A study of food microorganism which are involved in food borne illness

This Thesis paper submitted to the Department of Pharmacy, East West University for the partial fulfillment for the Degree of Bachelor of Pharmacy.

Submitted By

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This thesis paper is dedicated to my parents.

CERTIFICATE

This is to certify that, the thesis "Identification Of Microorganisms in Street Foods around East West University" submitted to the Department of Pharmacy, East West University, Mohakhali, Dhaka, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm) was carried out by Md. Mahi Uddin (ID# 2005-2-70-087) under our guidance and supervision and that no part of the thesis has been submitted for any other degree. We further certify that all the sources of information and laboratory facilities availed of in this connection is duly acknowledged.

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Abstract

Food borne diseases remain responsible for high levels of morbidity and mortality in the general population, but particularly for at-risk groups, such as infants and young, children, the elderly and the immuno compromised. In order to reduce the incidence and economic consequences of food borne diseases, the WHO Department of Food Safety has been assisting the Member States to establish and strengthen their programmes for assuring the safety of food from production to final consumption. In the area around East West University, street vended food is very popular among the students which are Microbiologically risks in food. The street vended food is relatively cheap. But the food is not very hygienic. Data from East West University medical center showed that a lot of students suffer from diarrhea, dysentery, viral hepatitis and enteric fever. In this study a total of 3 different food items (Sugar cane, pakura, piazu) were tested to see the contamination level. The food samples were first separately soaked in saline water for an hour. Then the liquid portion was the filtered. After that measured quantities of the liquid portion from each sample was inoculated in nutrient media and selective media (Nutrient Agar, MacConkey Agar, TCBS Agar, XLD Agar and SS agar). The bacterial growths in the media plates were checked. The colony morphology gave us a rough idea about what kinds of bacteria were present.. The colonies from all the plates were taken and biochemical tests were performed on them to identify the different bacteria. For further confirmation of the identity of the bacteria, agglutination test for serotyping was performed in which specific antibody was used to identify the specific bacteria. Among the food items tested pakura showed the highest level of contamination with bacteria including E. coli, Salmonella and Shigella and also coliform contamination.. Street vendors lack access to clean water, toilet facilities and operate under poor hygienic conditions.

The government should take steps to ensure that the standard of safety of street vended food. The government authorities must enforce regulations during the preparation and selling of street vended foods.

Chapter 1

1. Introduction

Food borne disease or illness is also commonly known as food poisoning which is caused by microorganisms rather than chemical or natural toxins. Food poisoning is a common illness. It is usually mild, but sometimes can be deadly. Typical symptoms include nausea, vomiting, abdominal cramping, and diarrhea that occur suddenly (within 48 hours) after consuming a contaminated food or drink. Depending on the contaminant, fever and chills, bloody stools, dehydration, and nervous system damage may follow. Food borne diseases usually occur because of the improper handling, preparation or storage of food. A lack of knowledge or the incorrect application of sound hygiene practices by food handlers are potential causes of serious outbreaks of food borne disease. Data from World Health Organization (WHO) indicates that food borne diseases affect 5-10% of the population of developed countries. A high proportion of these food borne diseases occurred as a result of incorrect food handling practices **(Maria-Jose Santos *et al.* 2008).**

"Street-vended foods" define as foods and beverages prepared or sold by vendors in streets and other public places for immediate consumption or consumption at a later time without further processing or preparation. This definition includes fresh fruits and vegetables which are sold outside authorized market areas for immediate consumption **(WHO 1996).** .

A panel of food safety experts considered washing hands before handling foods to be the single most important way of preventing food borne diseases. Incorrect use of temperatures (heat or cold) is one factor commonly associated with food borne diseases. Improper cooking, inadequate reheating, high temperatures in freezing and chilling, and cooked food being stored for too long are the main failings detected. Training food handlers to correctly use temperature is one of the measures recommended in the Codex Alimentarius (1993), and in the USA, this is reinforced by official guidelines **(Maria-Jose Santos *et al.* 2008).**

Food-borne diseases are a serious public health problem, even in developed countries. The number of outbreaks in the past decade has been high. Certain contributing factors were: modified eating patterns; importation of food from developing countries; newly emerging

pathogens; particularly in food pathogens; employment of unskilled laborers; pathogen that have grown resistance to antibiotics; an increase in the number of vulnerable individuals (**Patience Mesah *et al.* 2002).**

Street-vended foods also assure food security for low-income urban population and livelihood for a significant proportion of the population in many developing countries **(WHO et al. 1996).** In spite of these potential benefits, street foods have been reported to be contaminated by pathogens and have also been implicated in food borne epidemics **(BEAN et al. 1990).** Poor sanitary practices during preparation and sale, multiple routes of entry, and high ambient temperatures (28 °C) especially in topical environments have been described as the major factors responsible for facilitating the access and multiplication of bacterial contaminants **(Mankee et al. 2003)** in street foods.

However, the unhygienic conditions under which street food vendors operate, lack of basic food safety training of the street vendors, paucity of information concerning incidences of food borne disease related to these street foods, and the fact that none of these foods undergo any processing either before or during preparation and consumption **(Bryan et al. 1997, Mensah etal. 2002)** makes these foods more vulnerable to bacterial contaminants and deserve immediate attention on their microbial quality **(Moushumi Ghosh,et al. 2007.)**

Previous reports on street vended raw salad vegetables, sprouts and fruits have shown the presence of Staphylococcus aureus and Shigella spp. **(Vishwanathan P. et al. 2001)** in India. Staphylococcus is an important food borne pathogen

Street food may be consumed where it is purchased or can be taken away and eaten elsewhere. In selling snacks, complete meals, and refreshments at relatively low prices, they provide an essential service to workers shoppers, travelers and low incomes people. Low income People dependent on such food are often more interested in it convenience then in question of its safety, quality and hygiene **(Mesah et al. 2002).**

The street vendors are conveniently situated either in the living areas, near the workplace or on route for thousands of commuters, and they provide a source of inexpensive, convenient and comparatively nutritious food **(FAO 1997; Tinker 1997; Azanza et al. 2000)** Because street vending has proved to be a good source of income, requiring low capital investment, their numbers continue to increase **(Canet & N diaye 1996 ; WHO 1996; Tinker 1997)**

1.2 Global impact of food borne disease

The increased production of food as well as trade in modern times has increased the potential risk of food contamination. Many outbreaks of food-borne diseases that were once contained within a small community may now take place on global dimensions. The risk of pathogens traveling across borders has become evident during the recent outbreak of avian influenza worldwide. However diseases such as *Salmonella*, *Campylobacter* and *E. coli* have a higher rate of incidence and much larger impact on consumer health and the economy. Food safety authorities all over the world have acknowledged that ensuring food safety must not only be tackled at the national level but also through closer linkages among food safety authorities at the international level. In modern times, rapid globalization of food production and trade have increased the potential likelihood of food contamination. Many outbreaks of food borne diseases that were once contained within a small community may now take place on global dimensions. Food safety authorities all over the world have acknowledged that ensuring food safety must not only be tackled at the national level but also through closer linkages among food safety authorities at the international level. This is important for exchanging routine information on food safety issues and to have rapid access to information in case of food safety emergencies.

Food contamination creates an enormous social and economic strain on societies.In the U.S, diseases caused by the major pathogens are estimated to cost up to US $35 billion annually (1997) in medical costs and lost productivity.The re-emergence of cholera in Peru in 1991 resulted in the loss of US $500 million in fish and fishery product exports that year. **(WHO et al. 1999).**

1.3 Statistics of food-borne diseases in different countries

Every year there are about 76 million food-borne illnesses in the United States (26,000 cases for 100,000 inhabitants), 2 million in the United Kingdom (3,400 cases for 100,000 inhabitants) and 750,000 in France (1,210 cases for 100,000 inhabitants).

United States

In the United States, using FoodNet data from 1996-1998, the CDCP estimated there were 76 million food-borne illnesses (26,000 cases for 100,000 inhabitants).

- 325,000 were hospitalized (111 per 100,000 inhabitants);
- 5,000 people died (1.7 per 100,000 inhabitants.).
- Major pathogens from food borne illness in the United States cost upwards of US $35 billion in medical costs and lost productivity (1997).

France

In France, for 750,000 cases(1,210 per 100,000 inhabitants):

- 70,000 people consulted in the emergency department of an hospital (113 per 100,000 inhab.);
- 113,000 people were hospitalized (24 per 100,000 inhabitants);
- 400 people died (0.9 per 100,000 inhabitants).

Table 2: Causes of food-borne illness in France

Cause	**Annual cases**	**Rate (per 100,000 inhabitants)**
Salmonella	~8,000 cases	13
Campylobacter	~3,000 cases	4.8
Parasites	~500cases	0.8

incl. Toxoplasma	~400 cases	0.65
Listeria	~300 cases	0.5
Hepatitis A	~60 cases	0.1

Table 3: Causes of death by food-borne illness in France

Cause	**Annual**	**Rate (per 100,000 inhabitants)**
Salmonella	~300 cases	0.5
Listeria	~80 cases	0.13
Parasites	~37 cases	0.06 (95% due to toxoplasma)
Campylobacter	~15 cases	0.02
Hepatitis A	~2 cases	0.003

Australia

In Australia, there are an estimated 5.4 million cases of food-borne illness every year, causing:

- 18,000 hospitalizations
- 120 deaths
- 2.1 million lost days off work
- 1.2 million doctor consultations
- 300,000 prescriptions for antibiotics

1.2.0 Food standards:

FAO/WHO Food Standards Programme which was established by an FAO Conference resolution in 1961 and a World Health Assembly resolution, WHA 16.42, in 1963. Its principle objective is to protect the health of consumers and to facilitate the trade of food by setting international standards on foods (**i.e. Codex Standards**) and other texts which can be recommended to governments for acceptance.

CAC (Codex Alimentarius Commission) is an intergovernmental body to implement the Joint. The CAC is open to the governments of all member nations, or associate members of FAO and/or WHO. It currently has 176 Member States.

Badly stored food in a fridge

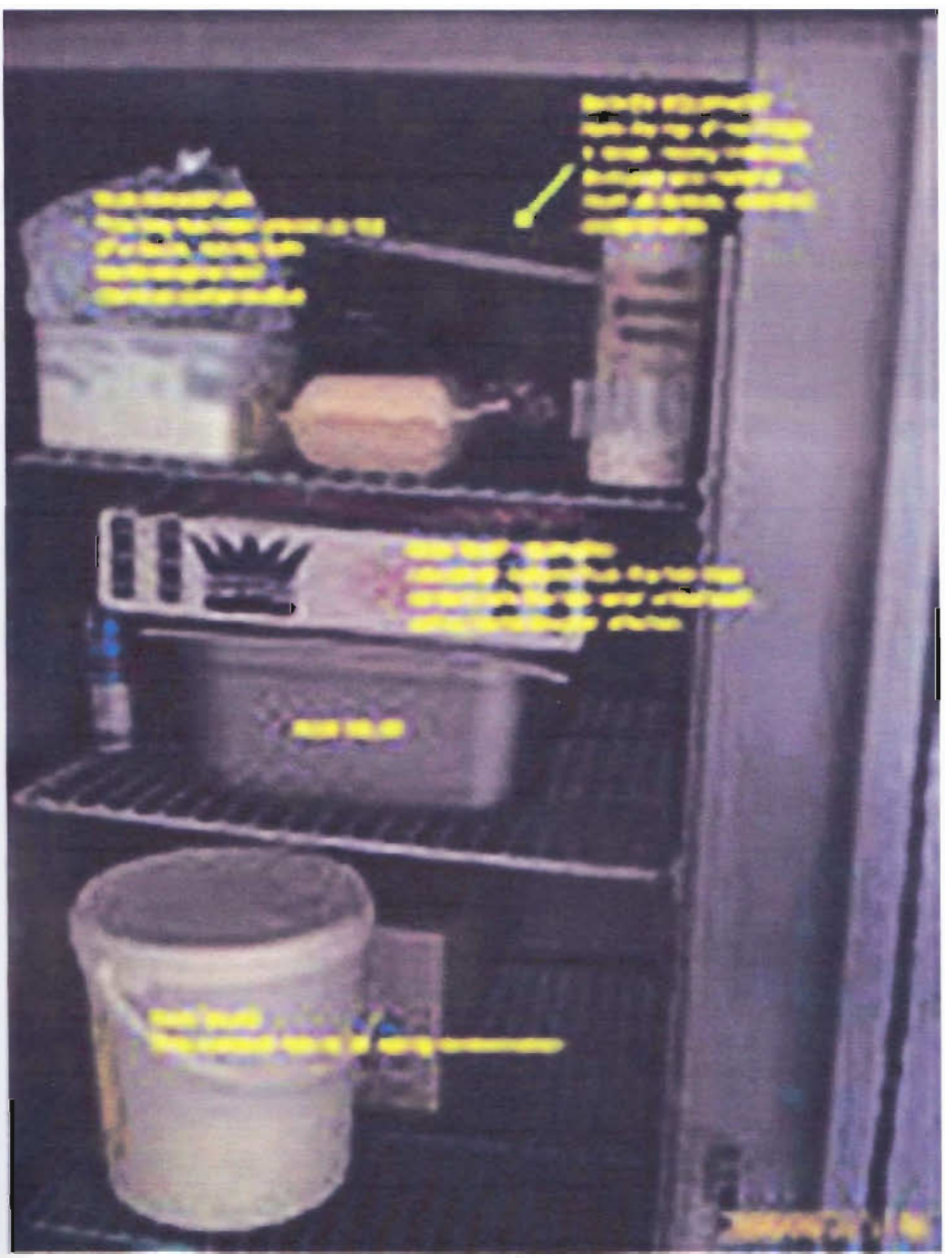

figure 1: Badly stored food in a fridge

Foodborne illness usually arises from improper handling, preparation, orfood storage. Good hygiene practices before, during, and after food preparation can reduce the chances of contracting an illness. There is a general consensus in the public health community that regular hand-washing is one of the most effective defenses against the spread of foodborne illness. The action of monitoring food to ensure that it will not cause foodborne illness is known as **food safety**. Foodborne disease can also be caused by a large variety of toxins that affect the environment. For foodborne illness caused by chemicals, see Food contaminants.

Description of some emerging food and water born pathogens

1.1 *Salmonella* serotypes

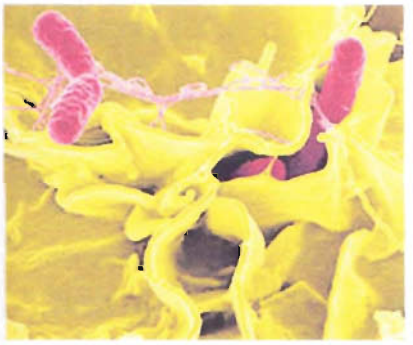

Figure 2:- *Salmonella*

There are more than 2200 different *Salmonella serotypes* distributed in a wide range of animal host, e.g. domestic and wild animals, rodents, reptiles etc. Most of this is capable of causing human infections. Only a few *serotypes* are predominant. *S. enteritidis* has come to be recognized as an emerging etiological agent of food borne infections of global significance over the past two decades. In USA, *Salmonella serotypes* accounted for 6% of reported Salmonella infections in human in 1980. The prevalence of *Salmonella serotypes* in India is not known in a recent study in Ahmednagar District in Maharashtre. Its incidence was found to be 2.43% (**H. C. Gugnani.et al. 1999).**

1.2 *Escherichia coli*

Escherichia coli have recently emerged as virulent pathogens causing haemorrhagiccolitis through consumption of food and water and by human-to-human transmission. Infection with *E. coli* was first described in 1982 as a cause of two outbreaks of bloody diarrhoea in USA As observed by **Aycicek (2005)** who found *Escherichia coli* on the hand of food handlers and their gloves during routine preparation ready to eat meal.

Bloody diarrhea is the major cause of uracmic syndrome and kidney failure in USA (**H. C. Gugnani.et al. 1999).**

Enteric *E. coli* (EC) are classified on the basis of serological characteristics and virulence properties. Virotypes include:

Enterotoxigenic *E. coli* (ETEC): causative agent of diarrhea (without fever) in humans, pigs, sheep, goats, cattle, dogs, and horses.

Enteropathogenic *E. coli* (EPEC): causative agent of diarrhea in humans, rabbits, dogs, cats and horses. Like ETEC, EPEC also causes diarrhea, but the molecular mechanisms of colonization and etiology are different. This virotype has an array of virulence factors that are similar to those found in *Shigella*, and may possess a shiga toxin.

Enteroinvasive *E. coli* (EIEC): found only in humans. EIEC infection causes a syndrome that is identical to Shigellosis, with profuse diarrhea and high fever. EIEC are highly invasive, and they utilize adhesin proteins to bind to and enter intestinal cells. They produce no toxins, but severely damage the intestinal wall through mechanical cell destruction.

Enterohemorrhagic *E. coli* (EHEC): found in humans, cattle, and goats. The sole member of this *virotype* is strain O157:H7, which causes bloody diarrhea and no fever. EHEC can cause hemolytic-uremic syndrome and sudden kidney failure.

1.3 *Listeria monocytogenes*

Listeriosis is a food borne disease. *Listeria* is the most important pathogenic species causing listeriosis in animal and man. Recently it has been isolated in low number from raw or proceeds food, including dairy product, meat, vegetable, sea food etc (**H. C. Gugnani. et al. 1999).**

1.4 *Campylobacter jejunii*

Campylobacter jejunii is widely distributed in both wild and domestic birds and mammals as well as untreated water. At present 15 species of *Campylobacter jejunii* are recognized and eight of them are of clinical importance. It is the First recognize human pathogen in 1970s. It causes food borne bacterial infections and it is more common then *salmonella* poisoning in some part of the world **(H. C. Gugnani.et al. 1999).**

1.5 *Yersinia enterocolytica*

This organism is a parasite to certain domestic animal, e.g. pigs, cattle and rodents. During the past ten years it has been increasingly recognized as a significant invasive enteric pathogen to men. Infection often involving intestinal lymph nodes. This is also capable of causing human intestinal disease **(H. C. Gugnani.et al. 1999).**

1.6 *Aeromonas* Species

Aeromonas often distributed in natural habitats such as soil, stagnant or flowing water, sewage etc. Natural disease due to *Aeromonas* occur in amphibians, reptiles and fish. It causes extra intestinal and wound infections in humans **(H. C. Gugnani.et al. 1999).**

1.7 Acquatic *Vibrio*

Vibrio are inhabitant of aquatic environment. They occasionally infect humans causing intestinal or extra intestinal disease. The mostly reported are cholera and the form of acute gastro enteritis **(H. C. Gugnani.et al. 1999).**

1.8 *Shigella*

Shigella species are members of the family of *Enterobacteriaceae*. *Shigellae* are Gram-negative, non-spore-forming, facultatively anaerobic, non-motile bacteria.

S. dysenteriae, spread by contaminated water and food, causes the most severe dysentery because of its potent and deadly Shiga toxin, but other species may also be dysentery agents.

Further complications of the disease may be seizures, toxic mega colon, reactive arthritis and hemolytic uremia syndrome. Transmission of the pathogen is by the fecal-oral route, commonly through food and water. *Shigellae* are one of the leading causes of bacterial food borne illnesses and can spread quickly within a population. *Shigella* species are an important cause of diarrhea disease in developing countries **(Varges et al. 1999).**

1..9 *Calicivirus*

This virus is an extremely common cause of food borne illness, though it is rarely diagnosed, because the laboratory test is not widely available. It causes an acute gastrointestinal illness, usually with more vomiting than diarrhea that resolves within two days. Unlike many food borne pathogens that have animal reservoirs, it is believed that Norwalk-like viruses spread primarily from one infected person to another.

1.10 Diarrhea

In medicine, diarrhea is frequent loose or liquid bowel movements. Although for many people diarrhea is merely unpleasant, diarrhea that is both acute and severe is a common cause of death in developing countries and the second most common cause of infant deaths worldwide. It is often due to gastroenteritis.

Causes of diarrhea

Diarrhea is most commonly caused by viral infections, parasites or bacterial toxins. In sanitary living conditions where there is ample food and a supply of clean water, an otherwise healthy patient usually recovers from viral infections in a few days. However, for ill or malnourished individuals diarrhea can lead to severe dehydration and can become life-threatening without treatment.

Types of diarrhea

There are at least four types of diarrhea: secretory diarrhea, osmotic diarrhea, motility-related diarrhea, and inflammatory diarrhea.

Secretory diarrhea

Secretory diarrhea means that there is an increase in the active secretion, or there is an inhibition of absorption. There is little to no structural damage. The most common cause of this type of diarrhea is a cholera toxin that stimulates the secretion of anions, especially chloride ions. Therefore, to maintain a charge balance in the lumen, sodium is carried with it, along with water.

Osmotic diarrhea

Osmotic diarrhea occurs when too much water is drawn into the bowels. This can be the result of maldigestion (e.g., pancreatic disease or Coeliac disease), in which the nutrients are left in the lumen to pull in water. Osmotic diarrhea can also be caused by osmotic laxatives (which work to alleviate constipation by drawing water into the bowels). In healthy individuals, too much magnesium or vitamin C or undigested lactose can produce osmotic diarrhea and distention of the bowel. A person who does not have lactose intolerance can have difficulty absorbing lactose after an extraordinarily high intake of dairy products. In persons who do not have fructose malabsorption, excess fructose intake can still cause diarrhea. High-fructose foods that also have a high glucose content are more absorbable and less likely to cause diarrhea. Sugar alcohols such as sorbitol (often found in sugar-free foods) are difficult for the body to absorb and, in large amounts, may lead to osmotic diarrhea.

Motility-related diarrhea

Motility-related diarrhea is caused by the rapid movement of food through the intestines (hypermotility). If the food moves too quickly through the GI tract, there is not enough time for sufficient nutrients and water to be absorbed. This can be due to a vagotomy or diabetic neuropathy, or a complication of menstruation. Hyperthyroidism can produce hypermotility and lead to pseudodiarrhea and occasionally real diarrhea. Diarrhea can be treated with antimotility agents (such as loperamide).

Inflammatory diarrhea

Inflammatory diarrhea occurs when there is damage to the mucosal lining or brush border, which leads to a passive loss of protein-rich fluids, and a decreased ability to absorb these lost fluids. Features of all three of the other types of diarrhea can be found in this type of diarrhea. It can be

caused by bacterial infections, viral infections, parasitic infections, or autoimmune problems such as inflammatory bowel diseases. It can also be caused by tuberculosis, colon cancer, and enteritis.

The Diarrheal Disease Control (CDD) programme of the World Health Organization has advocated the following strategy for diarrhea control:-

- Improved case management, with particular emphasis on the early use of oral rehydration therapy in acute diarrhea and on appropriate feeding during illness and convalescence.
- Improved maternal and children health care, with particular emphasis on breast feeding, weaning practices, personal and domestic hygiene, and maternal nutrition
- Improved use and maintenance of drinking water and sanitation facilities, and improved food hygiene
- Detection and control of epidemics

(R. G. Feachem, *et al.* 1983)

1.11 Cholera

Cholera, sometimes known as **Asiatic cholera** or **epidemic cholera**, is an infectious gastroenteritis caused by the bacterium *Vibrio cholerae*. Transmission to humans occurs through ingesting contaminated water or food. The major reservoir for cholera was long assumed to be humans themselves, but considerable evidence exists that aquatic environments can serve as reservoirs of the bacteria.

Vibrio cholerae is a Gram-negative bacterium that produces cholera toxin, an enterotoxin, whose action on the mucosal epithelium lining of the small intestine is responsible for the characteristic of massive diarrhoea of the disease.

In its most severe forms, cholera is one of the most rapidly fatal illnesses known, and a healthy person may become hypotensive within an hour of the onset of symptoms; infected patients may die within three hours if treatment is not provided. In a common scenario, the disease progresses

from the first liquid stool to shock in 4 to 12 hours, with death following in 18 hours to several days without oral rehydration therapy. Analysis of clinical and environmental data have revealed significant correlations of water temperature, water depth, rainfall, conductivity, and copepod counts with the occurrence of cholera toxin-producing bacteria (presumably V. cholera).

The coastal ecosystem of the Bay of Bengal is said to be a significant reservoir for the epidemic serogroups of V. cholera. **(Alam M. *et al.* 2006)**

Symptoms of cholera

The diarrhea associated with cholera is acute and so severe that, unless oral rehydration therapy is started promptly, the diarrhea may within hours result in severe dehydration, or even death.

Other symptoms include rapid dehydration, rapid pulse, dry skin, tiredness, abdominal cramps, nausea, and vomiting.

Traditionally, Cholera was widespread throughout third world countries, however more recently outbreaks have occurred in more rural parts of England and the United States' mid-west region.

Prevention of cholera

Although cholera can be life-threatening, prevention of the disease is straightforward if proper sanitation practices are followed. In the first world, due to advanced water treatment and sanitation systems, cholera is no longer a major health threat. The last major outbreak of cholera in the United States occurred in 1911. Good sanitation practices, if instituted in time, are usually sufficient to stop an epidemic. There are several points along the transmission path at which the spread may be halted:

- Sterilization: Proper disposal and treatment of the germ infected fecal waste (and all clothing and bedding that come in contact with it) produced by cholera victims is of primary importance. All materials (such as clothing and bedding) that come in contact with cholera patients should be sterilized in hot water using chlorine bleach if

possible. Hands that touch cholera patients or their clothing and bedding should be thoroughly cleaned and sterilized.

- Sewage: Treatment of general sewage before it enters the waterways or underground water supplies prevents undiagnosed patients from spreading the disease.
- Sources: Warnings about cholera contamination posted around contaminated water sources with directions on how to decontaminate the water.

Water purification: All water used for drinking, washing, or cooking should be sterilized by boiling or chlorination in any area where cholera may be present. Boiling, filtering, and chlorination of water kill the bacteria produced by cholera patients and prevent infections from spreading.

Killed oral cholera vaccines are internationally licensed for older children and adults, but not for infants and young children. Vaccination of older age groups was ssociated eith protection of children too young to be vaccinated. The pronounced herd protection of young children associated with vaccination of adult women suggests that adult women may play a prominent role in the transmission of cholera to young children. **(Ali M. *et al.* 2008)**

Other common bacterial food borne pathogens are:

- *Bacillus cereus*
- *Escherichia coli*, other virulence properties, such as enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroaggregative (EAEC or EAgEC)
- *Listeria monocytogenes*
- *Shigella* spp.
- *Staphylococcus aureus*
- *Streptococcus*
- *Vibrio cholerae*, including O1 and non-O1
- *Vibrio parahaemolyticus*
- *Vibrio vulnificus*
- *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

Less common bacterial agents:

- *Brucella* spp.
- *Corynebacterium ulcerans*
- *Coxiella burnetii* or Q fever
- *Plesiomonas shigelloides*

2.1 Exotoxins

In addition to disease caused by direct bacterial infection, some foodborne illnesses are caused by exotoxins which are excreted by the cell as the bacterium grows. Exotoxins can produce illness even when the microbes that produced them have been killed. Symptoms typically appear after 1–6 hours depending on the amount of toxin ingested.

- *Clostridium botulinum*
- *Clostridium perfringens*
- *Staphylococcus aureus*
- *Bacillus cereus*

For example *Staphylococcus aureus* produces a toxin that causes intense vomiting. The rare but potentially deadly disease botulism occurs when the anaerobic bacterium *Clostridium botulinum* grows in improperly canned low-acid foods and produces botulin, a powerful paralytic toxin. *Pseudoalteromonas tetraodonis*, certain species of *Pseudomonas* and *Vibrio*, and some other bacteria, produce the lethal tetrodotoxin, which is present in the tissues of some living animal species rather than being a product of decomposition.

2.2 Viruses

Viral infections make up perhaps one third of cases of food poisoning in developed countries. In the US, more than 50% of cases are viral and noroviruses are the most common food borne illness, causing 57% of outbreaks in 2004. Food borne viral infections are usually of intermediate (1–3 days) incubation periods, causing illnesses which are self-limited in otherwise healthy individuals, and are similar to the bacterial forms.

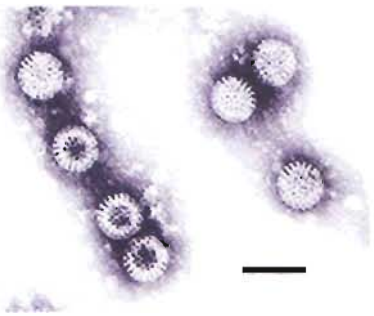

Figure 3:- Viruses

Rotavirus:

- Enterovirus
- Hepatitis A is distinguished from other viral causes by its prolonged (2–6 week) incubation period and its ability to spread beyond the stomach and intestines, into the liver. It often induces jaundice, or yellowing of the skin, and rarely leads to chronic liver dysfunction. The virus has been found to cause the infection due to the consumption of fresh-cut produce which has fecal contamination.
- Hepatitis E
- Norovirus
- Rotavirus

2.3 Natural toxins

Several foods can naturally contain toxins, many of which are not produced by bacteria. Plants in particular may be toxic; animals which are naturally poisonous to eat are rare. In evolutionary terms, animals can escape being eaten by fleeing; plants can use only passive defenses such as poisons and distasteful substances, for example capsaicin in chili peppers and pungent sulfur compounds in garlic and onions. Most animal poisons are not synthesized by the animal, but acquired by eating poisonous plants to which the animal is immune, or by bacterial action.

- Alkaloids
- Ciguatera poisoning
- Grayanotoxin (honey intoxication)
- Mushroom toxins

- Shellfish toxin, including paralytic shellfish poisoning, diarrhetic shellfish poisoning, neurotoxic shellfish poisoning, amnesic shellfish poisoning and ciguatera fish poisoning

The International Food Safety Authorities Network (INFOSAN)

This network is intended to complement and support the existing WHO Global Outbreak Alert and Response Network (GOARN) which includes a Chemical Alert and Response component.

World Health Organization Food Safety Department

The WHO provides scientific advice for organizations and the public on issues concerning the safety of food. It serves as a medium linking the food safety systems in countries around the world. Food safety is currently one of WHO's top ten priorities.

Food Safety is one of the major issues in our world today, and the Organization calls for more systematic and aggressive steps to be taken to significantly reduce the risk of food-borne diseases

Figure 4:- Food Safety Department

Statistics of students affected by food borne disease in EWU

Data taken from EWU medical centre showed us the number of students affected by food-borne diseases. Total number of students suffering from food borne disease from the month of August 2008 to August 2009 was 133.

This is the number of students who have reported to the medical center. It has been noted that a lot of students do not come to the medical center when they are ill, and some students prefer to go to a hospital or clinic. **(East West University medical center records)**

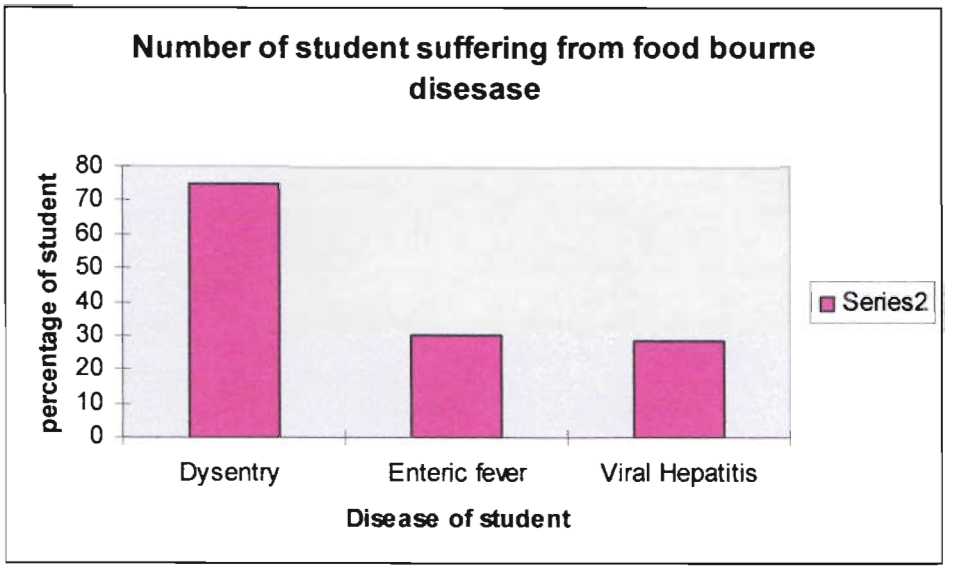

Figure 5:- total number of students suffering from food borne disease

Total number of students suffering from diarrhea or dysentery from the month of April 2008 to April 2009 was 88.

Total number of students suffering from viral hepatitis from the month of April 2008 to April 2009 was 37.

Total number of students suffering from enteric fever from the month of April 2008 to April 2009 was 8.

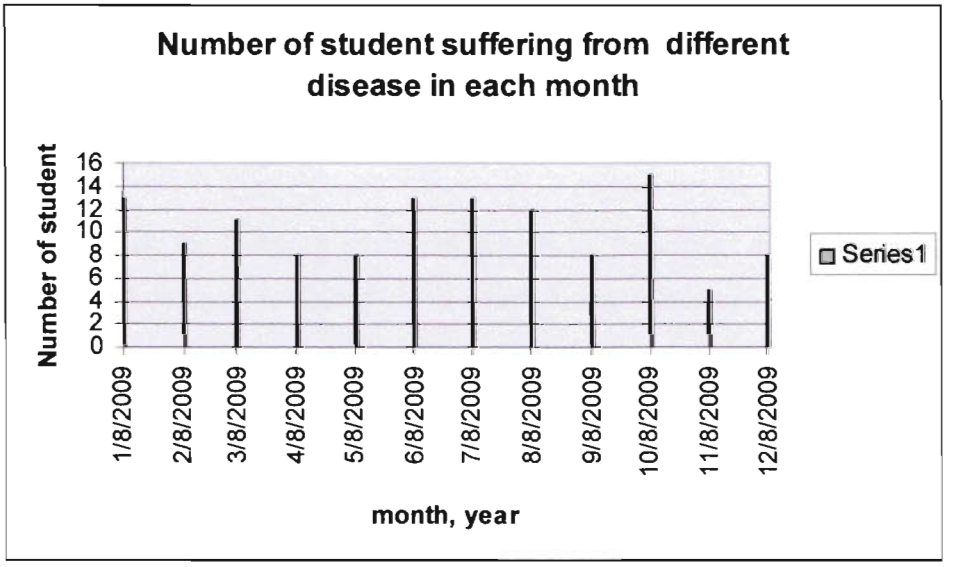

Figure 6:- Number of students suffering from different diseases in each month

Comparing the data for the different months show a higher occurrence of diarrhea and dysentry in the summer time then in winter. The data also shows a high occurrence of viral hepatitis in Macrh and April 2009.

Purpose of the study

The objective of this research was to identify the presence of microorganisms in street foods around the East West University.

Different types of food samples (pakura, piazue, sugarcan etc) were collected from the raw street vendors around East West University for identifying food microorganisms.

Gram staining was performed to find out food microorganisms. Two type of microorganism was found which is gram positive or gram negative.
The purpose of the study is to maintain adequate hygiene during preparing foods.

Significance of Study

The study of food borne disease will assure food security for the low income urban people and livelihood of the population in many developing countries.

Lack of basic food training of the street vendors, paucity of information concerning incidences of food-borne diseases related to these street foods, then steps can be taken to bring awareness to the consumers and to the street venders themselves.

Any processing either before or during preparation and consumption makes these foods more vulnerable to bacterial contaminations

Chapter 2

Methodology

1 Materials required for inoculation in media plates:-

- Petri dish
- Beaker
- Measuring cylinder
- Media preparation bottle
- Funnel
- Analytical balance
- Autoclave
- Hot air oven
- Laminar air flow chamber
- Micropipette

Materials required for inoculation in media plates

- Micropipette tips
- Test tubes
- Aluminum foil
- Nutrient agar
- MacConkey agar
- XLD agar
- TCBS agar
- SS agar
- Normal saline
- Distilled water

2 Materials required for Gram staining:

Crystal violet
Gram's io Ethyl alcohol dine

required for Gram staining

Safranin
Glass Slide
Bacterial colony in nutrient agar media.

3 Materials required for Biochemical tests

Catalase test

- Hydrogen peroxide, 3% H_2O_2
- sterile wooden stick or a glass rod
- test tube

This test demonstrates presence of the enzyme catalase which can catalize release of oxygen from H_2O_2 the test is done by pouring H2O2 over the colonies of the organisms under test.

Citrate utilization test

- Simmon's citrate agar
- Test tubes
- straight wire

Coagulase test

- EDTA anticoagulated human plasma or rabbit plasma or Oxalate or heparin plasma can also be used
- Glass slide

Indole test

- Tryptophan
- Kovac's or Ehrlich's reagent which contains 4 (p)-dimethylamino-benzaldehyde
- bijou bottle

Oxidase test/Cytochrome oxidase test

- oxidase reagent containing phenylenediamine
- filter paper
- petri dish
- stick or glass rod

Kliglar Iron Agar test (KIA test)

- Kliglar's Iron Agar
- Straight wire

Motility test

- semi solid agar medium
- Straight wire

Procedures

3.1 Sample collection from site

Food samples were collected from street venders in the area around East West University, Mohakhali, Dhaka. Samples of burger, pizza, patis and chop were collected.

3.2 Sample preparation

- 10 gm of each sample was weighed and taken in a sterilized beaker.
- 75 ml of sterilized normal saline water was added to the beaker.
- The beaker was covered and kept for 60 minutes.
- After 60 minutes the liquid portion was filtered using sterile cotton and a sterile funnel.
- The liquid was collected in a sterile test tube.
- This liquid was ready for inoculation in the different culture media.

3.3 Sterilization method

Sterilization was done using autoclave and/or hot air oven. Beakers, funnel, measuring cylinders, micropipette tips and test tubes, normal saline water and the culture media (the media that require autoclave) were sterilized using autoclave at 121 °C for 15 minutes. Petri dishes were sterilized in hot air oven at 200 °C for 90 minutes. All the items were wrapped in aluminum foil during sterilization. This is to ensure that the glassware do not get contaminated after sterilization when they are transferred from the sterilizer to the laminar air flow chamber. After sterilization the items are kept in the laminar air flow chamber and all the activities are done here to prevent contamination.

3.4 Pour plate method:-

1. The media is prepared according to the instructions.
2. A measures amount of sample is placed in the petri dish.
3. The media is poured in the petri dish up to 5mm.
4. The petri dish is swirled gently to mix the sample with the media.
5. After mixing the media is allowed to solidify.
6. After the media becomes solid the petri dish is incubated.

3.2.5 Gram Staining

The most important differential stain used in bacteriology is the Gram stain, named after Dr. Christian Gram. It divides bacterial cells into two major groups, gram positive and gram negative, which makes it an essential tool for classification and differentiation of microorganism. Gram positive and gram negative bacteria stain differently because of fundamental differences in the structure of their cell walls. Gram negative staining is based on the ability of bacteria cell wall to retain the crystal violet dye during solvent treatment. The cell walls for gram positive microorganisms have a higher peptidoglycan and lower lipid content than gram negative bacteria.

The gram stain uses four different reagents:

Crystal violet: It is used as the primary stain. Its function is to impart its color to all cells.

Gram's iodine: This reagent serves as mordant, a substance that forms an insoluble complex by binding to the primary stain. The resultant crystal violet-iodine (CV-I) complex serves to intensify the color of the stain and all the cells will appear purple at this point. Iodine is added as a mordant so that the dye cannot be removed easily. This step is commonly referred to as fixing the dye.

Ethyl alcohol: 95% ethyl alcohol is a decolorizing agent. This reagent dissolves the lipid layer from the gram-negative cells. The removal of the lipid layer enhances the leaching of the primary stain from the cells into the surrounding solvent. This facilitates release of the unbound CV-I complex, leaving these cells colorless or unstained. In contrast the solvent dehydrates the thicker

Gram-positive cell walls, closing the pores as the cell wall shrinks during dehydration. As a result, the primary stain cannot be removed and the cells remain purple.

Safranin: Safranin is a counter stain that is used to stain red/pink those cells that have been previously decolorized. Since only gram negative cells undergo decolorization, they may now absorb the counter stain. Gram positive cells retain the purple color of the primary stain.

Procedure for Gram staining:

a) A glass slide was cleaned and wipe with alcohol.
b) Using sterile technique, prepare a smear of organism. To do this, place a drop of water/normal saline on the slide and then transfer organism to the drop of water on the slide with a sterile, cooled loop. Mix and spread organisms by means of a circular motion of the inoculating loop.
c) Allow the smear to air dry and then heat fix.
d) Flood smear with crystal violet and then let stand for 1 minute.
e) Wash with tap water.
f) Flood the smear with Gram's iodine mordant and let stand for 1 minute.
g) Wash with tap water.
h) Decolorized with 95% ethanol for 40 seconds to 1 minute.
i) Wash with tap water.
j) Flood with safranin for 45 seconds.
k) Wash with tap water.

Air dry the slide and examine under microscope

Culture media used and methods of preparation

Selective media favor the growth of particular microorganisms. Media such as tryptic soy broth and tryptic soy agar are called general purpose media because they support the growth of many microorganisms. Bile salts or dyes like basic fuchsin and crystal violet favor the growth of gram negative bacteria by inhibiting the growth of gram positive bacteria without affecting the gram negative organisms. Endo agar, erosin methylene blue agar, and MacConkey agar, three widely uses for the detection of E. coli and related bacteria in water supplies.

1 Nutrient Agar

Nutrient agar is a microbiological growth medium commonly used for the routine cultivation of non-fastidious bacteria.

Nutrient agar typically contains

- 0.5 % peptone
- 0.3 % beef extract
- 1.5 % agar
- pH adjusted to neutral at 25 °C.

This formulation provides the nutrients necessary for the replication of a large number of microorganisms that are not excessively fastidious. The beef extract contains water soluble substances including carbohydrates, vitamins, organic nitrogen compounds and salts. Peptones are the principle sources of organic nitrogen, particularly amino acids and long chained peptides.

Nutrient Agar, is still specified in current compendia of methods for the microbiological examination of a broad spectrum of materials. Additionally, it is used in the laboratory for the cultivation and maintenance of nonfastidious species.

Directions for Preparation from Dehydrated Product

1. Suspend 23 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

2 MacConkey agars

MacConkeys Agar is a special bacterial growth medium that is selective for Gram- bacteria and can differentiate those bacteria that are able to ferment lactose.When bacterial cultures are grown in a laboratory; they are growing in a captive environment, somewhat like a tiny microbial zoo. These captive-bred bacteria are totally dependent on people to provide the proper environment for their survival and growth. A nutrient-rich media is required in order to grow bacteria in the lab.

If a bacterial growth medium is selective, that means that it grows only certain types of microbes while inhibiting the growth of others. Agar is considered a differential growth medium if, when specific microbes are present, the medium or bacterial colonies themselves exhibit a color change that provides information about their identity.

MacConkey's contains two additives that make it differential; neutral red (a pH indicator) and lactose (a disaccharide).

Bacteria, known as "lactose fermenters", eat the media's lactose, and, in the process, create an acidic end product that causes the pH indicator, neutral red, to turn pink. With MacConkey's, it is not the media that changes color, but rather the actual colonies of lactose fermenting bacteria that appear pink. Non-lactose fermenting bacteria will be colorless (or, if they have any color, will be their natural color rather than pink).

Whenever bacterial colonies are growing on MacConkey's Agar, they are Gram-negative bacteria (since Gram+ do not grow on this type of medium). If the colonies are pink, they are Gram- lactose-fermenting bacteria.

These pink colonies are typically coliform bacteria in the family Enterobacteriaceae, inlcuding the genera *Escherichia, Klebsiella, Enterobacter, Hafnia* and *Citrobacter*. Non-lactose fermenting, non-coliform members of Enterboacteriaceae include the genera *Proteus, Morganella, Providencia, Edwardsiella, Salmonella, Shigella* and *Yersenia* (plague bacteria).

Directions for Preparation from Dehydrated Product

1. Suspend 50 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45 - 50°C and dispense into sterile petri dishes.

Expected results:

Table 2:- Cultural response on MacConkey Agar at 37°C after incubation for 24 hours.

Microorganism	Response	Reactions	Bile ppt
Enterococcus faecalis	marked to complete inhibition	-	-
Escherichia coli	growth	pink colonies	+
Proteus mirabilis	growth with partial inhibition of swarming	colorless colonies	-
Salmonella typhimurium	growth	colorless colonies	-

1 TCBS agar

Environmental bacteria grown on TCBS agar plates (TCBS strains) were investigated for the presence of *Vibrio cholerae* in aquatic environments. TCBS strain counts were 0.01 – 0.001 times the total viable counts in pairs of the same samples. The TCBS strains were of two types which required N_aCl (salt strain) and did not require N_aCl (non-salt strain) to grow in peptone water. Non-salt strains made up 85.3 – 92.1% of TCBS strains isolated from river water. TCBS strains isolated from an estuary contained 40.9% of non-salt strains and 57.4% of salt strains. Salt strains made up 69.2 – 86.8% of TCBS strains isolated from seawater. The percentages of*Vibrio* species in TCBS strains were 11.9 – 47.9%. V.*alginolyticus* and *V. parahaemolyticus* were isolated from seawater. *V.* vulnificus was only isolated from estuary water.*V. Cholerae* non-Ol was isolated from both river water and estuary water which had low salinity.*V. Fluvialis* was isolated from all three aquatic environments. This investigation suggests that*Vibrio* species were present in each sample station and that*V. Cholerae* existed in river water.

Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS Agar) is used for the selective isolation of *cholera vibrios* and *Vibrio parahaemolyticus* from a variety of clinical and nonclinical specimens.

The alkaline pH of the medium enhances the recovery of *V. cholerae*. Thymol blue and bromthymol blue are included as indicators of pH changes.

Directions for Preparation from Dehydrated Product

1. Suspend 89 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Cool to 45-50°C and use immediately. DO NOT AUTOCLAVE.
4. Test samples of the finished product for performance using stable, typical control cultures.

Expected Results

Table 3:- Typical colony morphology on TCBS Agar

Organism	Response
V. cholerae	Large yellow colonies
V. parahaemolyticus	Colonies with blue to green centers.
V. alginolyticus	Large yellow colonies
Proteus/Enterococci	Partial inhibition. If growth, colonies are small and yellow to translucent.
Pseudomonas/Aeromonas	Partial inhibition. If growth, colonies are blue

2 XLD agar

Xylose lysine deoxycholate agar (XLD agar) is a selective growth medium used in the isolation of *Salmonella* and *Shigella* species from clinical samples and from food. It has a pH of approximately 7.4, leaving it with a bright pink or red appearance due to the indicator phenol red. Sugar fermentation lowers the pH and the phenol red indicator registers this by changing to yellow. Most gut bacteria, including *Salmonella*, can ferment the sugar xylose to produce acid; *Shigella* colonies cannot do this and therefore remain red. After exhausting the xylose supply

Salmonella colonies will decarboxylate lysine, increasing the pH once again to alkaline and mimicking the red *Shigella* colonies. Salmonellae metabolise thiosulfate to produce hydrogen sulfide, which leads to the formation of colonies with black centers and allows them to be differentiated from the similarly coloured *Shigella* colonies.

Other Enterobacteria such as *E. coli* will ferment the lactose and sucrose present in the medium to an extent that will prevent pH reversion by decarboxylation and acidify the medium turning it yellow.

Salmonella species: red colonies, some with black centers. The agar itself will turn red due to the presence of Salmonella type colonies.

Shigella species: red colonies.

Coliforms: yellow to orange colonies.

Pseudomonas aeruginosa: pink, flat, rough colonies.

This type of colony can be easily mistaken for Salmonella due to the colour similarities.

XLD agar contains:

Yeast extract	3g/l
L-Lysine	5g/l
Xylose	3.75g/l
Lactose	7.5g/l
Sucrose	7.5g/l
Sodium deoxycholate	1g/l
Sodium chloride	5g/l
Sodium thiosulfat	6.8g/l
Ferric ammonium citrate	0.8 mg/l
Phenol red	0.08g/l
Agar	12.5g/l

Expected Results

Table 4:- Typical colony morphology on XLD Agar

Organism	Response
Salmonella species	red colonies, some with black centers. The agar itself will turn red due to the presence of salmonella type colonies
Shigella species	red colonies.
Coliforms	yellow to orange colonies
Pseudomonas aeruginosa	pink, flat, rough colonies. This type of colony can be easily mistaken for salmonella due to the color similarities

Directions for Preparation from Dehydrated Product

1. Suspend 56.68g in 1 liter distilled water.
2. Heat with frequent agitation until the medium boils. Do not autoclave or overheat.
3. Transfer immediately to a water bath at 50 C. After cooling, pour into sterile petri dishes.

2.3.5 SS agar/*Salmonella Shigella* Agar

SS Agar and *Salmonella Shigella* Agar are designated as moderately selective media based upon the degree of inhibition of gram-positive microorganisms that they inhibit due to their content of bile salts, brilliant green and citrates.Brilliant green, ox bile and high concentrations of thiosulfate and citrate largely inhibit the accompanying microbial flora. Sulfide production is detected by using thiosulfate and iron ions, the colonies turn black. The presence of coliform bacteria is established by detecting degradation of lactose to acid with the pH indicator neutral red.SS Agar and *Salmonella Shigella* Agar are moderately selective and differential media for the isolation of pathogenic enteric bacilli, especially those belonging to the genus *Salmonella*. This formulation is not recommended for the primary isolation of *Shigella*.

The sodium thiosulfate and ferric citrate enable the detection of hydrogen sulfide production as evidenced by colonies with black centers.

Directions for Preparation from Dehydrated Product

1. Suspend 60g of powder in 1 liter of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Do not autoclave
3. Cool the medium to approximately 45 to 50 C and pour into Petri dishes.
4. Allow the plates to dry for approximately 2 hours with the covers partially removed.

Expected Result:-

Table 5:- Typical colony morphology on SS Agar

Bacteria	Colony color	H_2S(black center)
Enterococcus faecalis	colorless	-
Escherichia coli	Pink to red	-
Salmonella choleraesuis	colorless	+
Shigella flexneri	colourless	-
Proteus	colourless	+
Pseudomonas	Irregular, slight growth	

Procedure for inoculation in media plates

- All glass ware are sterilized using autoclave and/or hot air oven.
- 10 gm of the sample was weighed and taken in a sterilized beaker.
- 75 ml saline water was added to the beaker. The beaker was covered and kept for 60 minutes.
- The liquid portion was filtered using sterilized cotton and a sterilized funnel.
- 0.5 ml of the liquid portion was inoculated into media plate using pour plate method.
- The plate was incubated for 24 hours at 37° C
- The plates were observed and the results were recorded.
- Colony counting was done for the nutrient agar plates.
- Gram staining was done on a few randomly selected colonies on the nutrient agar plates.

The following steps are carried out in a laminar air flow chamber and all the glassware and other materials used were sterilized prior to use.

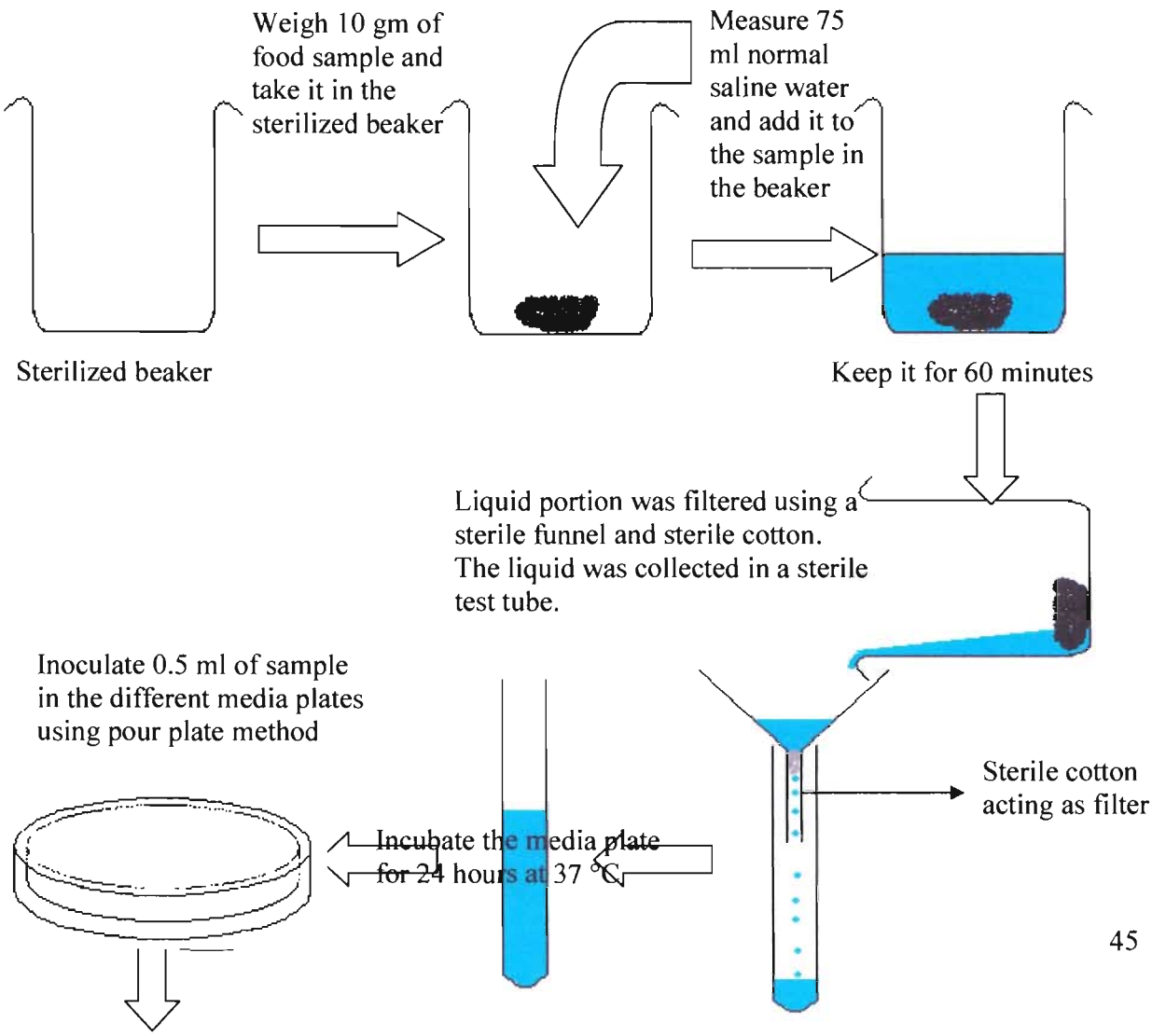

Figure 7:- Procedure for inoculation in media plates

2.5 Biochemical tests

2.5.1 Catalase test

This test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as *streptococci.*

Principle

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hours old.

Required

Hydrogen peroxide, 3% H_2O_2

Method

1. Pour 2-3 ml of the hydrogen peroxide solution into a test tube.
2. Using a sterile wooden stick or a glass rod *{not* a nichrome wire loop), remove several colonies of the test organism and immerse in the hydrogen peroxide solution.

   Important: Care must be taken when testing an organism cultured on a medium containing blood because catalase is present in red cells. If any of the blood agar is removed with the organism, a false positive reaction may occur.
3. Look for immediate bubbling.

Expected Results

Active bubbling ..... Positive catalase test

No bubbles.....Negative catalase test

2.5.2 Citrate utilization test

This test is one of several techniques used occasionally to assist in the identification of *enterobacteria*. The test is based on the ability of an organism to use citrate as its only source of carbon.

Citrate method using Simmon's citrate agar

1. Prepare slopes of the medium in bijou bottles as recommended by the manufacturer (store at 2-8 °C).
2. Using a sterile straight wire, first streak the slope with a saline suspension of the test organism and then stab the butt.
3. Incubate at 35 °C for 48 hours. Look for a bright blue color in the medium.

Expected Results

Bright blue	Positive citrate test
No change in color of medium	Negative citrate test

2.5.3 Coagulase test

This test is used to identify *S. aureus* which produces the enzyme coagulase.

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of *S. aureus:*

- Free coagulase which converts fibrinogen to fibrin by activating a coagulase-reacting factor present in plasma. Free coagulase is detected by clotting in the tube test.
- Bound coagulase (clumping factor) which converts fibrinogen directly to fibrin without requiring a coagulase-reacting factor. It can be detected by the clumping of bacterial cells in the rapid slide test.

A tube test must always be performed when the result of a slide test is not clear, or when the slide test is negative and *Staphylococcus* has been isolated from a serious infection. A tube test is required to detect some MRSA (methiciliin resistant S. *aureus)* strains. Before performing a coagulase test, examine a Gram stained smear to confirm that the organism is a Gram positive coccus.

Material Required

EDTA anticoagulated human plasma (preferably pooled and previously HIV and hepatitis tested) or rabbit plasma. The plasma should be allowed to warm to room temperature before being used. Plasma: Oxalate or heparin plasma can also be used. Do not use citrated plasma because citrate-utilizing bacteria e.g. *enterococci*, *Pseudomonas* and *Serratia* may cause clotting of the plasma (in tube test). Occasionally, human plasma may contain inhibitory substances which can interfere with coagulase testing. It is therefore essential to test the plasma using a known coagulase positive S. *aureus*. The plasma can be stored frozen in amounts ready for use.

Slide test method (detects bound coagulase)

1. Place a drop of distilled water on each end of a slide or on two separate slides.
2. Emulsify a colony of the test organism (previously checked by Gram staining) in each of the drops to make two thick suspensions.
   Note: Colonies from a mannitol salt agar culture are not suitable for coagulase testing. The organism must first be cultured on nutrient agar or blood agar.
3. Add a loopful (not more) of plasma to one of the suspensions, and mix gently. Look for clumping of the organisms within 10 seconds.
   No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping.

Expected Results

Clumping within 10 secs	S. *aureus*
No clumping within 10 secs	No bound coagulase

Controls

Positive coagulase control: *Staphylococcus aureus*

Negative coagulase control: *Escherichia coli* or *Staphylococcus epidermidis*

Tube test method (detects free coagulase)

1 Take three small test tubes and label:

T = Test organism (18-24 h broth culture)

Pos = Positive control (18-24 h *S. aureus* broth culture)

Neg = Negative control (sterile broth)

2 Pipette 0.2 ml of plasma into each tube.

3 Add 0.8 ml of the test broth culture to tube T.

Add 0.8 ml of the *S. aureus* culture to the tube labelled 'Pos'.

Add 0.8 ml of sterile broth to the tube labelled 'Neg'.

4 After mixing gently, incubate the three tubes at 35-37°C. Examine for clotting after 1 hour. If no clotting has occurred, examine after 3 hours. If the test is still negative, leave the tube at room temperature overnight and examine again.

Note: When looking for clotting, tilt each tube gently.

Expected Results

Clotting of tube content or fibrin clot in tube ..... S. aureus

No clotting or fibrin clot .....Negative test

Note: There should be no clotting in the negative control tube.

2.5.4 Indole test

Testing for indole production is important in the identification of enterobacteria. Most strains of *E. coli, P. vulgaris, P. rettgeri, M. morganii,* and *Providencia* species break down the amino acid tryptophan with the release of indole.

Principle

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dimethylamino-benzaldehyde. This reacts with the indole to produce a red colored compound. Kovac's reagent is recommended in preference to Ehrlich's reagent for the detection of indole from enterobacteria.

Ways of performing an indole test:

- As a single test using tryptone water and Kovac's reagent.
- As a combined beta-glucuronidase-indole test using a Rosco PGUA/Indole identification tablet and Kovac's reagent. This is useful when identifying E. coli.
- As a combined lysine decarboxylase-indole test using a Rosco LDC/Indole identification tablet. This is useful in helping to identify salmonellae and *shigellae*

Detecting indole using tryptone water

1. Inoculate the test organism in a bijou bottle containing 3 ml of sterile tryptone water.
2. Incubate at 35-37 °C for up to 48 h.
3. Test for indole by adding 0.5 ml of Kovac's reagent. Shake gently. Examine for a red colour in the *surface layer* within 10 minutes.

Expected Results

Red surface layer Positive indole test

No red surface layer Negative indole test

Detecting indole using Rosco PGUA/Indole tablet

1. Prepare a dense suspension of the test organism in 0.25 ml physiological saline in a small tube.
2. Add a PGUA/Indole tablet and close the tube. Incubate at 35-37°C for 3-4 hours (or overnight).
3. First read the beta-glucuronidase (PGUA) reaction:

Expected Results

Yellow colour	Positive PGUA test
Colourless	Negative PGUA test

4. Add 3 drops of Kovac's reagent (Rosco code 920-31 or other Kovac's reagent) and shake.
5. Wait 3 minutes before reading the indole reaction. Examine the colour of the *surface layer.*

Expected Results

Red surface layer Positive indole test

Yellow surface layer Negative indole test

Note: About 94% of E. coli strains are PGUA positive and 99% are indole positive.

Detecting indole using Rosco LDC/indole tablet

1. Prepare a dense suspension of the test organism in 0.25 ml physiological saline in a small tube.
2. Add an LDC/indole tablet. Add 3 drops of paraffin oil and close the tube.
   The oil layer provides the anaerobic conditions required for the LDC reaction.
3. Incubate at 35-37 °C for 3-4 hours (or overnight).
4. First read the lysine decarboxylase (LDC) reaction:

Expected Results for LDC test

Blue/violet color*	Positive LDC test
Yellow, green or grey color	Negative LDC test

*If examining after overnight incubation, a positive test is indicated by a *strong* blue or violet color.

5. Add 3 drops of Kovac's reagent (Rosco code 920-31 or other Kovac's reagent) and shake.
6. Wait 3 minutes before reading the indole reaction. Examine the color of the surface layer.

Expected Results for indole test

Red surface layer	Positive indole test
Yellow surface layer	Negative indole test

2.5.5 Oxidase test/Cytochrome oxidase test

The oxidase test is used to assist in the identification of *Pseudomonas, Neisseria, Vibrio, Brucella,* and *Pasteurella* species, all of which produce the enzyme cytochrome oxidase.

Principle

A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. Alternatively an oxidase reagent strip can be used. When the organism is oxidase-producing the phenylenediamine in the reagent will be oxidized to a deep purple color.

Occasionally the test is performed by flooding the culture plate with oxidase reagent but this technique is not recommended for routine use because the reagent rapidly kills bacteria. It can however be useful when attempting to isolate *N. gonorrhoeae* colonies from mixed cultures in

the absence of a selective medium. The oxidase positive colonies must be removed and subcultured within 30 seconds of flooding the plate.

Important: Acidity inhibits oxidase enzyme activity, therefore the oxidase test must not be performed on colonies that produce fermentation on carbohydrate-containing media such as TCBS or MacConkey agar. Subinoculation on nutrient agar is required before the oxidase test can be performed. Colonies tested from a medium that contains nitrate may give unreliable oxidase test results.

Material Required

Oxidase reagent, freshly prepared, or use an oxidase reagent strip

Note: Fresh oxidase reagent is easily oxidized. When oxidized it appears blue and must not be used.

Stable oxidase reagent strips

Method (fresh reagent)

1. Place a piece of filter paper in a clean petri dish and add 2 or 3 drops of freshly prepared oxidase reagent.
2. Using a piece of stick or glass rod (not an oxidized wire loop), remove a colony of the test organism and smear it on the filter paper.
3. Look for the development of a blue-purple color within a few seconds as shown in color.

Expected Results

Blue-purple color (within 10 seconds)	Positive oxidase test
No blue-purple color (within 10 seconds)	Negative oxidase test

Note: Ignore any blue-purple color that develops after 10 seconds.

Method using an oxidase reagent strip

1. Moisten the strip with a drop of sterile water.

2. Using a piece of stick or glass rod (not an oxidized wire loop) remove a colony of the test organism and rub it on the strip.
3. Look for a red-purple color within 20 seconds.

   Red-purple color positive oxidase test.

Note: When using a Merck reagent strip, follow the manufacturer's instructions on how to perform the test.

Controls

Positive oxidase control: *Pseudomonas aeruginosa*

Negative oxidase control: *Escherichia coli*

2.5.6 Kliglar Iron Agar test (KIA test)

This test was carried out by using Kliglar's Iron Agar slant with a deep butt. The butt was stabbed inoculated and the slant was streaked. After incubation at optimum temperature for up to seven days, production of hydrogen sulphide was shown by blackening of thee medium. After inoculation if the slant is irregular containing bubbles then the inoculated microorganism is gas positive. If the slant is smooth then the microorganism is gas negative. In the test tube the top portion can be made acidic and the butt can be made alkaline. If the microorganism grows in the top the bacteria is can grow in acid. If the growth is observed in the butt then the microorganism can grow in alkaline pH.

2.5.7 Motility test

For motility test, tubes of semi solid agar medium were inoculated by stabbing the medium to a depth of 5 mm. The tubes were incubated at 37 °C for 24 hours. Growth all throughout the medium indicates the motility of the inoculated microorganism. The non-motile microorganism will be confined to the stab.

2.5.8 Agglutination test for serotyping

For final identification, organism in typical colonies is subjected to agglutination test using several polyvalent sera. Using a glass pencil, a glass slide is divided into several parts. Physiological saline is placed in each of the parts. Then the bacteria to be tested are taken with

platinum loop and mixed with the saline. Then the serum is added to the bacteria. The slide is tilted back and forth to observe the agglutination. Only strong agglutination occurring within one minute is a positive result.

.6 Overall Procedure:-

- All glass ware are sterilized using autoclave and/or hot air oven.
- 10 gm of the sample was weighed and taken in a sterilized beaker.
- 75 ml saline water was added to the beaker. The beaker was covered and kept for 60 minutes.
- The liquid portion was filtered using sterilized cotton and a sterilized funnel.
- 0.5 ml of the liquid portion was inoculated into media plate using pour plate method.
- The plate was incubated for 24 hours at 37° C
- The plates were observed and the results were recorded.
- Colony counting was done for the nutrient agar plates.
- Gram staining was done on a few randomly selected colonies on the nutrient agar plates.
- The bacterial were isolates and cultured in new media plates
- Biochemical tests were performed on the isolated bacteria

7 Data Analysis

All the data were checked after collection and analyzed. Then data was entered in to computer, with the help of MS word and MS excel. An analysis plan was developed keeping in view the objectives of the identification of microorganism in street vended foods around East West University. The results were presented in tabulated form and also in figures

Chapter 3

Results

pakura

Figure 8:- pakura inoculated XLD media agar plate

Pakura was inoculated in XLD media. Growth was seen. Yellow colony with black center was seen. The media was discolored. Yellow colony indicates the presence of *Coliforms.* Colony with black center indicated the presence of *Salmonella* in the inoculated sample.

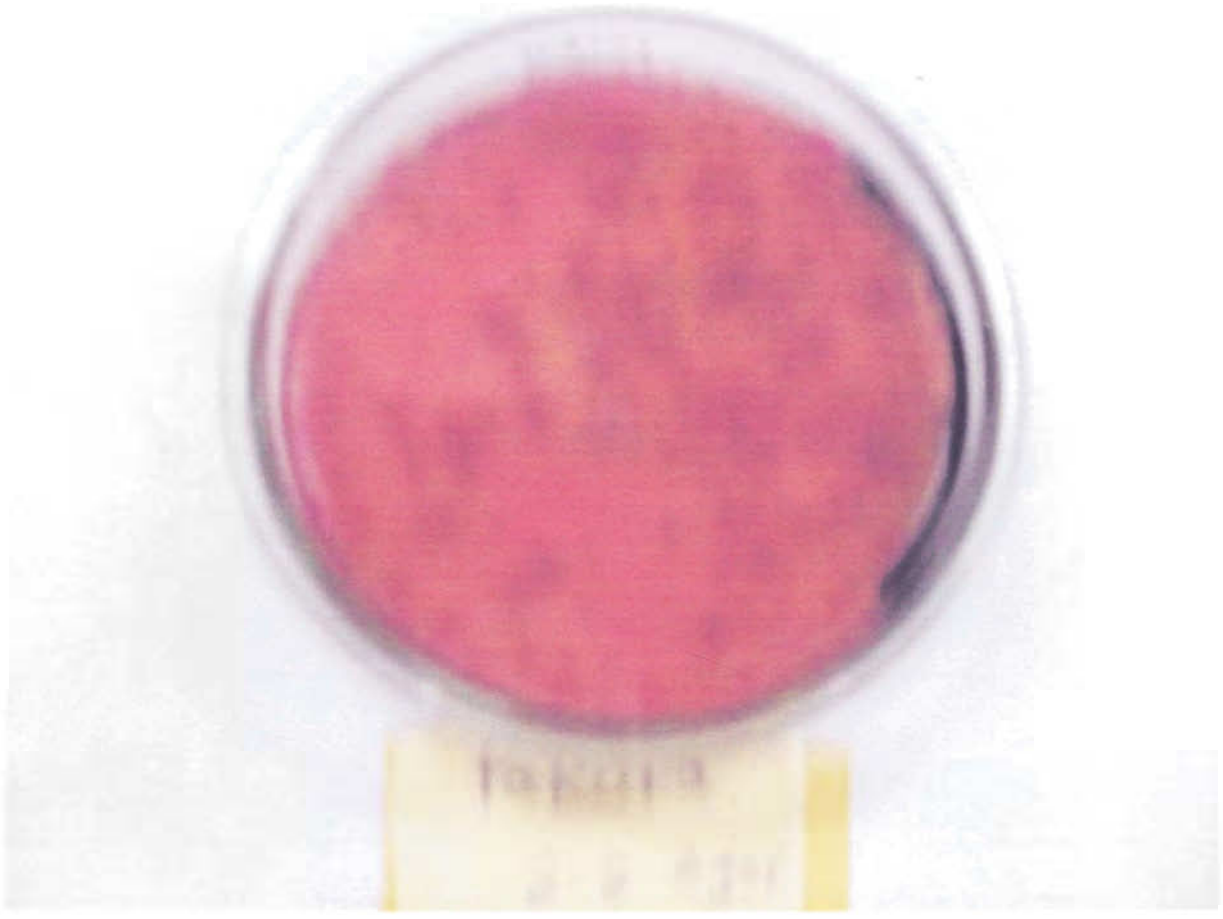

Figure 9:- pakura inoculated S.S agar plate

Pakura was inoculated in SS agar media. Heavy growth was observed (Figure 2). Pink and purple colonies were observed. Colonies with black center was observed. Discoloration was observed in part of the plate. Red colonies may be *E. coli*. Colonies with black center may be *Salmonella*.

Figure 10:- pakura inoculated MacConKey agar media plate

Pakura was inoculated in MacConkey agar media. Heavy growth was observed (Figure 3). Pink and purple colonies were observed. Colonies with black center was observed. Discoloration was observed in part of the plate. Red colonies may be *E. coli*. Colonies with black center may be *Salmonella*.

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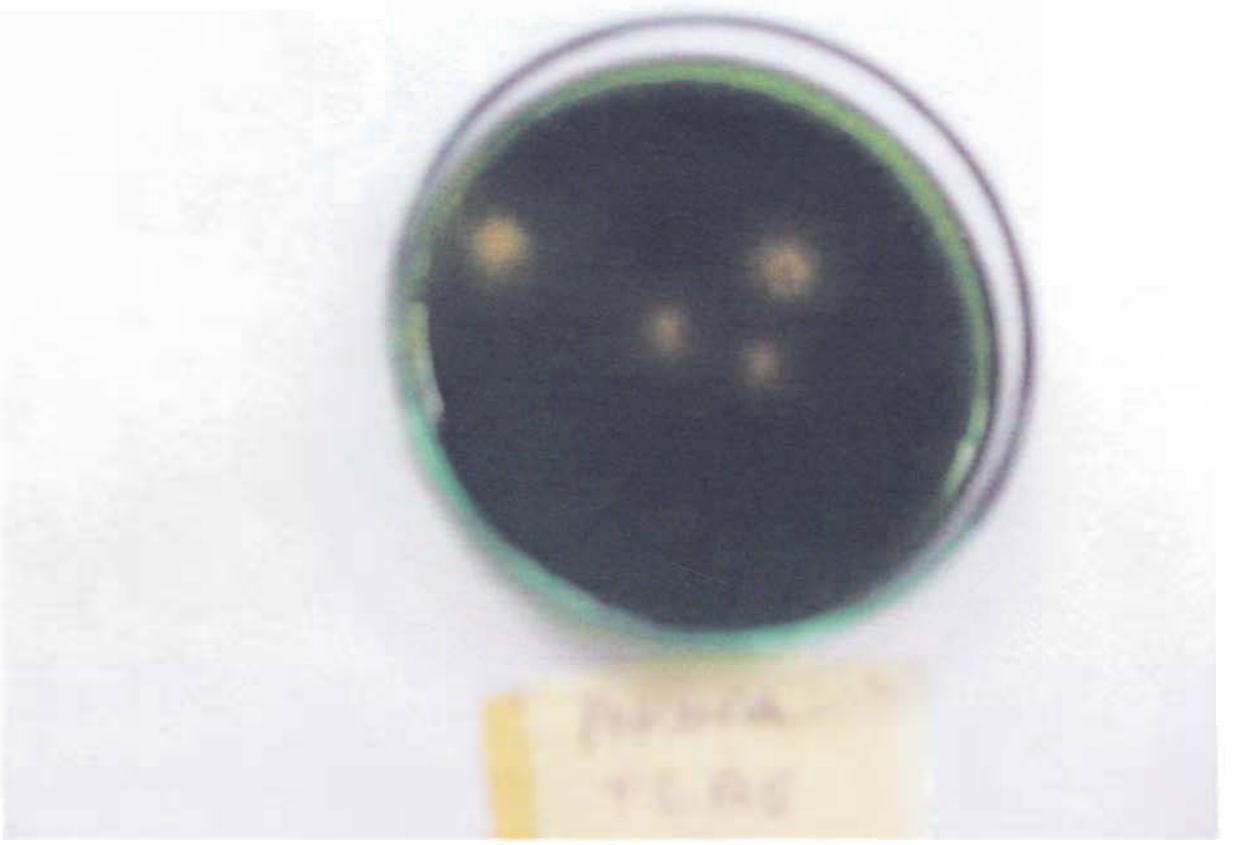


Figure 11:- pakura inoculated TCBS media plate

pakura was inoculated in TCBS agar media. growth was seen. This means Vibrio species was present in the inoculated sample.

3.2 piazue

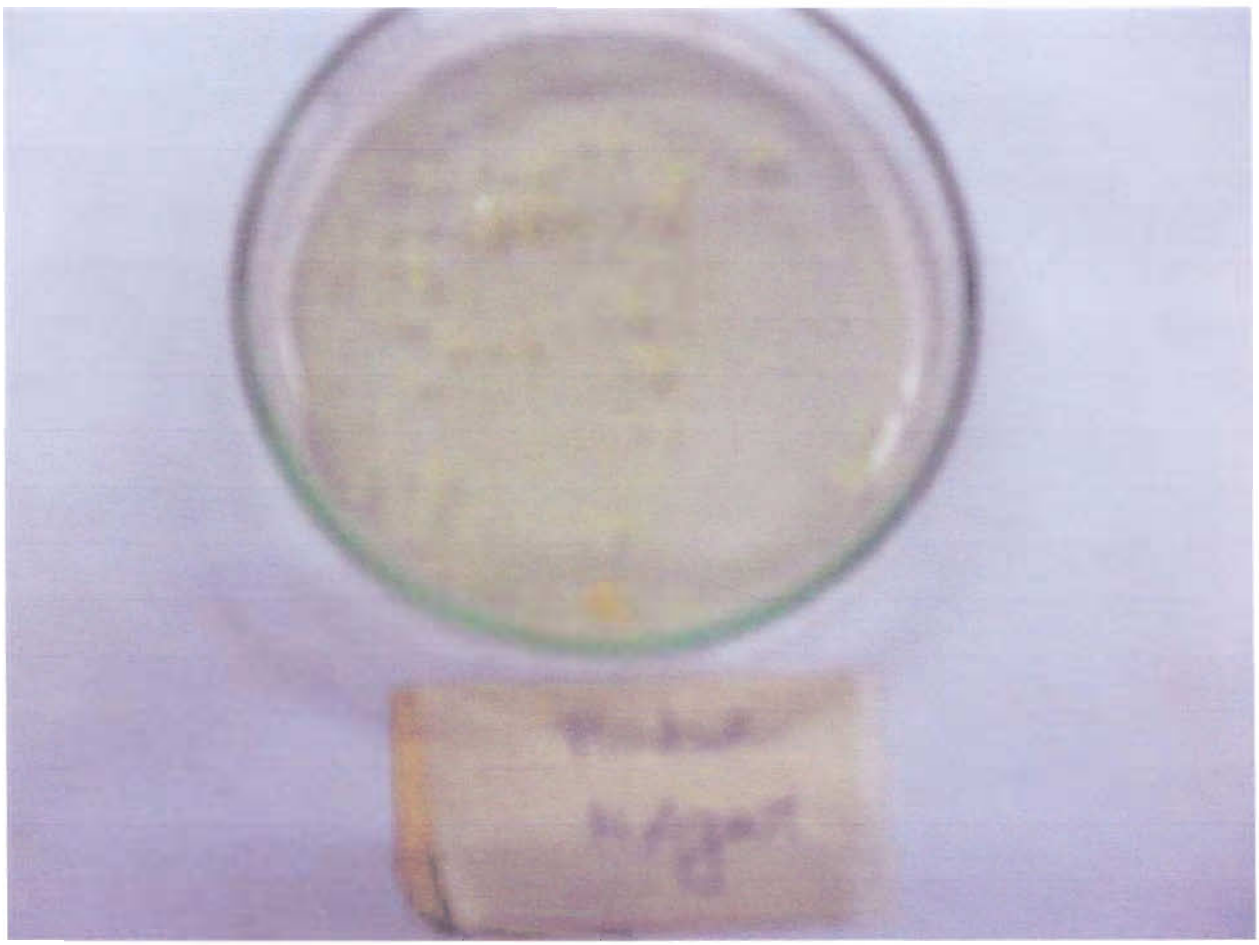


Figure12:- piazue inoculated S.S agar plate

Piazue was inoculated in nutrient agar media. Bacterial growth was observed. Colony counting showed 380 colonies in the entire plate(Figure 7). A few large colonies were seen but most of the colonies were small in size. Gram staining was performed on 5 randomly selected colonies. 4 of the 5 colonies were Gram positive(Figure 8 B) bacteria and one of the colonies were a gram negative bacteria (Figure 8 A).

3.3 Sugarcane

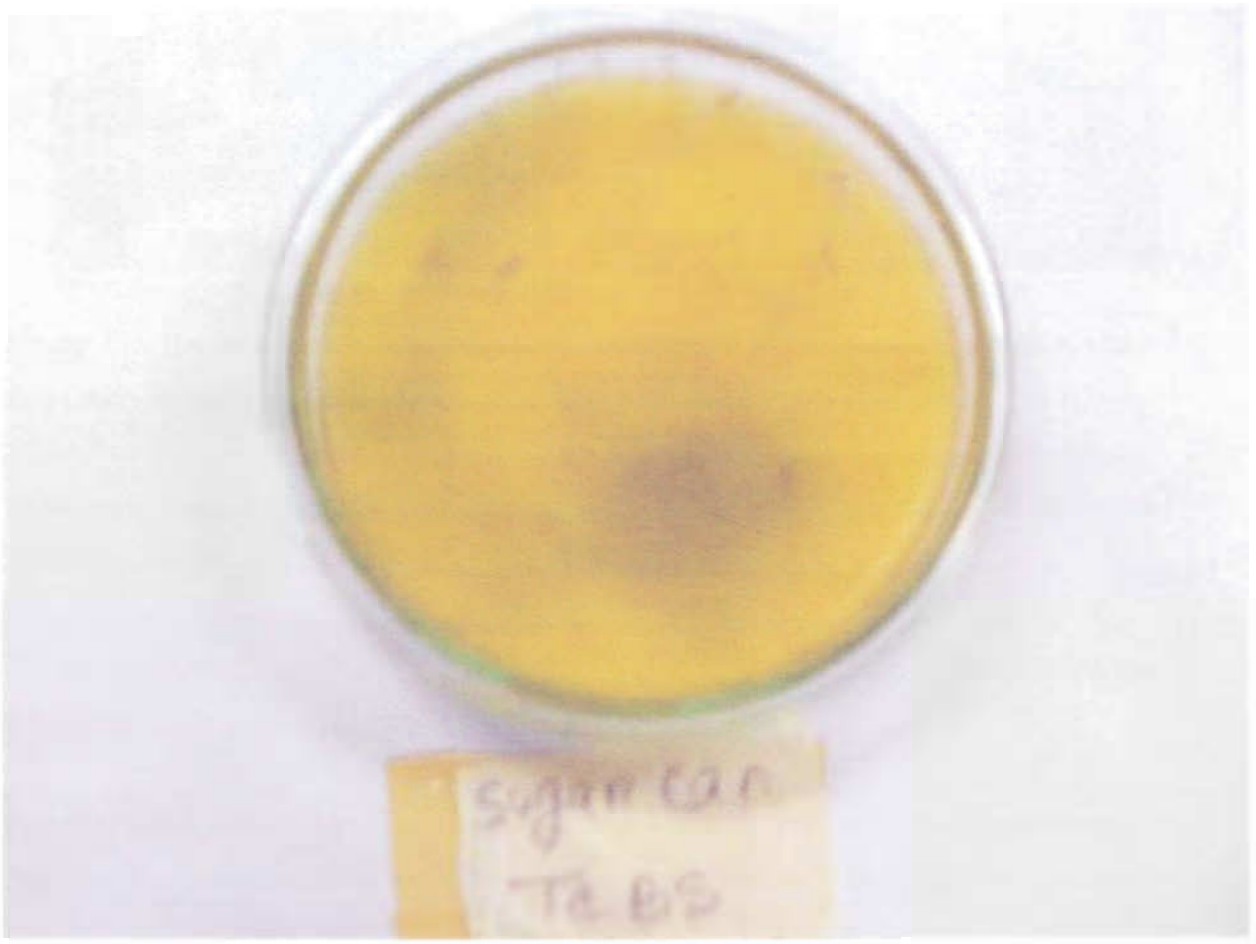
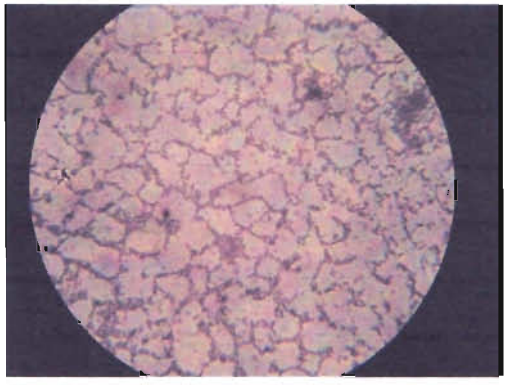


Figure 13:- Sugarcan inoculated TCBS media plate

Sugar cane was inoculated in TCBS media. No growth was seen. This means *Vibrio* species was not present in the inoculated sample

Microscopic observation

A



B

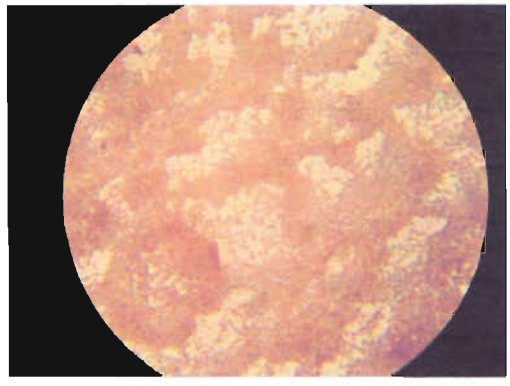


Figure 14:- Bacteria from pakura inoculated nutrient agar plate, viewed under microscope after Gram Staining. **A**- Gram positive bacteria; **B**- Gram Negative Bacteria

pakura was inoculated in MacConkey agar media. No growth was seen. This indicates that Gram negative bacteria were not present in the inoculated sample.

Pakura was inoculated in TCBS media. No growth was seen. This means *Vibrio* species was not present in the inoculated sample.

Pakura was inoculated in XLD media. No growth was seen. This indicates that *Salmonella, Shigella, Coliforms*, and *Pseudomonas aeruginosa* were not present in the inoculated sample.

Pakura was inoculated in SS agar media. No growth was seen. This indicates that the inoculated sample did not contain *Salmonella* or *Shigella*.

Table 8: Presence of microorganisms in different media

Number	Food Samples	Nutrient Agar	MacConkey Agar	XLD	TCBS	SS Agar
1	pakura	+ +	+ +	+ +	+ +	+ +
2	Piazu	+	-	-	-	-
3	Sugar cane	–	+	-	+	+

Note: (+)= Presence of microorganism

(-)= Absence of microorganism

Table 9: Gram Staining result in nutrient agar media

Number	Food Samples	Nutrient Agar	
		Gram +Ve	Gram -Ve
1	pakura	-	+
2	piazue	+	-
3	Sugar can	-	+

4.1 Discussion and Conclusion

This research was conducted on food samples of pakura, Piazue, Sugarcan. The results of this study showed that some of the food items are highly contaminated with microorganisms while others are relatively safer. In pakura I observed different microbial growth like *E. coli, Salmonella, Shigella, V. cholerae*. Pakura is made by the vegetables which contains moisture that may be the main cause of microbial growth. In piazue and Sugarcane were low growths of microorganism seen because they do not not remain for long time for selling. Piazues are made in boiled oil and there is every chance of the death of bacteria in boiled oil. Microbial growth can occur in the food by different sources like: During preparation of the food, When foods are being served, during the cleaning and washing of dishes and from the vendor's hand.

Most of the street vendors of Bangladesh are illiterate. They hardly know about food safety and hygiene practice. Some of the vendors use same water to clean the dishes repeatedly. This can be a source of contamination. They need to use clean and fresh water to clean dishes and prepare foods. So it is important to make them aware of the consequences of food-borne diseases. To prevent the contamination proper hygiene practice needs to be maintained.

Contamination can also occur because of improper storage conditions. The item is usually open to the air and it can be easily contaminated with dust particles and insects. Pathogens can be passed by flies. *Salmonella typhimurium* and *Shigella* can multiply in the gut of the housefly and can be excreted for weeks or longer **(Levine OS. *et al.* 1991).**

The use of soap to wash hands and crockery reduce the level of bacteria; Gram negative bacilli such as *S. typhi* are fairly susceptible to soap made from saturated fatty acids but resistant to soaps made from unsaturated fatty acids. Most microorganisms die after coming in contact with soap but their susceptibilities vary **(Banna EA. *et al.* 1965).**

Some samples were contaminated by microorganisms which lead to diseases like dysentery, diarrhea, enteric fever etc. Student should need to be more careful while purchasing food from the street venders.

Rules and regulation should be taken by the government, so that the level of contamination can be reduced as it is a serious issue for public health. Government agencies must enforce regulation on sanitary practice during the preparation and selling of street food. Non-Governmental organizations (NGOs) and the press could play key roles in educating the vendors on food and personal hygiene.

Preparation of food and good hygienic practice are essential to improve the safety and quality of street-vended food. Training of food handlers regarding the hazards about their product is essential. University can also take some steps against substandard food seller. Otherwise the possibility of food borne diseases spreading cannot be ignored.

The result of the study showed that Pakura has a high possibility of spreading food borne diseases among the students. Sources of contamination can be contaminated raw materials, improper or in adequate cooking, or improper storage. Sugarcane showed low growth as well because it is not remain for long time for selling purpose. As a result bacterial growth is not favored. In our country, consumers are often unaware of the relationship between contaminated food and food borne diseases. Consumers provide perhaps the strongest motivating force for vendors to alter their food handling practices.

Regulation is a common approach to controlling the street-vended food sector. Registering or licensing vendors can be a good way to regulate the food safety.

Common facilities are required like potable water and electric supplies, waste disposal services, drainage, toilets, etc. In addition common utensils can be centrally supplied and cleaned. There is also a need for improving the design and construction of the street vending stalls.

Our study reveals the potential hazard about three street vended foods: pakura, sugarcane piazue around the East West University.

Reference

Aycicek H,CakirogluS.Stevenson TH. 2005 Incidence of Staphylococcus aureus in ready-to-eat meals from military cafeteria in Ankara,Turkey.Food control.16531-534.

Ali M, Emch M, Yunus M, Sack D, Lopez AL, Holmgren J, Clemens J. 2008. Vaccination Protection of Bangladeshi infants and young children against cholera: implications for vaccine deployment and person-to-person transmission. *Pediatr Infect Dis J.* **27**(1) 33-7

Bryan FL, Jermini, M,Schmitt R,et al. 1997. Hazardas associated with holding and reheating foods at vending site in a small town of Zambia. *J Food port* **57**:895..

Bean NH, Grifin PM, Goulding JS, Ivey CB,1990. Food borne disease out breaks, A five year summary 1983-1987.

Canet & N diaye 1996,Street food in Africa. *J food nutrit Agric* **17/18**:4-13.

East West University medical center records.

Food And Agriculture organization.1997.Percentage of Woman street food vendors (cited 20Nov 2004)http//www.fao.org.

Gupta S, et al. Verocytopanic activity of *Escherichia coli* 0157:H7 and "O" serogroups isolated from patients of diarrhea. *Indian J Med Res.* 1992; **95**: 71-76.

H. C. Gugnani. 1999. Some emerging food and water borne pathogens. *J. Commu. Dis.* **31(2):** 65-72.

Hart CA, Tress J, and Duerden B. Zoonosis. *J. Med. Microbial.* 1997; **46**: 4-33.

Maria-Jose Santos, Jose Rocha Nogueira, Luis Patarata, Olga Mayan. 2008. Knowledge levels of food handles in Portuguese school canteens and their self-reported behavior towards food safety. *International Journal of Environmental Health Research.* **18(6).** 387-388.

Moushumi Ghosh, Sidhi Wahi, Mukesh Kumar, Abhijit Ganguli. 2007. Prevalence of enterotoxigenic Staphylococcus aureus and Shigella spp. In some raw street vended Indian foods. **17(2).** 151-152

Mankee A,Ali S,Chin A,et al.2003.Bacteriological quality of doubles sold by street-vended in Trinidad and the attitudes, knowledge and perception of the public about its consumption and health.

Mensah P, Yeboah-manu D,Owusu-Darko K,Ablordey A,2002.Street food in Accra,Ghana/WHO **80**:546

Patience Mesah, Dorothy Yeboah-Manu, Kwaku Owusu-Darko, Anthony Ablordey. 2002. Street foods in Accra, Ghana: how safe are they. *Bulletin of the World Health Organization.* **80(7).** 546

Tinker 1997. Street foods,Urban food and employment in developing countrys.Berkely:Oxford University press.

Vargas M,Gascon J,de anta JMT.1999. Prevalence of shigella enterotoxin1 And 2 among Shigella Strains isolated from patient with travelers diarrhea .*J Clin Microbial* **37**:3608-3611.

Who 1996.Essential safety requirement for street vended food. Geneva: WHO. pp 2-11.

Warren Levinson. 2004. Medical microbiology and immunology. 8th edition. **131.** (6). 4096-104)

R. G. Feachem, R. C. Hogan, M. H. Merson. 1983. Diarrhoeal disease control:reviews of potential interventions. *Bulletin of the World Health Organization*. **61**(4). 638 Retrieved on 2007-07-28.

Vishwanathan P, Kaur R. 2001. Prevalence and growt of pathogens an salad vegetables, fruits and sprouts. *Int J Hyg Environ Health*. **203**. 205-213

World Health Organization (WHO) 1996. Essential safety requirements for street vended foods (revised edition). Geneva: WHO pg 2-11

Who 1996. Essential safety requirement for street vended food. Geneva: WHO.

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APPENDIX I

Formula for nutrient agar media

Approximate Formula per Liter

Beef Extract ..... 3.0 g

Peptone ..... 5.0 g

Agar ..... 15.0 g

Formula for MacConkey agar media

Approximate Formula per Liter

Enzymatic Digest of Gelatin.....17 g

Enzymatic Digest of Casein.....1.5 g

Enzymatic Digest of Animal Tissue.....1.5 g

Lactose.....10 g

Bile Salts Mixture.....1.5 g

Sodium Chloride.....5 g

Neutral Red.....0.03 g

Crystal Violet.....0.001 g

Agar.....13.5 g

Final pH: 7.1 ± 0.2 at 25°C

Formula for TCBS agar media

Approximate Formula per Liter

Yeast Extract.....5.0 g

Proteose Peptone No. 3 .....10.0 g

Sodium Citrate .....10.0 g

Sodium Thiosulfate.....10.0 g

Oxgall .....8.0 g

Saccharose..... 20.0 g

Sodium Chloride .....10.0 g

Ferric Ammonium Citrate.....1.0 g

Bromthymol Blue	0.04 g
Thymol Blue	0.04 g
Agar	15.0 g

Formula for XLD agar media

Approximate Formula per Liter

Yeast extract	3 g
L-lysine	5 g
Xylose	3.7 g
Lactose	7.5 g
Sucrose	7.5 g
Sodium deoxycholate	1 g
Sodium chloride	5 g
Sodium thiosulfate	6.8 g
Ferric ammonium sulfate	0.8 g
Phenol red	0.08 g
Agar	12.5 g

Formula for SS agar media

Difco ™ SS agar

Approximate formula per liter

Beef extract	5g
Protease peptone	5g
Lactose	10g
Bile salt no. 3	8.5g
Sodium citrate	8.5g
Ferric citrate	1g
Agar	13.5g
Brilliant green	0.33g
Neutral red	25mg

BBL ™ Salmonella Shigella agar

Approximate formula per liter

Beef extract	5g
Pancreatic digest of casein	2.5g
Peptic Digest of Animal tissue	2.5g
Lactose	10g
Bile salt no. 3	8.5g
Sodium citrate	8.5g
Ferric citrate	1g
Agar	13.5g
Brilliant green	0.33g
Neutral red	25mg