Microbial profile testing of ready-to-eat street vended foods collected from different university premises

A research paper is submitted to the Department of Pharmacy, East West University in conformity with the requirements for the degree of Bachelor of Pharmacy.

Submitted by

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Declaration by the Candidate

I, Shahnaz Siddiqua, hereby declare that the dissertation entitled "Microbial profile testing of ready-to-eat street vended foods collected from different university premises" submitted by me to the Department of Pharmacy, East West University and in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, work carried out by me during the period 2016 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.

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List of Abbreviations:

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

Abstract:

"Street foods" describes as a wide range of ready-to-eat foods and beverages sold and sometimes prepared in public places, notably streets. The objective of this study was to isolate, identify and test microbial profile of enteric bacteria (*Escherichia coli*, *Klebsiella spp*, *Shigella* spp, *Salmonella spp* and *Vibrio* spp) in different street vended foods collected from different private universities in Dhaka city. Thirty food samples were collected from fixed and mobile vendors from area around 10 private universities in Dhaka city. The tested samples were laddu, singara, somucha jhal-muri, fuchka, vhel-puri, panipuri, bun, cake, danish, chola, peaju, sweet, sheek-kabab, etc. Sterile polythene bags were used to collect 3 different samples each day from each university. They were tested for the presence of microorganisms following conventional microbiological processes. Biochemical tests were done for the confirmation of *Escherichia coli*, *Klebsiella spp*, *Shigella* spp, *Salmonella* and *Vibrio* spp. Out of 30 foodsamples, six (60%) were suspected to contain *Klebsiella spp,* three (30%) were suspected to contain *Escherichia coli*, and one (10%) was suspected to contain *Vibrio spp*. All these enteric pathogens could be the potential cause for foodborne illnesses and provision of education to the vendors would improve quality of street foods.

Key Words: Street foods,microbial profile test,*Escherichia coli*,*Klebsiella Spp*,*Shigella spp, Vibrio spp,* Dhaka city, Private University.

1.1 Street foods:

"Street foods" describes as a wide range of ready-to-eat foods and beverages sold and sometimes prepared in public places, notably streets. The final preparation of street foods occurs when the customer orders the meal which can be consumed where it is purchased or taken away, like fast foods. Street foods and fast foods are low in cost compared with restaurant meals and offer an attractive alternative to home-cooked food. In spite of these similarities, street food and fast food enterprises differ in variety, environment, marketing techniques and ownership (FAO Corporate document Repository, 2007). Various attempts have been made to define them, but the most widely cited definition is that of FAO:

"Street foods are ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers especially in streets and other similar public places" (FAO 1989).

Fig1.1: Street vended foods

In unhygienic conditions the street vended foods are prepared and displayed where the foods are open to a high degree of contamination. Street foods are sometimes stored at improper temperatures and sold from vending sites which includes kiosks, make-shift accommodation, and push carts as well as other temporary structures. In most cases running water is not available at vending sites, washing of hands and crockery are done in bowls or buckets and sometimes without soap. Thus from the health point of view, selling foods in the street is very controversial (Bereda et al., 2016). These foods can endanger public health by causing various acute and chronic food borne diseases through pathogenic microbes or toxic substances present in them (Nazni. P & Jaganathan. A, 2014)

1.2 Types of Street Foods:

Dhaka is a very popular place for many kind of street Foods. Some examples are chop,nuts,Buts,cake,Tea,Puri,Vhapapitha,Chitoipitha,Achaar,Egg,Vegetableroll,Golgappa, Banana etc. The most popular and traditional street-vended foods in Bangladesh include jhalmuri, fuchka, vhel-puri, panipuri, bun, cake, danish, betel-leaf, chola, peaju, sweet, sheek-kabab, laddu, singara, somucha etc (Rahman, Rahman &Ansary, 2014).

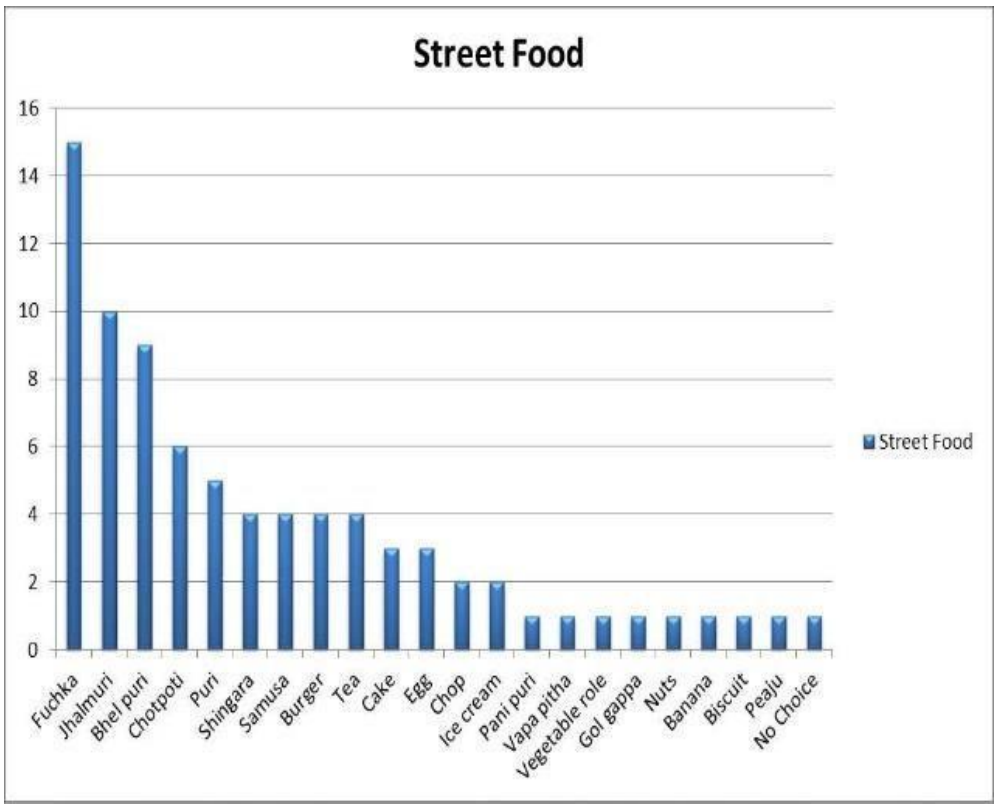


Figure 1.2: A Bar Diagram Showing Most Favorite Street foods in Bangladesh

1.3 Factors that Affect the Preference Levels of Consumers Regarding Street:

Food Consumer preferences can be termed as the individual taste which is measured by the various levels of stimulus which instigate a consumer to buy certain products and services. There may be demographic factors, social, psychographic factors related to this preference level.

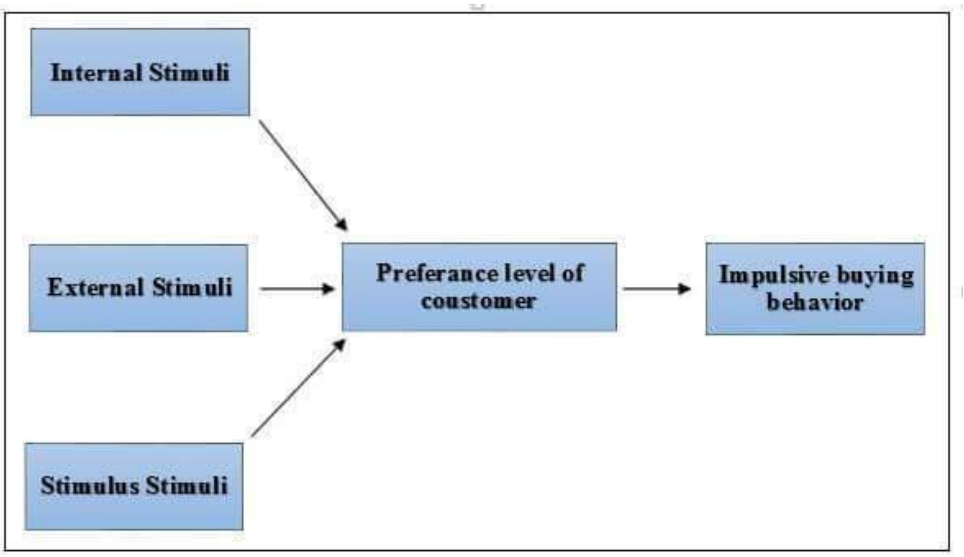


Figure 1.3: Criteria Affecting Consumer Preferences (proposed model)

It can be said that consumers prefer the street food for this aspect shown in this graph. But to be precise large scale research need to be conducted to analyze the street food consumers, according to their demographic situation as well as their preference criteria (N.Kasem, 2005).

1.4 Street Vendors:

Today, vending is an important source of employment for a large number of urban poor because it requires low skills and small financial inputs. A street vendor is a person who offers goods or services for sale to the public without having a permanent built-up structure but with a temporary static structure or mobile stall (or head-load). Street vendors could be stationary and occupy space on the pavements or other public/private areas, or could be mobile, and move from place to place carrying their wares on push carts or in cycles or baskets on their heads, or could sell their wares in moving buses. The Government of India has used the term 'urban vendor' as inclusive of traders and service providers, stationary as well as mobile, and incorporates all other local/region specific terms used to describe them (WIEGO, Empowering Informal Workers, Securing Informal livelihood).

1.5 Sources and quality of raw foods and ingredients:

Vendors to maximize the profit or to make street foods affordable for the consumers, some vendors patronize cheap and unsafe ingredients that may be detrimental to the health of the consumers.

Results of survey conducted by Omemu and Aderoju showed that street food vendors in Nigeria considered the volume (94%) and the price (93%) than the freshness and cleanliness when buying raw foods to be cooked or vended. In the study conducted in India, (Choudhury et al.)observed that procurement habits of food items by street vendors differ according to the size of the establishments and was significantly influenced by the vendors type, ownership and average monthly income. The study reported that all the mobile vendors and owners of small restaurant procure unlabeled and unpacked food grains and semi-processed ingredients from grocery shops. While majority (87%) of owners of small restaurants procures labeled and packed condiments, dry fruits and spices from grocery, most (44%) of the mobile food vendors purchase condiments and spices, nuts and dry fruits from traditional weekly or daily markets with 37% of them prepared, dried and powdered their own ingredients at home. Close to 56% of mobile vendors used unlabeled and unpacked condiments and spices. Studies have shown that homemade cereal flour and condiments used in street foods preparations are contaminated with *Bacillus cereus* which was reported to be responsible for outbreak of food borne illness. Some street food vendors use leftover perishable raw materials for next day preparation without storage facility. Not a single small restaurant owners interviewed by (Choudhury et al.) had refrigeration facility, whereas 20%, 93%, 97% and 30% of them stored left-over green vegetables, raw food materials, canned/bottled foods, and milk and milk products, respectively for more than 24h.Poisoned fish from chemically treated ponds, meat and milk from sick and old animals, use of substandard slaughter facility, and vegetables and crops with heavy chemical residues are often use for food preparation in some developing countries. These practices were encouraged by weak regulatory and inspection facilities in these countries. Inadequate cooking of ingredients with heavy microbial loads could results in the survival of pathogens of significant health importance to the consumers (B.A Alimi, 2016).

1.6 Food Poisoning:

Food poisoning is globally important, as they result in considerable morbidity, mortality, and economic costs. Food poisoningis infections or irritations of the gastrointestinal (GI) tract caused by eating contaminated food or beverages. Infectious organisms including bacteria, viruses and

parasites or their toxins are the most common causes of food poisoning. Infectious organisms or their toxins can contaminate food at any point of processing, production, growing, harvesting, storing, shipping or preparing (Kirk et al., 2015). Microbiological contamination of street foods has become a major public health concern (WHO, 2002). The majority of street food vendors are uninformed of good hygiene practices (GHP) (Mensah et al., 2002), which poses increased risk of contamination for most of the food products involved In most parts of the world, food-borne disease incidences are more commonly associated with Salmonella serotype enteritidis (SE),

Vibrio cholera, *Escherichia coli* serotype 0157:H7, *Listeria monocytogenes* and food-borne trematodes. The emergence of the above mentioned disease outbreaks is mainly linked to globalization of food supply that introduces pathogens into new geographical areas, exposure of travelers, refugees and immigrants to unfamiliar foodborne hazards, mutations in microorganisms, changes in the human population and changes in peoples' life styles (D. Mugampoza, 2013).

1.6.1 Primary influences contributing to pathogenic contamination:

Vending Location: Food Handling and Waste Disposal-

The conditions under which some street vendors operate are reported to be unsuitable for the preparation and selling of food. The two major sources from where the contaminants can enter the preparation area are: Improper food handling and waste disposal.

Waste Disposal -

Few vendors congregate in overcrowded areas where there are high numbers of potential customers, which usually provide limited access to basic sanitary facilities. Hence, the contamination of street foods is often linked to the waste generated by food processing, that is usually dumped near the vending site. The lack of facilities for liquid drainage and wastewater and garbage disposal encourages wastes to be thrown into nearby streets and gutters. Such areas act as habitats for rodents, breeding points for flies and media for growth of microorganisms (Rane, 2011).

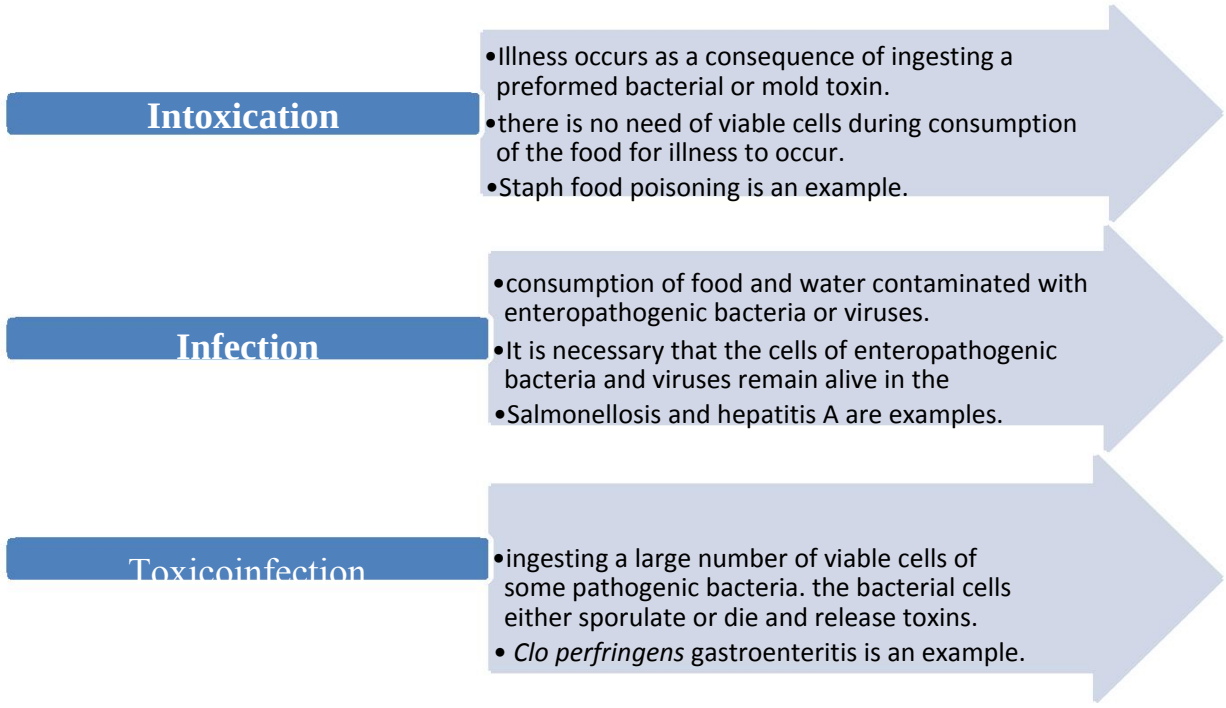
Food Handling-

Unsanitary handling of street foods by the some of the vendor has been commonly found to be the source of contamination. The vendors can be carriers of pathogens like Escherichia coli, Salmonella, Shigella, Campylobacter and S. aureus who eventually transfer these food borne

hazards to the consumers. The hands of the food handlers are the most important vehicle for the transfer of organisms from faeces, nose, and skin to the food (Rane, 2011).

1.6.2 Types of diseases caused by food poisoning:

Diseases caused due to viable pathogenic bacteria cells, spores in the case of infant botulism or food containing toxins produced by toxigenic bacteria and molds. On the basis of mode of illnesses, these can be arbitrarily divided into three groups: intoxication or poisoning, infection, and toxicoinfection (Ray, 2004).



1.6.3 Persons susceptible for Food Poisoning:

The probability of the illness of a person after eating contaminated foods depends on the organism, the amount of exposure, age and health. Anyone can get a food borne illness. However, some people are more likely to develop food borne illnesses than others. High-risk groups include:

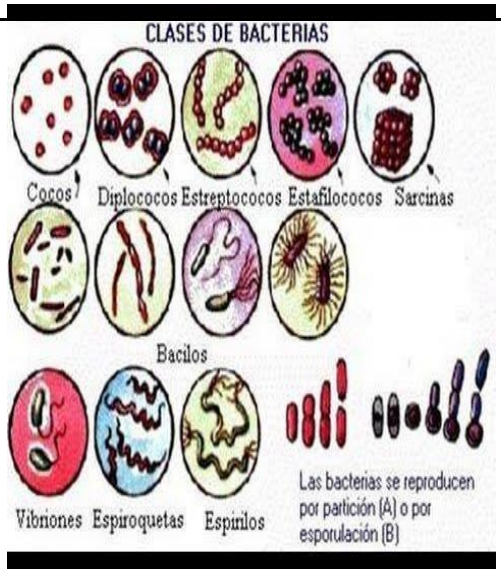
- **Older adults:** As anyone gets older, immune system may not respond as quickly and as effectively to infectious organisms as when younger.
- **Pregnant women**: During pregnancy, changes in metabolism and circulation may increase the risk of food poisoning. The reaction may be more severe during pregnancy.

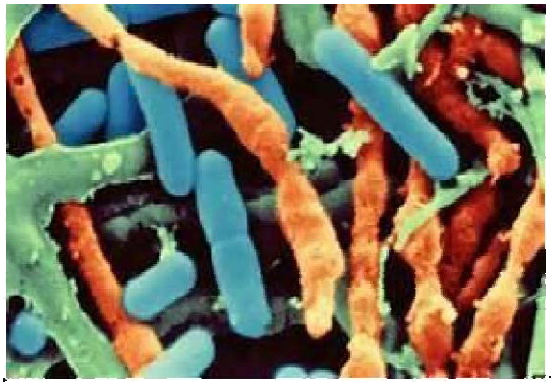
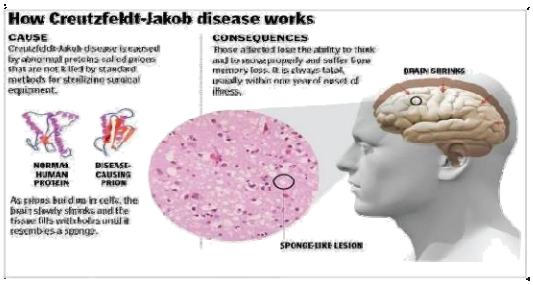
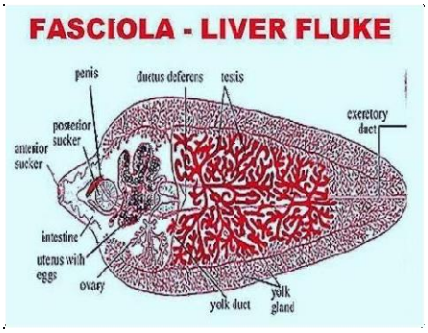
  Rarely, the baby may get sick, too.
- **Infants and young children:** Their immune systems haven't fully developed.
- **People with chronic disease:** Having a chronic condition such as diabetes, liver disease or AIDS or receiving chemotherapy or radiation therapy for cancer reduces immune

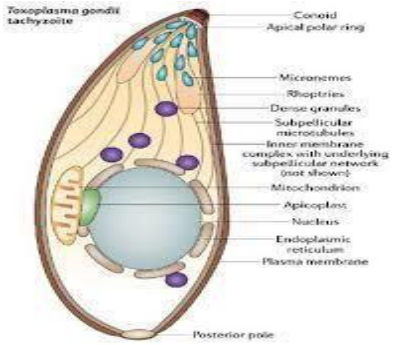
  response (Niddk.nih.gov, 2016).

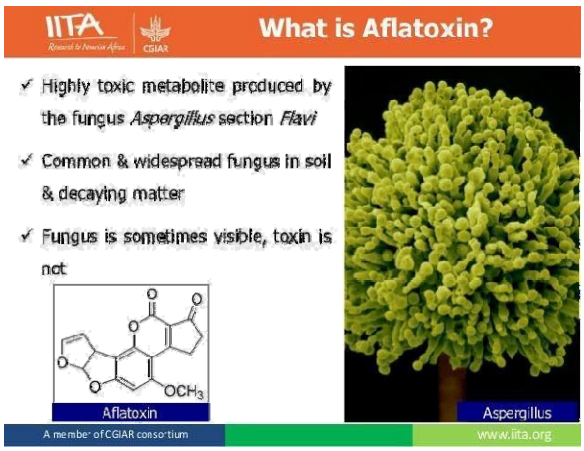
1.6.4 Reasons for Food Poisoning:

Under certain circumstances a number of different infectious pathogen may be contracted from foods, some exclusive or predominant pathogens that are contracted from the consumption of food products. The recognized food borne pathogens include bacteria, viruses, multicellular animal parasites, protozoa, fungi, and possibly prions. Some harmful microorganisms may already be present in foods when they are purchased. Raw foods including meat, poultry, fish and shellfish, eggs, unpasteurized milk and dairy products often contain microorganisms that cause food poisoning.

Pathogens	Examples	Figure
1. Bacteria	Gram positive*Staphylococcus, Bacillus cereus, Clostridium botulinum, C. perfringens, Listeria monocytogenes, Mycobacterium paratuberculosis* Gram negative- *Salmonella, Shigella, Escherichia, Yersinia, Vibrio, Campylobacter,*	 **Fig 1.6.4: Gram(+ve) and Gram (-ve) Bacteria**

2. Listeria	*Listeria monocytogenes, Listeria ivanovii L.seeligeri* Twotypically nonhemolytic species- (*L. innocua* and *L. welshimeri*) (Brian D. Sauders et. Al. 2012)	 **figure 1.6.4:Listerium**
3. Viruses.	Hepatitis A, - Small round structured viruses (SRSVs), -Rotaviruses	 **Figure 1.6.4: Viruses**
4.Prions	Creutzfeldt-Jakob disease	 **figure 1.6.4: prions for causing Creutzfeldt-Jakob disease**
5.Flatworms Flukes-	Fasciola,Fasciolopsis, Paragonimus, Clonorchis	 **Figure 1.6.4: *Fasciola Hepatica***

Pathogens	Examples	Figure
6. Roundworms Pseudoterranova	Trichinella, Ascaris, Anisakis, Toxocara,	 **figure 1.6.4: *Ascarislumbricoides***
7. Tapeworms-	Diphyllobothrium, Taenia	 **figure 1.6.4: *Taeniasolium***
8. Protozoa	Giardia, Entamoeba, Toxoplasma, Sarcocystis, Cryptosporium, Cyclospora	 **Figure 1.6.4: *Toxoplasma gondii***

Pathogens	Examples	Figure
9. Fungi	mycotoxin producers Aflatoxins, Fumonisins, Alternaria toxins, Ochratoxins	 **figure 1.6.4: *Aspergillusflavi***
10. Toxigenic phytoplanktons	Paralytic shellfish poison, Domoic acid, Pfiesteriapiscicida, Ciguatoxin	 **figure 1.6.4: plantothrix**

1.7 Influencing factors affecting Microbial growth:

There are some intrinsic and extrinsic parameters that help to prevent or retardant of the microbial growth. Plants and animals are major sources of the food and plants and animals have its own mechanisms to prevent the invasion ad proliferation of microorganisms and causing diseases. Though diseases occurs if the raw materials are not fresh, and those have not the ability to prevent the microbial growth (Jay et al., 2005).

1.7.1 Intrinsic Parameters:

In plants and animal tissues which parts are inheriting parts of the tissues are referred as intrinsic parameters to prevent microbial growth (Jay, et al., 2005).

1.7.2 Extrinsic Parameters:

The extrinsic parameters of foods are those properties of the storage environment that affect both the foods and their microorganisms (Jay, et al., 2005).Those of greatest importance to the welfare of food borne organisms are as follows:

Table 1.7 Intrinsic and Extrinsic parameters:

Intrinsic Parameters	Extrinsic Parameter
▪ pH	▪ Temperature of storage
▪ Moisture content	▪ Relative humidity of environment
▪ Oxidation-reduction potential (Eh)	▪ Presence and concentration of gases
▪ Nutrient content	▪ Presence and activities of other microorganisms
▪ Antimicrobial constituents	
▪ Biological structures	

(Jay et al., 2005).

The food processor reduces potential problems from microorganisms in several ways:

- Minimizing microbial growth on equipment, by cleaning and sanitizing, and in the product itself by adjusting storage temperature, pH, and other environmental factors
- Removing or destroying them by trimming, washing, heating, pickling, by adding chemicals, or by encouraging competition by acid- or alcohol-forming organisms.
- Minimizing contamination from equipment, people, the environment, and from unprocessed food (Ray, 2004).

1.8 Microorganisms Causing Food Borne Illnesses:

1.8.1 *Escherichia coli*

Escherichia coli (or E. coli) are the most prevalent infecting organisms in the family of gramnegative bacteria known as enterobacteriaceae. E. coli bacteria normally live in the intestines of healthy people and animals. The bacteria are rod shaped, non-spore forming, motile with peritrichous flagella or nonmotile. They can grow under aerobic and anaerobic conditions and do not produce enterotoxins (Adams & Moss, 2008).

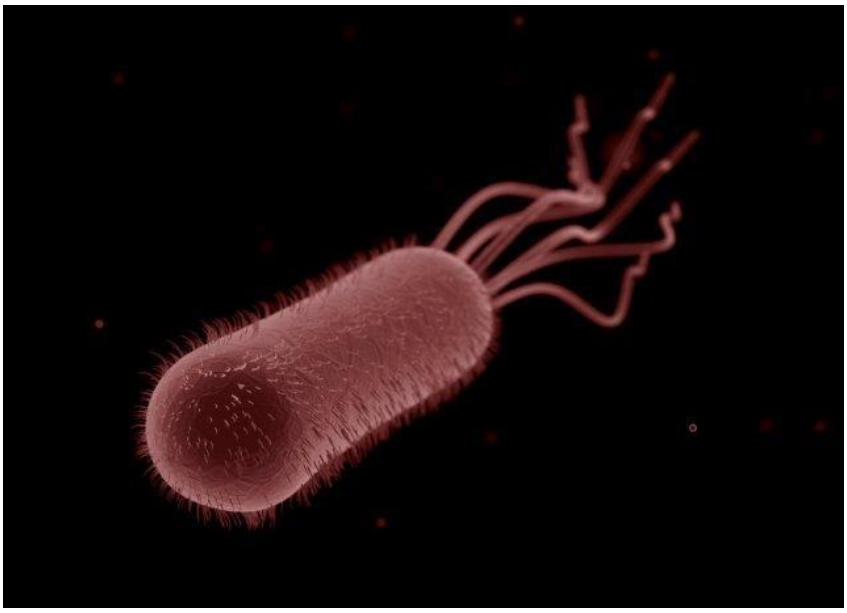


Figure: 1.8.1 *Escherichia coli*

1.8.1.1 Pathogenesis of *Escherichia coli*:

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

1. 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 containing blood, mucus, and faecal leukocytes. The infective dose of EIEC appears to be substantially higher than for *Shigella*and this is thought to be a reflection of the organism's greater sensitivity to gastric acidity.
2. 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
3. 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.
4. 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).

(Adams & Moss, 2008)

1.8.1.2 Source of contamination:

Pathogenic *Escherichia coli,* or *E. coli*, will be accepted on most accioli live in the intestines about cows. Specific serotypes of *E. coli*, for example, such that e. Coli O157:H7, bring likewise been discovered in the intestines of chickens, deer, sheep, What's more pigs. These microscopic organisms make human sickness when they would ingested, Also could prompt e. Coli spoiling through Different modes from claiming transmission, including through nourishment and water sources, animal-to-human contact, Furthermore individual to-individual contact done daycares Furthermore other settings. Some other sources are:

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

(Clark, 2016)

1.8.2 Salmonella Spp:

Salmonella is an Gram-negative, rod-shaped, motile bacilli which moves for the utilization about its peritrate flagella. Those class salmonella might a chance to be separated under two species (S. Enterica What's more encountered with urban decay because of deindustrialization, engineering imagined, government lodgin. Bongori), dependent upon their phenotypic profile (Acharya, 2013) The Salmonella family includes over 2,500 serotypes of bacteria - they are microscopic one-celled organisms (WHO, 2016). The class salmonella may be a part of the family enterobacteriaceae. Salmonella may be a standout amongst those the greater part normal reason for nourishment borne ailment in the reality (T.Acharya, 2013).

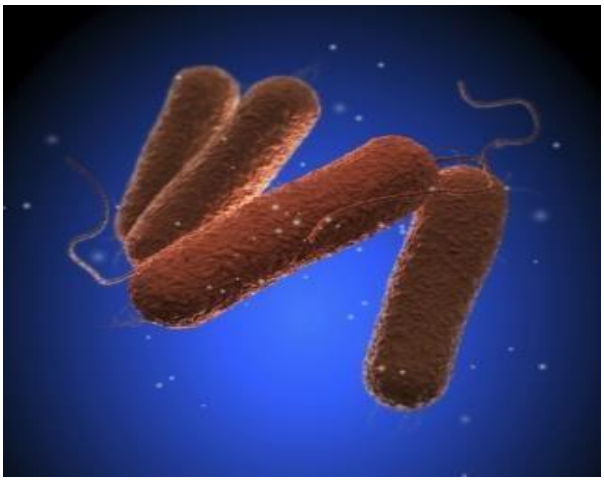


Figure 1.8.2: *Salmonella* spp

1.8.2.1Pathogenesis:

Salmonella spoiling will be a standout amongst a significant number time permits reason for gastroenteritis (also known as ‘gastro’). There are many distinctive sorts from claiming salmonella microscopic organisms and they happen in a number down home and wild animals, including birds, frequently creating ailment clinched alongside them. Two particular sorts from claiming salmonella could foundation typhoid Also paratyphoid fever, which reasons an alternate ailment. Concerning illustration their name infers salmonella enterica would includedOn creating maladies of the intestines (enteric implies pertaining of the intestine). Those three principle serovars for salmonella enterica would Typhimurium, Enteritidis, FurthermoreTyphi. Each of these will be talked about further the following.

Main types of *Salmonella enterica*serovars	**Description of the serovars**
• salmonellaentericaserovarTyphi.	Also known as *Salmonella typhi* or abbreviated with encountered with urban decay because of deindustrialization, engineering imagined, government lodgin. Typhi. This bacterium will be those causative agenize from claiming
Main types of *Salmonella enterica*serovars	**Description of the serovars**

	typhoid fever. It makes a serious, regularly deadly mishap sickness. The indications of typhoid fever incorporate nausea, vomiting, fever and passing.
• salmonella entericaserovarTyphimurium	Also called salmonella typhimurium or abbreviated on encountered with urban decay because of deindustrialization, engineering concocted, government lodgi. The ailment may be described Toward diarrhea, abdominal cramps, spewing and nausea, What's more by keeps up dependent upon 7 days.
• salmonella entericaserovarEnteritidis	Also called salmonella enteritidis. Many people have blamed the recent increase in the rise of *S.* Enteritidis infections on the use of mass production chicken farms.

(SA health, 2016)

1.8.2.2 Typical Symptoms of Salmonella infection:

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

- Common enterocolitis may result without enteric fever characterized by headache, abdominal pain, nausea, vomiting, diarrhea, dehydration (Adams & Moss, 2008).

Typhoid Fever Symptoms:
Symptoms of typhoid fever appear between 8 and 14 days after eating contaminated food and last anywhere from 3 to 60 days. They include a fever of 104 F, weakness, lethargy, abdominal pain, coughing, nosebleeds, delirium, and enlarged organs. Typhoid fever is a serious illness that can result in death (Adams & Moss, 2008).

1.8.2.3 Source of contamination:

- *Salmonella* infection usually results from ingestion of the bacteria from contaminated food, water or hands.
- Eggs, milk, meat or poultry are particularly high risk foods.
- Fruit and vegetables may also be contaminated, especially if manure has been used as fertilizer.
- People may become infected if they transfer animal faecescontaining *Salmonella* bacteria from their hands to their mouths, for example, if eating after touching animals and failing to wash their hands.
- Person-to-person spread may occur when hands, objects or food become contaminated with faeces from people who are infected and the bacteria are then taken in by mouth by another person.

1.8.3 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. *Sh. dysenteriae* has been responsible for epidemics of severe bacillary dysentery in tropical countries but is now rarely encountered in Europe and North America where *Sh. sonnei* is more common. *Sh. sonnei* causes the mildest illness, while that caused by *Sh. boydii* and *Sh. flexneri* is of intermediate severity (Adams & Moss, 2008).*Shigella*sare members of the family Enterobacteriaceae. They are nonmotile, nonsporeforming, Gram-negative rods which are catalase positive (with the exception of

Shiga's bacillus, S. dysenteriae serotype 1), 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 are typical mesophileswith 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 (Adams & Moss, 2008).

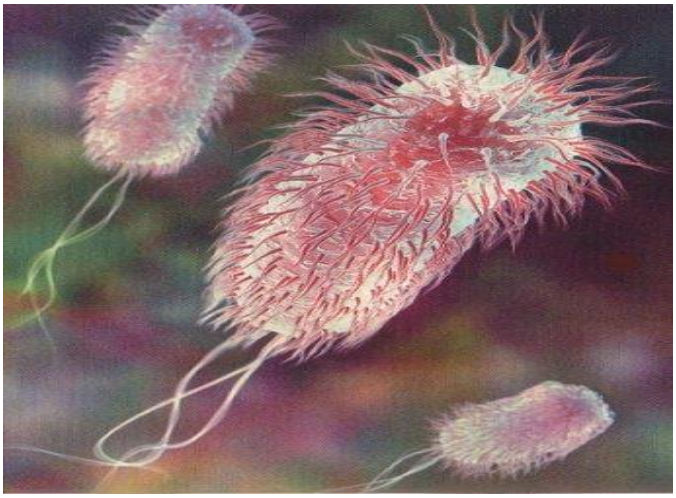


Figure 1.8.3: *Shigella Spp*

1.8.3.1 Pathogenesis:

Shigellas cause bacillary dysentery in humans and other higher primates. Studies with human volunteers have indicated that the infectious dose is low; of the order of 10–100 organisms. 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 and a carrier state may develop which can persist for several months. 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 (Adams & Moss, 2008).

1.8.3.2 Source of contamination:

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

1.8.4 *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 19^{th} century. It was Robert Koch who firmly established the causal link between *Vibrio cholerae* and cholera when working in Egypt in 1886.*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%.

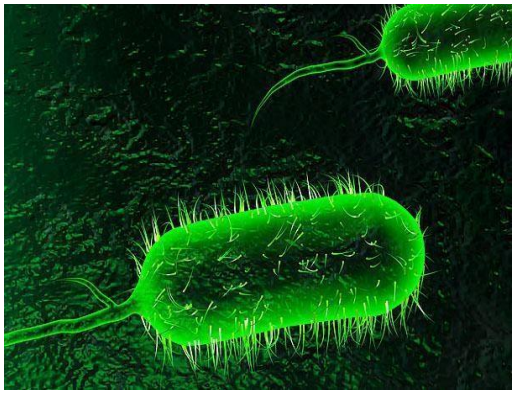


Figure1.8.4: *Vibrio Spp*

1.8.4.1 Pathogenesis:

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).

1.8.4.2 Source of contamination:

- Cholera is regarded primarily as a waterborne infection, though food which has been in contact with contaminated water can often serve as the vehicle.
- 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).

1.8.5 *Klebsiella spp*:

Klebsiella is a gram-negative, non-motile, lactose fermenting, rod-shape organism. K. pneumoniae is able to grow either with or without free oxygen, deeming it a facultative anerobe which is usually found in the normal flora of skin, mouth, and intestines. This organism is also surrounded by a capsule, which increases its virulence by acting as a physical barrier to evade the

host's immune response (Puspanadan et al., 2012). Increasingly, *Klebsiella* bacteria have developed antimicrobial resistance, most recently to the class of antibiotics known as carbapenems. In healthcare settings, *Klebsiella* infections commonly occur among sick patients who are receiving treatment for other conditions. Patients whose care requires devices like ventilators (breathing machines) or intravenous (vein) catheters, and patients who are taking long courses of certain antibiotics are most at risk for Klebsiella infections. Healthy people usually do not get *Klebsiella* infections. *Klebsiella* is a type of bacteria that can cause different types of healthcare-associated infections, including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis (CDC, 2016).

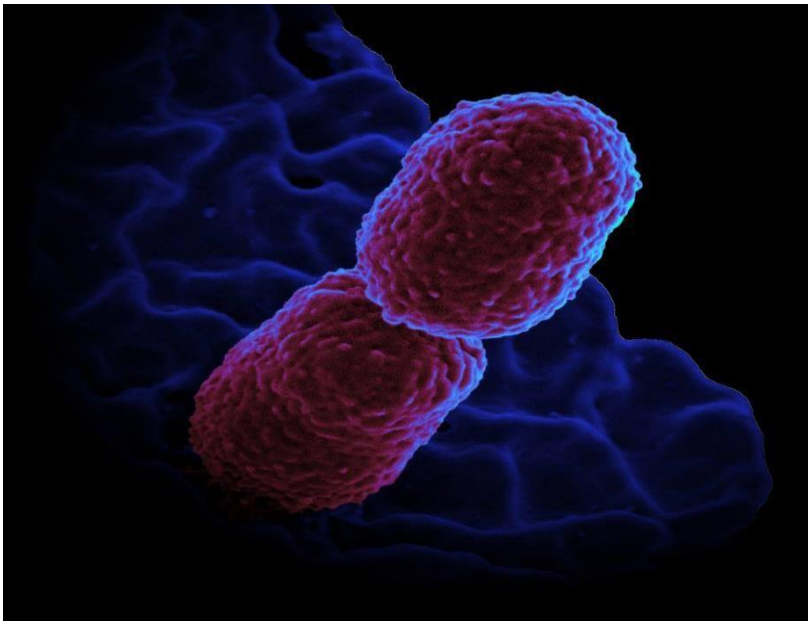


Figure1.8.5: *Klebsiella Spp*

1.8.5.1 Pathogenesis:

The symptoms of a *K. pneumoniae* infection differ depending on where the infection is located, and are similar to symptoms of the same diseases caused by other microbes. For instance, meningitis from *K. pneumoniae* produces the hallmark symptoms of bacterial meningitis, including

- Fever,
- Confusion,
- Neck stiffness, and
- Sensitivity to bright lights.

Bloodstream infections (bacteremia and sepsis) from *Klebsiella* cause:

- Fever,
- Chills,
- Rash,
- Light-headedness, and Altered mental states.

Pneumonia from K. pneumoniae can result in:

- Fevers and chills
- flu-like symptoms
- Cough, which may produce mucus that's yellow, green, or bloody Breathing issues (Bennington-Castro, 2016).

1.8.5.2 Source of contamination:

- To get a *Klebsiella* infection, a person must be exposed to the bacteria. For example, *Klebsiella* must enter the respiratory (breathing) tract to cause pneumoniae, or the blood to cause a bloodstream infection.
- In healthcare settings, *Klebsiella* bacteria can be spread through person-to-person contact (for example, from patient to patient via the contaminated hands of healthcare personnel, or other persons) or, less commonly, by contamination of the environment. The bacteria are not spread through the air. Patients in healthcare settings also may be exposed to Klebsiella when they are on ventilators (breathing machines), or have intravenous (vein) catheters or wounds (caused by injury or surgery).
- Unfortunately, these medical tools and conditions may allow *Klebsiella* to enter the body and cause infection (CDC, 2016).

1.9 Epidemiological study of food borne illness in various countries in World:

Expression of the similar symptoms or sickness by two or more of the individuals after consumption of the same contaminated food is labeled as an outbreak of food-borne illness. The description of outbreak includes time, place, and person distribution (Jahan, 2012).

It is important that food-borne illness outbreaks are investigated timely and proper environmental assessments are done so that appropriate prevention strategies can be identified. According to CDC, the etiology of majority (68%) of reported food-borne illness outbreaks is unknown due to lack of timely reporting and lack of resources for investigations. In addition, persons who do not

seek health care and limited testing of specimens are also the contributory factors in failure to determine the cause of food-borne illness outbreak (Lynch et al., 2009).

A number of food-borne illness outbreaks are reported from various parts of the world. Worldwide, a total of 4093 food-borne outbreaks occurred between 1988 and 2007. It was found that

Salmonella Enteritidis outbreaks were more common in the EU states and eggs were the most frequent vehicle of infection. Poultry products in the EU and dairy products in the United States were related to *Campylobacter* associated outbreaks. In Canada, *Escherichia coli* outbreaks were associated with beef. In Australia and New Zealand, *Salmonella typhiumurium* outbreaks were more common (Greig& Ravel, 2009).

Daniels and colleague (2002) conducted a study in the United States, to describe the epidemiology of food-borne illness outbreaks in schools, colleges and universities. The data from January 1, 1973, to December 31, 1997 was reviewed. In majority (60%) of the outbreaks the etiology was unknown. Among the outbreaks with a known etiology, in 36% of outbreak reports Salmonella was the most commonly identified pathogen. However, the highest mortality was caused by

Listeriamonocytogenes. Viral pathogens were responsible for 33% of the outbreaks. Among the viral pathogens, norovirus was the most common causative agent (Lynch et al., 2006).

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

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

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

In a study carried out from October 2004 to October 2005 in Catalonia, Spain, 181 outbreaks were reported; 72 were caused by *Salmonella* and 30 by norovirus (NoV) (Crespo et al., 2005). In 2002, in

the Netherlands a national study of food-borne illness outbreaks was performed. A total of 281 food-borne illness outbreaks were included. Most of these outbreaks were reported from nursing homes, restaurants, hospitals and day-care centers. The causative agents included norovirus (54%), Salmonella spp. (4%), rotavirus (2%), and Campylobacter spp. (1%) (Duynhoven et al., 2005).

A study conducted in Qassim province, Saudi Arabia, analyzed the food-borne illness surveillance data for the year 2006. During the study period, 31 food-borne illness outbreaks comprising of 251 cases, were reported. The most common etiologic agent was *Salmonella*spp, followed by *Staphylococcus* aureus(Jahan, 2012).

1.10 Now-a-days Affection for Street Foods in Dhaka city:

In Dhaka streets, food vending is everywhere; however the vendors in Bangladesh lack education regarding the basic food safety issues. Vendors generally use carts and stands, where they do not have easy access to running water, furthermore dish and hand washing is done using the same bucket, sometimes even without soap. Garbage and waste water is typically discarded in the streets nearby and thus attracting and providing food for rodents and insects. Toilets are not available nearby in several cases thus forcing the vendors to eliminate their body wastes in nearby areas and return to their vending sites without washing their hands. Environmental condition and practices like this often lead to contamination of cooked food. Vendors may purchase raw materials from doubtful sources which may either be contaminated with food borne pathogens or be unfit for consumption due to other reasons (Rahman, Rahman &Ansary, 2011). Foods sold by street vendors in Dhaka city are contaminated with pathogenic bacterial organisms, which are likely to pose a potential hazard to consumers, an issue that needs to be addressed. Provision of health education to the street food vendors on personal hygiene, safe food handling practice and proper disposal of waste would improve food quality and thereby reduce the risk of contamination of street-sold food. Infrastructure development for access to potable water, public toilet, washing and waste disposal facilities also would reduce the health hazards to consumers. Although there is a growing demand for these food products, enough information is not available regarding the microbiological quality of these products in Dhaka city, Bangladesh; there are some limitations in the isolation and confirmation of the presence of other microorganisms present in the food samples. Therefore, future studies will be needed to determine the presence of various microorganisms responsible for food-borne illnesses and their confirmation in the laboratory (Islam et al., 2015).

Objective of this study:

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

- To isolate and identify the presence of enteric bacteria (*Escherichia coli*, Klebsiella spp, Shigellaspp, Salmonella and Vibrio spp) in different street vended foods collected from different private universities.

3 METHODOLGY:

3.1 Bacteriological Subculture:

3.1.1 Sample Collection:

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

3.1.1.1 Sample Category:

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

3.1.2 Sample Processing:

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

3.1.3 Enrichment of the Organisms:

3.1.3.1 Enrichment of E. coli and Klebsiellaspp:

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

3.1.3.2 Enrichment of Salmonella spp and Shigellaspp:

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

3.1.3.3 Enrichment of Vibrio spp:

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

Fig.3.1: Enrichment of the Organisms

3.1.4 Selective Growth of the Organisms:

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

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

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

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

3.1.4.3 Selective Growth of *Vibrio spp*:

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

3.1.5 Sterilization Procedure:

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

Fig. 3.2: Autoclave and Hot air Oven

Fig. 3.3: Laminar Air Flow Cabinet

3.1.6 Preparation of Petri dishes:

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

Fig. 3.4: Petri dishes preparation

3.1.7 Incubation:

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

Fig. 3.5: Incubator

Table 3.1: Standard Colony Morphology of Suspected Organisms:

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

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

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

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

Agar:

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

Enrichment Broth:

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

3.2 Biochemical Tests:

3.2.1 Kliglar Iron Agar Test (KIA Test) :

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

3.2.1.2 Inoculation for KIA Test:

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

Fig. 3.6: Preparation of test tubes for KIA test

3.2.2 MIO Test:

3.2.2.1 Test Tube Preparation for MIO Test:

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

3.2.2.2 Inoculation for MIO Test:

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

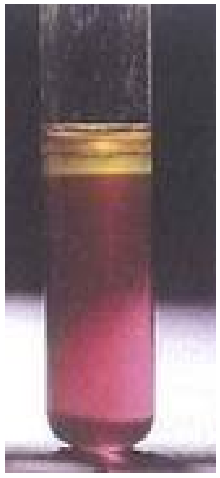


Fig. 3.7: Preparation of test tubes for MIO test

3.2.3 Citrate Test:

3.2.3.1 Test Tube Preparation for Citrate Test:

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

3.2.3.2 Inoculation for Citrate Test:

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

Fig. 3.8: Preparation of test tubes for Citrate test

3.2.4 Urease Test:

3.2.4.1 Test Tube Preparation for Urease Test:

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

3.2.4.2 Inoculation for Urease Test:

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

Fig. 3.9: Preparation of test tubes for Urease test

3.2.5 Oxidase test:

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

3.2.6 Apparatus & reagent used for Biochemical Tests

- Laminar air flow cabinet (ESCO, Singapore)
- Screw cap test tubes
- Autoclave (HIRAYAMA, Japan)
- Hot air oven (FN-500, Niive)
- Straight wire
- Spirit burner
- 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 Gas production	Black color Bubble production	No Black color No bubble in test tube

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

3.3 Colony Counting Methodology:

3.3.1. Cell counting and serial dilutions:

3.3.1.2. Theory:

In quantitative microbiology, we are concerned with determining the concentration of colony forming units (CFUs) in our sample – i.e., the number of CFUs per ml or per gram of the sample. More realistically, the concentration of CFUs in the sample could have been considerably greater. Counting the colonies on a plate inoculated with one ml of sample may be impossible. We would like to have "countable" plates – containing between 30 and 300 colonies. If fewer

than 30, we run into greater statistical inaccuracy. If greater than 300, the colonies would be tedious to count and also would tend to run together.

So we now get into "dilution theory" to accomplish the equivalent of plating out succeedingly smaller amounts of sample. Making serial decimal dilutions (i.e., successive 1/10 dilutions, each made by adding one part of inoculum to 9 parts of diluent) and inoculating one ml into each of the plates, we can construct a plating procedure that is equivalent to the above.

3.3.1.3. Materials Required:

1. Tubes
2. Micropipette with tips
3. Distilled water
4. Bacteria sample
5. Nutrient agar
6. Petri dishes
7. Water bath
8. Alchohol
9. Colony counter
10. Conical Flask 11. Labeling Tape

3.3.1.4 Procedure:

There are four major steps in the procedure:

- preparation of serial dilutions
- mixing the serial dilutions into agar
- counting the resulting bacterial colonies
- Calculation of total numbers of viable bacteria from these counts.

3.3.1.5. Preparation of Serial Dilutions

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

Tubes	**Dilution**	**Dilution**	**Dilution Factor**
1	10^{-1}	1/10	10^{1}
2	10^{-2}	1/100	10^{2}
3	10^{-3}	1/1,000	10^{3}
4	10^{-4}	1/10,000	10^{4}

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.

3.3.1.5. Counting bacterial colonies

1. After an appropriate incubation period the plates were examined for colonial growth.
2. Colonies will form on the top of the agar as well as in the agar. Those on top of the agar will be larger but all colonies must be counted.
3. Plates were selected that appear to have between 30 - 300 colonies in and on the agar as this gives the best statistical representation of the number of bacteria in the undiluted sample.
4. Using a light box or colony counter (if one is available) and marker pen (put a dot above each colony as you count it), the number of colonies were counted in each of the dilutions having between 30 - 300 colonies.

4. Result:

4.1 Bacterial colony morphology:

Table 4.1: Bacterial colony morphology isolated from different street-vended food

Name of University	Sample	Plates				
		MacConkey	TBX	BGA	XLD	TCBS
Ahsanullah University of Science and Technology (AUST)	KimaPuri	Pink	No growth	White	Yellowish	Yellow
	Plan cake	No growth	White	No growth	Whitish	No growth
	Bun	No growth	White	No growth	No growth	Yellow
Green University of Bangladesh	Nimki	Mucoid pink	blue	White	No growth	No growth
	Jhal bon	Mucoid pink	White	No growth	Whitish	No growth
	Double Layer Cake	No growth	No growth	Whitish	Yellow	No growth
East West University (EWU)	somucha	Mucoid pink	blue	No growth	No growth	Yellow
	Danish	No growth	No growth	No growth	No growth	No growth
	jilapi	Oval pink	No growth	No growth	No growth	No growth
United International University (UIU)	Roll	Mucoid pink	White	White	No growth	No growth
	Velpuri	Colorless	Colorless	No growth	No growth	No growth
	Cake 3	No growth	No growth	No growth	No growth	Yellow
University of Liberal Arts Bangladesh (ULAB)	AmerKoli	No growth	No growth	No growth	No growth	Yellow
	Panipuri	Pink	Blue	No growth	No growth	No growth
	TetulerAchar	Colorless	No growth	No growth	No growth	No growth

Table 4.1 Around 15 food samples were collected from five different private universities in Dhaka city. In total 11 samples show growth of different pathogenic or non pathogenic microorganisms. Of which, 5 samples show positive growth of our suspected organisms (*E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp.)* and sample shows no growth in these agar media. The reason for observing no growth in sample may include the following: a) sometimes fresh foods were collected early in the

morning so no contamination occurred yet, b) sometimes food were hot which prevented growth of bacteria.

Table 4.2: Bacterial colony morphology isolated from different street vended food samples

Name of University	Sample	Plates				
		MacConkey	TBX	BGA	XLD	TCBS
Prime Asia University	Kabab 2	pink	Blue	No growth	No growth	No growth
	Misty bar	No growth	White	No growth	Yellow	Yellow
	Cup cake	Colorless	No growth	Yellowish	Yellow	No growth
University of Asia Pacific (UAP)	kalojam	Mucoid pink	blue	No growth	No growth	No growth
	Vegetable roll	No growth	No growth	No growth	No growth	No growth
	LalMohon	No growth	No growth	Yellowish	Yellow	No growth
Bangladesh University of Professional (BUP)	Badammakha	Colorless	No growth	No growth	Yellow	No growth
	kabab	No growth	No growth	Whitish	Yellow	Yellow
	Misty Bun	No growth	White	Yellowish	No growth	No growth
Popular Medical College	Roll	Mucoid pink	White	No growth	No growth	No growth
	Tehari	No growth	No growth	Yellowish	Yellow	yellow
	VhunaKhichuri	colorless	White	White	Yellow	Yellow & black
Bangladesh Islamic University	puri	Flat Pink	No growth	No growth	No growth	Yellow
	Chicken ball	Mucoid pink	blue	White	Red flat	Yellow
	Chomchom	No growth	White	Yellowish	No growth	No growth
	Vegetable roll	No growth	No growth	No growth	No growth	No growth
	LalMohon	No growth	No growth	Yellowish	Yellow	No growth

Table 4.2: (Bacterial colony morphology isolated from different street-vended food samples) shows bacterial colony morphology isolated from different street vended food samples. Around 15 food samples were collected from five different private universities in Dhaka city. In total, 10 samples show growth of different pathogenic or non pathogenic microorganisms. Of which,5 samples show positive growth of our suspected organisms (*E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp.*) and 1 sample shows no growth in these agar media. The reason for observing no growth in sample may include the following: a) sometimes fresh foods were collected early in the morning so no contamination occurred yet, b) sometimes food were hot which prevented growth of bacteria.

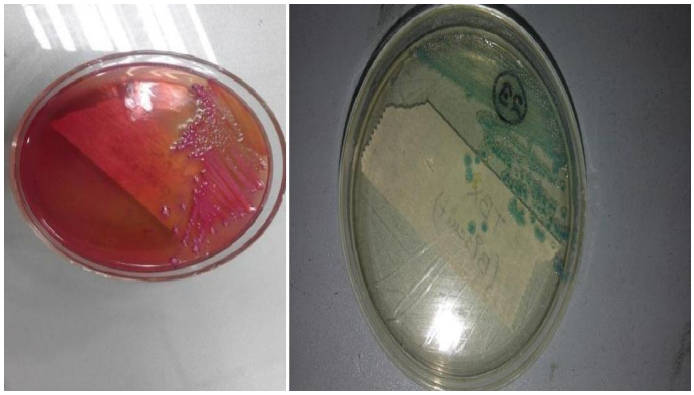


Figure 4.1: Bacterial colony on ager plate

Table 4.3: Number of food samples with growth of suspected organisms determined by colony morphology (n=30)

Name of Universities	No. of of samples with +ve growth *E.coli*	No. of of samples with +ve growth *klebsiella Spp*	No. of of samples with +ve growth *Vibrio spp*	No. of of samples with +ve growth *Salmonella*	No. of of samples with +ve growth *Shigella Spp*
AUST	1	0	0	0	0
GUB	1	1	0	0	0
EWU	0	1	1	0	0
UIU	0	1	1	0	0
ULAB	1	0	1	0	0
PAU	1	1	1	0	0
UAP	0	1	0	0	0
BUP	1	0	2	0	0
PMC	1	1	0	0	0
BIU	1	1	0	0	0

From total 30 food samples collected from street vendors, we found contamination in 26 (87%) samples (Table 4.1 and Table 4.2). Of which, 20 (67%) samples were suspected to be contaminated with our targeted organisms (*E coli, Klebsiella, Shigella, Salmonella* and *Vibrio* species).

In total 20 samples, 7 (35%) samples were suspected to be contaminated with *E coli*, 7 (35%) with *Klebsiella*, 6 (30%) with *Vibrio,* 0 (0%) with *Shigella* and 0 (0%) with *Salmonella* species.

4.2 Suspected Organisms from Biochemical Tests

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

Samples	Plates	Colony Morphology	M	I	O	Citrate	Urease	Oxidase	KIA			organism
									Slunt/ butt	gas	H_2S	
Somucha2	MacConkey	Pink	-	+	-	+	-	-	A/A	-	+	*KlebsiellaSpp*
Kalojam	TBX	Blue	-	+	-	+	-	-	A/A	-	+	
Chicken ball	MacConkey	Mucoid Pink	-	+	-	+	-	-	A/A	-	+	
Cake 3	MacConkey	Mucoid Pink	+	-	-	+	-	-	K/A	-	+	
Puri	MacConkey	Flat Pink	-	+	-	+	-	-	A/A	-	+	
Roll	MacConkey	Flat Pink	-	+	-	+	-	.	A/A	.	+	

Among 20 (70%) food samples were subjected for different biochemical test to identify our targeted organisms. Biochemical test results of about 10 (40%) food samples show similarities with the standard biochemical test results of our targeted organimsms (E.coli, Klebsiella spp., Vibiospp and Shigella spp. except Salmonella spp.) as compared.

Table 4.4 shows identification of the suspected organism (Klebsiella spp.) from different biochemical test. In total 6 (60%) food samples were identified to be contaminated with our suspected organism Klebsiella spp. from these biochemical tests.

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

Samples	Plates	Colony Morphology	M	I	O	Citrae	Urease	Oxidase	KIA			organism
									Slunt /butt	Gas	H_2S	
Nimki	TBX	Blue	+	+	-	+	-	+	A/A	-	+	
Kabab 2	MacConkey	Pink	+	+	-	+	-	-	A/A	-	+	*E.coli*
Panipuri	MacConkey	Mucoid Pink	+	+	-	-	-	-	K/A	-	+	
Kabab 1	TCBS	Yellow	+	+	+	+	-	-	K/A	-	+	*Vibrio spp*

Among 20 (70%) food samples were subjected for different biochemical test to identify our targeted organisms. Biochemical test results of about 10 (34%) food samples show similarities with the standard biochemical test results of our targeted organimsms (E.coli, Klebsiella spp., Vibiospp and Shigellaspp except Salmonella spp) as compared.

Table 4.5 shows identification of the suspected organism (E.coli, Vibio spp. and Shigella spp.) from different biochemical test. In total 3 (10%) food samples were identified to be contaminated with *E.coli*and 1 (3%) food sample was identified to be contaminated with *Vibio spp*. from these biochemical tests.

Fig. 4.3: Different Biochemical test

Table 4.6: Presence of suspected organisms in no of food samples from different university (n=10)

Name of University	*E.coli*	*Klebsiella spp.*	*Vibrio spp.*	*Shigella spp.*	*Salmonella spp.*
AUST	0	0	0	0	0
GUB	1	0	0	0	0
EWU	0	1	0	0	0
UIU	0	1	0	0	0
ULAB	1	0	0	0	0
PAU	1	0	0	0	0
UAP	0	1	0	0	0
BUP	0	0	1	0	0
PMC	0	1	0	0	0
BIU	0	2	0	0	0

Table 4.6 shows presence of suspected organisms in no of food samples from different university. In total 10 (34%) food samples from different university were suspected to be contaminated with our targeted organisms E.coli, Klebsiella spp., Vibiospp and Shigellaspp except Salmonella spp.

In AUST, no food samples were suspected to be contaminated withany spp of microorganisms. In GUB 1 food sample were suspected contaminated with*E.coli*. In EWU, 1 food samples were

suspected to be contaminated with *Klebsiella*spp. respectively. In UIU, 1 food sample was suspected to be contaminated with Klebsiella spp. In ULAB, 1 food sample was suspected to be contaminated with*E.coli*spp. In PAU, 1 food sample was suspected to be contaminated with *E.coli*. In UAP, 1 food samples were suspected to be contaminated with *Klebsiella* spp. In BUP, 1 food samples were suspected to be contaminated with*Vibrio* spp. In PMC, 1 food samples were suspected to be contaminated with *Klebsiella*spp, In BIU 2 food samples were suspected to be contaminated with Klebsiella spp.

Table 4.7: Incidence of food borne pathogens in various street vended food samples

Pathogen	Food Categories				
	Deep fried and fried items(n=9)	Spicy items (n=9)	Baked items (n=7)	Sweet items (n=5)	Total(n=30)
E.coli	2 (23%)	1 (12%)	Nd	Nd	3 (10%)
Klebsiella spp.	4(45%)	Nd	1 (15%)	1 (20%)	6(20%)
Vibrios pp.	1 (12%)	Nd	Nd	Nd	1 (3%)
Shigella spp.	Nd	Nd	Nd	Nd	Nd
Salmonella spp.	Nd	Nd	Nd	Nd	Nd

Table 4.7 shows the incidence of food borne pathogens in various street vended food samples. Among 9deep fried and fried items, 4 (45%) samples were suspected to contain *Klebsiella*spp, 1 (12%) sample was suspected to contain Vibrio spp and 2 (23%) sample was suspected to contain *E.coli*. Among 9 spicy items, 1 (12%) sample was suspected to contain *E.coli*. Among 7 baked items, 1 (15%) samples were suspected to contain *Klebsiella* spp. Among 5 sweet items, 1 (20%) samples were suspected to contain *Klebsiella* spp.

Table 4.8: Colony counting of various samples:

Sample Name	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Misty Shingara	Uncountable	Uncountable	Uncountable	42
Plan cake	Uncountable	Uncountable	50	19
Dim chop	Uncountable	Uncountable	Uncountable	71
Misty bar	Uncountable	Uncountable	35	Uncountable
Kathikabab	40	10	8	6
Achar	Uncountable	Uncountable	70	28

For misty shingara 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:

42 colonies on plate 4 x dilution factor of 10,000 = 420,000 cells/ml.

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

50 colonies on plate 3 x dilution factor of 1000 = 50000 cells/ml.

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

71 colonies on plate 4 x dilution factor of 10,000 = 710,000 cells/ml.

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

35 colonies on plate 3 x dilution factor of 1000 = 35,000 cells/ml.

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

40 colonies on plate 1 x dilution factor of 10 = 400 cells/ml.

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

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

4.9. Table: Number of colonies per ml of sample:

Sample Name	Number of microoraganism (cells/ml)
Misty Shingara	420,000
Plan cake	50,000
Dim chop	710,000
Misty bar	35,000
Kathikabab	400
Achar	70,000

5. Discussion:

At present time, road sustenance distributing has turned into a noteworthy group medical problem and matter of sympathy toward every one of us. A ton of nourishment borne sickness episodes are happening each year around the world. The explanations for this incorporates absence of suitable information and supervision on road nourishment distributing, planning of sustenance under insanitary conditions and showing sustenance transparently which likewise prompt to further tainting by clean, creepy crawlies, rodents and hands of expecting purchasers.

The present research work was subsequently untaken to discover the nearness of enteric microscopic organisms extraordinarily E. coli, Klebsiella, Salmonella, Shigella and Vibrio species from various sorts of road distributed nourishment things gathered from various private colleges 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 utilized to watch the nearness of our focused on microorganisms in nourishment things. MacConkey and TBX agar were utilized for the distinguishing proof and confinement of E. coli and Klebsiella. TCBS Agar is very particular for Vibrio species detachment. XLD and BGA were utilized for seclusion of Salmonella and Shigella species from sustenance tests.
A study was led to survey microbiological wellbeing of road distributed nourishments from May to November, 2014 in Jigjiga City. One hundred thirty-two examples of road nourishments were aseptically gathered from four "kebeles" of Jigjiga City. The study uncovered that 95(72%) of the nourishment tests had pathogenic bacterial defilements. Three distinctive bacterial species were segregated: E. coli 68(51.5%), S. aureus 85(64.4%) and 26(19.7%) Salmonella species. The most noteworthy rate of S. aureus 23/33(69%) was seen in 'Sambusa'; the most elevated frequency of E. coli 24/33(73.5%) was seen in 'Pasta', while the most elevated Salmonella frequency was seen in "Ades" (Bereda et al., 2016).

A study has been done to dissect the microbiological nature of plates of mixed greens served alongside road sustenances of Hyderabad. An aggregate of 163 serving of mixed greens tests, 53 of carrot and 110 of onion tests, were gathered from four unique zones of Hyderabad. Around 74% and 56% had Staphylococcus aureus in carrots and onions, individually. Fifty-eight percent of carrots and forty-five percent of onions tests contained Salmonella, 68% of carrots and 24% of onions had Yersinia (Sabbithi et al., 2014).

A study was directed in Amravati, India. Forty water test of panipuri were aseptically gathered from eleven areas of Amravati City. Examination of the nourishment tests uncovered that 93% of panipuri water tests had high heaps of bacterial pathogens, for example, Escherichia coli (41%), Staphylococcus aureus (31%), Klebsiella spp. (20%), Pseudomonas spp. (5%) and yeast (3%). It is proposed that customary checking of the nature of road nourishments must be rehearsed to keep away from any sustenance borne contamination in future (Tambekar et al., 2011).

In this study, 30 distinctive sustenance tests were gathered from 10 private colleges. Among them, we discovered defilement in 26 (87%) specimens. Of which, 20 (67%) specimens were suspected to be debased with our focused on creatures (*E coli, Klebsiella, Shigella, Salmonella and Vibrio species*). In total 20 samples, 7 (35%) samples were suspected to be contaminated with *E coli*, 7 (35%) with *Klebsiella*, 6 (30%) with *Vibrio,* 0 (0%) with *Shigella* and 0 (0%) with *Salmonella* species. From the consequences of biochemical test we got 10 of our speculated microscopic organisms from 10 distinct examples. Altogether, we got 6 (60%) *Klebsiella*, 1 (10%) *Vibrio* species, 3 (30%) *E.coli*.

This study showed that the road distributed nourishments of Dhaka city are very tainted with pathogenic microscopic organisms which can add to potential wellbeing dangers for purchasers. The hazard variables to the pollution incorporate the low instructive foundation of the sellers, poor individual cleanliness, despicable taking care of and capacity routine of sustenances. The greater part of the sellers took care of sustenance with uncovered hand and didn't wear any gloves or hand cover while taking care of cash that can bring about cross-defilement by presenting organisms on safe nourishment.

5.1 Conclusions:

Handling and cooking of street foods is very essential to maintain the hygiene. Personal hygiene is also very much important for food safety because human are the largest source of contamination. So it is very important to maintain cleanliness. From this study, it clear that all the samples are microbiologically unacceptable to eat. Strict public health regulations should be established to control the situation. The maintenance of these street vended foods should be monitored cautiously. Incidences of food borne illness are increasing day by day in Bangladesh. The present study revealed that street vended foods in Dhaka city constitute an important potential hazard to human health which needs to be addressed. There were some limitations in this study. Only five organisms were to be identified due to lack of methods and facilities. New method of identification of organisms and assessment of food borne hazards should be implemented. There is a reasonable gap on food safety knowledge among street vendors. Due attention should be given by the government to improve knowledge about food safety and quality standards of street foods sold in the country. Most importantly, relevant agencies such as consumer protection rights and others need to ensure and enforce strict compliance to hazard analysis and critical control points in all food production sectors in Bangladesh.

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