospitals of Dhaka city. Among all 30 samples, we found contamination in 17 (56.6%) samples. Of which, 17 (56.6%) samples were suspected to be contaminated with our targeted

organisms (*E coli, Shigella,Aeromonous*and *Vibrio* species). Among them, 5 (29.4%) samples were suspected to be contaminated with *E coli*, 8(47.05%) with *Vibrio,* 4 (23.5%) with *Aeromonous s*pecies.

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 consul-tent 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 Hazard Analysis and Critical Control Points or HACCP, and procedures to ensure that suppliers of food and water have satisfactory food safety policies.(Lund & O'Brien, 2009).

The contamination include the low educational background of the vendors, poor personal hygiene, improper handling and storage practice of foods. Most of the hospitals food with bare hand and didn’t wear any gloves or hand 42.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).

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.

So it is important to control the contamination of hospital foods. Nutritious food should supply for the patients and visitors of hospitals.

Therefore, an understanding regarding the food safety in the hospital is necessary. It includes food preparation, handling and food storage. The source of the raw materials is a key point for food safety. Hygiene has to be ensured regarding the foods as well as the raw materials as well. If the hygiene in the foods is not properly maintained, patients may have additional infection and could be more vulnerable stage. In our study we have observed that some of the foods are contaminated with some enteric pathogens. Care should be taken by the hospital authority on the food safety in these hospitals and thereby ensure patients' health and wellbeing.

Chapter-6

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