MICROBIOLOGICAL EVALUATION OF GRAM NEGATIVE BACTERIA FROM DIFFERENT STREET FOODS RESPONSIBLE FOR ENTERIC DISEASES

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 Research Candidate

I, Md. Abu Bakar Siddique Bhuiyan, ID: 2013-1-70-020, hereby declare that the dissertation entitled— "Microbiological Evaluation Of Gram Negative Bacteria From Different Street Foods Responsible For Enteric Diseases'' submitted by me to the Department of Pharmacy, East West University in partial fulfillment of the requirement for the award of the degree of Bachelor of Pharmacy is a record of research work under the supervision and guidance of Dr. Sufia Islam, Professor and Nafisa Tanjia, Senior Lecturer, Department of Pharmacy, East West University, Dhaka.

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Certificate by the Supervisor

This is to certify that the thesis entitled "Microbiological Evaluation Of Gram Negative Bacteria From Different Street Foods Responsible For Enteric Diseases'' submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy is a bonafide record of original and genuine research work carried out by Md. Abu Bakar Siddique Bhuiyan, ID: 2013-1-70-020 in 2016 of her research in the Department of Pharmacy, East West University, under the supervision and guidance of me.

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This is to certify that the thesis entitled “Microbiological Evaluation Of Gram Negative Bacteria From Different Street Foods Responsible For Enteric Diseases" submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy is a bonafide record of original and genuine research work carried out by Md. Abu Bakar Siddique Bhuiyan, ID: 2013-1-70-020 in 2016 of her research in the Department of Pharmacy, East West University, under the co-supervision and guidance of me.

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Dedication

This research work is dedicated to my beloved parents and research supervisors.

Abstract

In this present study, we analyzed the microbial quality of street vended food samples. The main purpose of this study was to find out the incidence of enteric bacteria specially *Escherichia coli, Shigella, Vibrios*, *Klebsiella* and *Salmonella* in different food samples. Total 30 samples were collected from the street vended shops beside the 10 universities in Dhaka city, Bangladesh. Five different agar media were used for isolation and identification of our suspected organisms from the samples. The agar media were MacConkey, TBX (Tryptone Bile X-glucuronide Agar), BGA (Brilliant Green Agar), XLD (Xylose lysine deoxycholate) and TCBS (Thiosulfate citrate-bile salts sucrose). Microbial growth was observed in different media plates. In the study, among 12 fried items, 2(17%) samples were contaminated with *E.coli*, again 2(17%) were contaminated with *Klebsiella spp.* and 1(8%) were contaminated with *Vibrio spp.* Among 17 spicy items 2(12%) samples were contaminated with *Klebsiella spp.*, again 2(12%) were contaminated with *Vibrio spp.* among 1 baked items, only 1(100%) samples were contaminated with *Klebsiella spp.* No *Salmonella spp*. and no *Shigella spp.* were found from any food sample. Seven biochemical tests were performed for characterizing the organisms. We have also performed colony counting of additional six foods standards method. Among six samples, highly concentrated colonies were found in plain cake. Therefore, adequate measures and specific training should be provided for the street food venders, and should be concerned about it in the students community.

Key Words: Street vended food, Food-borne illness, *E.coli, Klebsiella spp.*, MacConkey Agar, TCBS (Thiosulfate citrate-bile salts sucrose), Urease Test, MIO (Mortility Indole Ornithine) Test.

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List of Abbreviations

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

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1.1 Street Foods

Street foods refer to various kinds of foods and beverages which are made and/or sold by vendors and hawkers especially in streets around trading centers and other public places for instant consumption or later consumption without further processing or making. Street-vended foods are induced to contaminations as they are sold in the open and are almost not coated. As street vendors make attraction to consume their products to their customers, they frequently handle from places such as bus terminals, industrial areas, schools, market places and streets. The street food industry plays a key role in developing countries in equitable with the food demands of the urban dwellers. Street foods feed millions of people daily with a various food which are comparatively inexpensive. However, there are some health problems, associated with street foods. Street foods are origins of nutrition for some ethnic groups at economical prices in large urban areas. These foods could be main vehicles for the transfer of serious and disastrous diseases which is life-threatening (Bereda et.al, 2016).

Fig1.1: Street vended foods

1.2 Types of street foods

The ingredients of Street food are dependent on particular area and largely unregimented. There are so many categories that it is difficult to provide a menu of all the various street foods taken around the world. In Bangladesh, street foods include chola boot (chickpeas), bhelpuri (puffed rice with potatoes), and samucha (deep-fried dough stuffed with vegetables and/or meat) and drinks like sugar-cane juice and lassi (yoghurt and water). Another popular snacks are ghugni

(boiled and mashed white peas with spices), singara (flour wraps stuffed with vegetables, spices, and occasionally liver) etc (Khairuzzaman, et.al, 2014).

There are also other foods like dim chop, puri, jhal muri, vel puri, chotpoti, fuska, achar, badam,peaju,chola bhut, pop corn, hot pattice, kima puri etc (Kasem, 2014).

1.3 Consumer of street foods

The consumption habits of street foods and their contribution to dietary intake are inadequate. According to FAO 2006, the main purchasers of street foods in many countries were members of the unofficial sector, like fellow hawkers and hustlers and casual wage laborers and another purchasers were included i.e. children and students, office workers, and housewives. In many countries, the prevalence and consistency of taken foods are changed. In several countries, street foods are purchased regularly & have become an elemental part of the diet. Particularly in Bangladesh, they seemed ancillary and small number of purchasers bought them regularly. Most of the people such as students, uneducated labors, homeless who buy food from the vendors. The cost of street foods are relatively cheaper than the rich foods from the retaurants and fast food outlets because large cost of gas, oil and other elements in municipal areas where inexpensive street foods are generated than the hoom made food (Khairuzzaman, et .al. 2014)

1.4 Factors that Affect the Preference Levels of Consumers Regarding Street Food

External stimuli

- Odor of the products
- Environment of the shop
- Food price
- Exhibition
- Environmental stimuli etc.

Internal Stimuli

- Pleasure
- Performance
- Attitude
- Spirits Of Enjoyment

- Passion
- Happiness etc.

Situational stimuli

- Time
- Product
- Category
- Word of mouth etc. (Kasem,2014)

1.5 Increasing Demand of Street Food

The metropolitan inhabitants in Bangladesh is growing swiftly. In the previous decade, the sum of individuals living in the country's capital named Dhaka nearly doubled from 5.3 to 9.3 million. This expansion has directed to a rise in the need for comparatively low-priced and readymade foods as many metropolitan inhabitants pass maximum time of the day outdoor and have tiny amount of time and money to expend on food. Quick urbanization also turned street food selling into a vital business. In Dhaka city, about 200,000 individuals earn their living by vending street foods. The little price, availability and convenience are the main features for the increasing demand of street foods. Women have a very crucial role in the growth of street food sector because of their direct or indirect contribution in the business. Furthermore an important number of street food sellers are woman-headed households. The variety that exists among street food sellers is mirrored in the variety of food they make and sell, their business scale, the method in which they are functioning, the places in which they make and sell food, the type of customers to whom they trade food, and so forth (Khairuzzaman, et .al. 2014).

1.6 Public Health Hazards in Street Foods of Bangladesh

Maximum number of handlers of street sold foods in underdeveloped countries and the growing world at large, are greatly unaware of simple food safety issues. In Bangladesh foodstuffs (from the uncooked material to the terminal stage) are often uncovered to sources of pollution such as soil, dust and sand. Additional common actual risk factors consist of time, temperature manipulations, hygiene and cleanliness of street food selling operations. Most of the traditional street foods are offered and supplied in the open area, without appropriate protective wrapping. Existing data specify that street foods contain great amount of microorganisms as well as pathogens (Rahman, 2011). The existence of pathogens such as coliform and salmonellae,

shigellae, staphylococci or enteropathogenic Escherichia coli in street foods are proved. Pathogens, indicator bacteria or groups and large numbers of aerobic microorganisms have also been collected from street-vended foods (Rahman, 2011).

1.7 Street Food Region:

Street foods display huge variation in components, making, ways of marketing and ingesting. They often reveal traditional native cultures and have an infinite variety including meals, snacks and drinks. There is much variety in the initial ingredients and in the technique of preparation of street foods. Additionally, there are alterations in the places of street foods preparation and can be largely divided as follows:

a) Food which are prepared in small food factories or traditional factories;
b) Food which are prepared in the house;
c) Food which are prepared in shops; and
d) Food which are prepared on the road.

These classifications replicate an increasing struggle to provide adequate infrastructure as well as environmental cleanliness to ensure the safe and nontoxic making of food. In many republics, street foods are prepared in shops and mostly food marketplaces. (WHO, 2010)

Such places normally do not satisfy food and safety needs (Rahman, Rahman & Ansary, 2014).

1.8 Foodborne Illness:

Foodborne illnesses are infections or irritations of the gastrointestinal (GI) tract triggered by food or beverages having destructive bacteria, parasites, viruses, or chemicals. The GI tract is a hollow organ, twisting tube from the mouth to the anus. Common symptoms of foodborne illnesses include vomiting, diarrhea, abdominal pain, fever, and chills. Most foodborne illnesses are acute, as they happen suddenly and last a short time, and most people recover on their own without treatment (NIH, 2014).

1.9 Major Source of Foodborne Illness:

1.9.1 Bacteria

Bacteria are minute organisms that can cause infections of the GI tract. All bacteria are not destructive to humans. Some destructive bacteria may already be present in foods when they are bought. Raw foods including meat, poultry, fish and shellfish, eggs, unpasteurized milk and dairy products, and fresh produce often contain bacteria causing foodborne illnesses. Bacteria

can contaminate food, making it destructive to eat during growth, harvesting or slaughter, processing, storage, and delivery. Foods may also be contaminated with bacteria during food preparation in a restaurant or home kitchen. If food preparers do not thoroughly wash their hands, kitchen utensils, cutting boards, and other kitchen surfaces that come into contact with raw foods, cross-contamination may occur. If hot food is not kept hot enough or cold food is not kept cold enough, bacteria may multiply. Bacteria multiply quickly when the temperature of food is between 40 and 140 degrees. Cold food should be kept below 40 degrees and hot food should be kept above 140 degrees. Bacteria multiply more slowly when food is refrigerated, and freezing food can further slow or even stop the spread of bacteria. However, bacteria in refrigerated or frozen foods become active again when food is brought to room temperature. Systematically cooking food kills bacteria.

Many types of bacteria cause foodborne illnesses. Examples include

- *Salmonella*, a bacterium found in many foods, including raw and undercooked meat, poultry, dairy products, and seafood. *Salmonella* may also be present on egg shells and inside eggs.
- *Campylobacter jejuni (C. jejuni),* found in raw or undercooked chicken and unpasteurized milk.
- *Shigella,* a bacterium spread from person to person. These bacteria are present in the stools of people who are infected. If people who are infected do not wash their hands thoroughly after using the bathroom, they can contaminate food that they handle or prepare. Water contaminated with infected stools can also contaminate produce in the field.
- *Escherichia coli (E. coli),* which includes several different strains, only a few of which cause illness in humans. *E. coli* O157:H7 is the strain that causes the most severe illness. Common sources of *E. coli* include raw or undercooked hamburger, unpasteurized fruit juices and milk, and fresh produce.
- *Listeria monocytogenes (L. monocytogenes),* which has been found in raw and undercooked meats, unpasteurized milk, soft cheeses, and ready-to-eat deli meats and hot dogs
- *Vibrio,* a bacterium that may contaminate fish or shellfish.
- *Clostridium botulinum (C. botulinum),* a bacterium that may contaminate improperly canned foods and smoked and salted fish.

1.9.2 Viruses

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

Common sources of foodborne viruses include

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

Common foodborne viruses include

- norovirus, which causes inflammation of the stomach and intestines
- hepatitis A, which causes inflammation of the liver

1.9.3 Parasites

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

1.9.4 Chemicals

Harmful chemicals that cause illness may contaminate foods such as

- Fish or shellfish, which may feed on algae that produce toxins, leading to high concentrations of toxins in their bodies. Some types of fish, including tuna and mahi mahi, may be contaminated with bacteria that produce toxins if the fish are not properly refrigerated before they are cooked or served.

- Certain types of wild mushrooms.
- Unwashed fruits and vegetables that contain high concentrations of pesticides.

1.10 Vending Place: Food Management and Waste Removal

The circumstances under which a number of street sellers function are described to be inappropriate for the making and vending of food. The food is prepared at home or at shops, which are situated on the street region and are made up of timber, polythene bags and tin. The location of preparation is not clean at all times and not distant from source of pollution. Preparation places used by few sellers have residues of foods prepared previously that can stimulate cross contamination. Many of these foods are uncovered and are wide-open to flies and dust, which may contain foodborne pathogens. In 70–90% of the situations, existence of animals, insects and wastes which are liquid in nature, in food preparation zones have been stated. The two main sources from where the pollutants can go into the preparation zone are: Improper handling of food and disposal of waste (Sartelli, et.al, 2016).

1.10.1 Handling of Foods:

Unhygienic management of street foods by various number of seller has been normally identified to be the source of pollution. The sellers can be transporters of pathogens such as *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter* as well as *S. aureus* who ultimately handover these food borne dangers to the clients. The hands of the food sellers are the most vital vehicle for the transmission of organisms from different body parts such as faces, nose, and skin to the food. The finding from investigation is that Salmonella, non-typhi salmonellae, *Campylobacter* and *E. coli* can persist on finger tips and other zones for different time periods and in some instances even after washing, supports the investigation reports of pollution of street vended food with pathogenic *S. aureus*, the most important being supportive injuries of humans and the environment (Sartelli,et.al,2016).

1.10.2 Waste Disposal

Few sellers who congregate in overpopulated zones where there are large numbers of potential consumers, which normally provide inadequate access to primary sanitary services. Therefore, the pollution of street foods is often associated with the waste created by food handling that is typically discarded near the selling region. The deficiency of services for liquid drainage, wastewater and garbage dumping encourages trashes to be thrown into neighboring roads and

gutters. Such zones act as habitations for rodents, flies and media for development of microorganisms. A study which has done in Africa discovered that 85% of the sellers prepared foods such as fish, fruit salads, roasted maize as well as chips in unhealthy conditions, given that trash and dirty left-over were evidently close to the shops. In these regions great amounts of junk gathers which provide habitation for insects and animal pests which are associated with enteric disease transmission such as *Shigella*, *Salmonella* and *E. coli* (Sartelli, et.al, 2016).

1.11 Quality of Raw Constituents: Water and Other Material

The quality of raw ingredients used in the making of street foods is very crucial as their pollution can continue through making and or cooking.

1.11.1 Water

Water is an important raw component in various street-sold processes. Polluted water can generate a public health hazard when it is used for drinking, cleaning of foods, integrated in the food as a component and used in the making of food or used for cleaning apparatus, utensils and hands. It is a well-known means of transmission for entero pathogens like *E. coli*, *Salmonella* spp. and *Campylobacter* spp. Research carried out in various regions of Asia, Africa and South America have often pointed the inaccessibility of potable water for different accomplishments at the selling region as a main concern. Due to the lack of clean water, many sellers re-use the water, mainly for cleaning apparatuses and used dishes. Research done to discover the bacteriological feature of the water used by numerous street sellers have exposed frequent pollution with coliforms and fecal coliforms. Once the street foods in Trinidad and Tobago were examined, it was stated that 35% of foods were polluted by *E. coli* while 57.5% of water which was used by sellers were polluted by coliforms. These reports were parallel to the results that the deposited water used by customers and sellers, at the selling region, exhibited heavy bacteriological pollution of fecal origin. These heavily polluted water is a key source of diarrheal diseases happens the street food consumers. When water samples from storage tanks used by some sellers were tested at various localities in Pune, India, it was exposed that 29.6% of the water were not following the WHO standards and had coliform sums of more than 16/100 ml, while fecal coliform sums were more than 16/100 ml in 15.5% of water samples, 4.5% of samples were proved positive for *E. coli* and 2.7% for enteropathogenic *E. coli*. In the same way, pathogens like *Salmonella* and *Shigella* have been identified in the water which is used by sellers for dishwashing (Sartelli, et.al, 2016).

1.11.2 Other Raw Materials

In addition to water, other raw ingredients are also crucial to the well-being of the street sold foods because of the biological, chemical as well as physical dangers that they may introduce. To retain prices low, some sellers buy inexpensive or adulterated materials containing unpermitted chemical flavorings from illegal suppliers which may increase the hazards associated with the food. Raw meat, poultry and vegetables are mainly polluted with huge numbers of bacteria, such as such as *B. cereus*, *C. perfringens*, *C. jejuni*, *E. coli*, *L. monocytogenes*, *Salmonella* and *S. Aureus*. Flavors are known to culture a great number of microorganisms which consist of members of the genus *Bacillus*, anaerobic sporeformers, enterococci, and members of Enterobacteriaceae, a variation of yeast and mould and pathogens similar to coagulase positive staphylococci. Pollution of foods by spices has been reported to food decomposition and may even lead to food poisoning. Sporeformers present in spices might lead to food decomposition, when they continue the cooking process and grow under favorable conditions.

In a study conducted in Calcutta, food samples that were supposed of adulteration were examined and in 30 of the 50 samples, illegal food additives were detected. Correspondingly, pathogens such as *B. cereus*, *S. aureus*, *C. perfringens*, *V. metschnikovii* and *E. coli* were found in raw chicken, salad and gravy raw materials. These organisms were possibly present in these foods at the time of purchase by sellers or may have been acquaint with cross contamination at the time of food handling or during making of food (Sartelli, et.al, 2016).

1.12 Utensils and Equipments: Chemical as well as Microbial Contaminants

Use of proper apparatuses for cooking and storage of ready food is often crucial to the safety of street sold foods. Poor quality of ingredients coupled with unsuitable practices can lead to formation of toxin, growth of pathogen or recontamination. The plan, assembly and preservation of equipments and utensils is very crucial to food safety, as their low maintenance may lead to the incapability to efficiently clean and sanitize zones. This may result in the formation of residues of food, assisting microbial growth, leading to a growing contamination. The proper use of equipment is also crucial to stop the cross contamination occurred from raw materials (Sartelli, et.al, 2016).

1.12.1 Chemical Contaminants

As many containers will leach dangerous chemicals such as copper, lead and cadmium into food, use of utensils unsuited with the food being handled, should be prevented. This has been observed principally with acidic food and beverages.

1.12.2 Microbial Contaminants

The serving utensils used at the vending site are adulterated with *Micrococcus* spp. and *Staphylococcus spp*. that may have initiated from the vendors hands when they touched the food preparation zones, dishcloths, or the water during dish washing or hand washing indicating cross contamination between dishwater, food preparation surfaces, and the food itself. It is informed that bacteria from dirty dish washing water and other causes adhere to the utensil surface and can start a risk during the food vending procedure. Microbiological analysis of utensils surface and knives have displayed the occurrence of *Salmonella* and *Shigella*. It is also informed that during the preparation of food, the raw material is cut and sliced using the same knife without in between cleaning and such knives are frequently occupied by flies (Sartelli,et.al, 2016).

1.13 Food Preparation: Storage and Reheating

A significant concern influencing food contamination and contributing to additional rise in contamination is food storage temperature. The preparation of food long before its ingestion, storage at atmospheric temperature, insufficient cooling and reheating, contaminated treated food, and undercooking are marked as the crucial factors leading to food poisoning outbreaks.

1.13.1 Storage

Keeping foods at high atmospheric temperatures for long periods of time have been conveyed to be a foremost contributor to the incidence of food poisoning outbreaks. Foods are frequently kept for numerous hours after cooking and this includes overnight keeping at atmospheric temperatures, until sold, and in this way, can harbor high microbial populations. Moreover, some of the foods are kept in the pans in which they are cooked, until sold or reheated, resulting in longer keeping time, therefore creating satisfactory circumstances for the growth of foodborne pathogens.

In this way, the counts of *Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens* are reported to be in height.

B. cereus was isolated from 42 (26.3%) samples of fried fish, tuwo, soup, and boiled rice and moin signifying that their spores subsisted the cooking process. The incidence of this bacterium attached with the storage of these foods at atmospheric temperatures for several hours under high temperature and high relative humidity revealed that the product could be risky. *B. cereus* has been answerable for outbreaks of foodborne illness as it goods heat stable and heat sensitive toxins when foods are seized under beneficial conditions for numerous hours. A number of pathogens like *E. coli*, *Salmonella typhimurium*, *Salmonella gallinarum*, *Shigella dysentriae*, *Pseudomonas fluorescens* and *Klebsiella pneumoniae* were also present in these samples (Sartelli, et.al, 2016).

1.13.2 Reheating

Time-temperature exposures during reheating requirement to be satisfactorily high or long to disable large amounts of infectious microorganisms that could improve during the extended keeping process. Some food vendors partially or fully cook products onward of time, store them and then reheat them when demanded by customers. Yet, this reheating is often insufficient to destroy bacteria that may be remain as this would let the foodborne pathogens that incubate from spores which subsisted cooking or that contaminate the food after cooking, to survive and multiply (Sartelli,et.al, 2016).

1.14 Personal Hygiene of the Vendors or Food Handlers

In accordance with WHO, food handling personnel play a significant role in confirming food safety throughout the chain of food production, processing, storage and preparation.

Mishandling and disregard of hygienic measures on the part of the food vendors may enable pathogens to come into contact with food and in some cases to subsist and multiply in satisfactory numbers to origin illness in the consumer.

Some food handlers may present biological hazards by cross contamination after handling raw materials when they suffer from definite diseases and physical hazards by careless food handling practices. Most of the vendors pack the food in polythene bags for their customers. During packing these foods, they blow air into the polythene bags to open them, in this process a number of pathogens can be handed on to the consumer.

A study in Santa Fe de Bogota, Colombia revealed that over 30% of a group of food handlers examined were carriers of pathogenic microorganism including *Salmonella typhi*, *Staphylococcus aureus*, *Salmonella enteritidis*, and *Shigella* (Sartelli,et.al, 2016).

1.15 Factors Affecting Growth of Microorganisms

1.15.1 Intrinsic Parameters

These parameters are as follows:

- pH
- Moisture content
- Oxidation-reduction potential (Eh)
- Nutrient content (water, source of energy, source of nitrogen, vitamins and related growth factors, minerals)
- Antimicrobial constituents
- Biological structures

1.15.2 Extrinsic Parameters

The extrinsic parameters of foods are those belongings of the storage environment that affect both the foods and their microorganisms. Those of greatest importance to the welfare of food-borne organisms are as follows:

- Temperature of storage
- Relative humidity of environment
- Presence and concentration of gases
- Presence and activities of other microorganisms

1.15.3 Implicit Factors

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

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

- Eradicating or destroying them by trimming, washing, heating, pickling, by addition of chemicals, or by encouraging competition by acid or alcohol-forming organisms.

- ❖ Lessening contamination from equipment, people, the environment, and from unprocessed food.
- ❖ Lessening microbial growth on equipment, by cleaning and sanitizing, and in the product, itself by fine-tuning storage temperature, pH, and other environmental factors (Ray, 2004).

1.16 Bacterial Agents of Food-borne Illness

1.16.1 *Salmonella* species

Salmonella is a significant bacterial genus, originating one of the most common forms of food poisoning worldwide. It is one of the most broadly studied bacterial species in terms of its physiology, genetics, cell structure, and development. It is also one of the most extensively characterized bacterial pathogens and is a chief cause of bacterial gastroenteritis. *Salmonella* is capable of causing a variety of disease syndromes: enteric fever, bacteremia, enterocolitis, and focal infections (Darwin, 1999).

Fig 1.2: *Salmonella* spp

1.16.2 Microbiological Characteristics

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

1.16.3 Pathogenesis and Clinical Features

Generalized systemic enteric fever, headache, malaise, anorexia, enlarged spleen, and constipation followed by more severe abdominal symptoms; rose spots on trunk in 25% of

Caucasian patients; complications include ulceration of Peyer's patches in ileum, can produce hemorrhage or perforation; Common enterocolitis may result without enteric fever; characterized by headache, abdominal pain, nausea, vomiting, diarrhea, dehydration may result; case fatality of 16% reduced to 1% with antibiotic therapy (Adams & Moss, 2008).

1.16.4 Association with Foods

Salmonellosis is described as a zoonotic infection since the major source of human illness is infected animals. Transmission is by the faecal–oral route whereby intestinal contents from an infected animal are ingested with food or water. Meat, milk, poultry, and eggs are primary vehicles; they may be undercooked, allowing the salmonellas to survive, or they may cross-contaminate other foods that are consumed without further cooking. Cross-contamination can occur through direct contact or indirectly via contaminated kitchen equipment and utensils. Human carriers are generally less important than animals in the transmission of salmonellosis. Human transmission can occur if the faecally contaminated hands of an infected food handler touch a food which is then consumed without adequate cooking, often after an intervening period in which microbial growth occurs (Adams & Moss, 2008).

1.17 *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).

Fig 1.3: *Shigella Spp*

1.17.1 Characteristics

Shigellas are members of the family Enterobacteriaceae. They are nonmotile, non-sporeforming, 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 mesophiles with a growth temperature range between 10–45 °C and heat sensitivity comparable to other members of the family. They grow best in the pH range 6–8 and do not survive well below pH 4.5 (Adams & Moss, 2008).

1.17.2 Pathogenesis and Clinical Features

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).

1.17.3 Isolation and Identification

Lack of interest in *Shigella* as a food-borne pathogen has meant that laboratory protocols for its isolation and identification from foods are relatively underdeveloped. A pre-enrichment procedure has been described based on resuscitation on a non-selective agar before overlaying with selective media. Selective enrichment in both Gram-negative broth and selenite broth has been recommended. Selective plating media used are generally those employed for enumerating the Enterobacteriaceae or Salmonella although neither are entirely satisfactory. Rapid techniques for identification based on immunoassays which detect the virulence marker antigen, and on the polymerase chain reaction to detect the virulence plasmid by DNA/DNA hybridization have also been applied (Adams & Moss, 2008).

1.17.4 Association with Foods

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

1.18 *Vibrio* Species

Historically, cholera has been one of the diseases most feared by mankind. It is endemic to the Indian subcontinent where it is estimated to have killed more than 20 million people in 19th century. It was Robert Koch who firmly established the causal link between *Vibrio cholerae* and cholera when working in Egypt in 1886.

1.18.1 Characteristics

Vibrios are Gram-negative pleomorphic (curved or straight), short rods which are motile with (normally) sheathed, polar flagella. Catalase and oxidase-positive cells are facultatively anaerobic and capable of both fermentative and respiratory metabolism. Sodium chloride stimulates the growth of all species and is an obligate requirement for some. The optimum level for the growth of clinically important species is 1–3%.

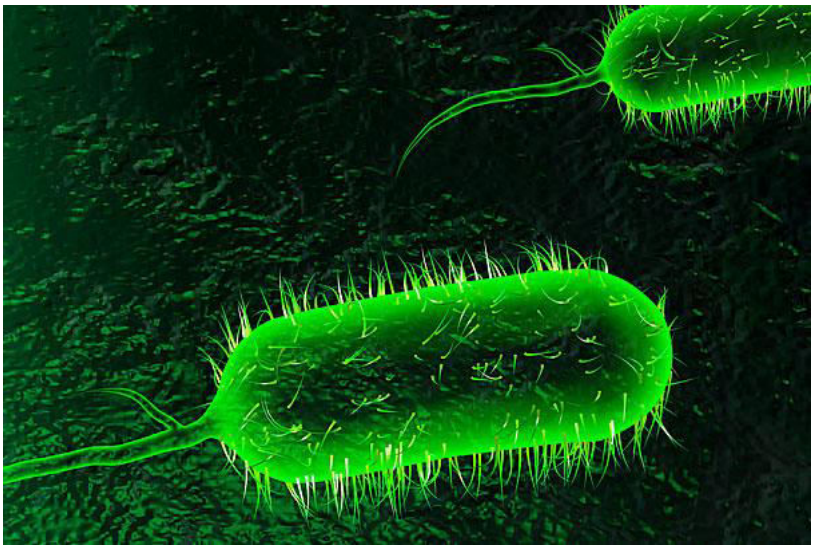

Fig 1.4: *Vibrio Spp*

1.18.2 Pathogenesis and Clinical Features

Cholera usually has an incubation period of between one and three days and can vary from mild, self-limiting diarrhoea to a severe, life threatening disorder. The infectious dose in normal healthy individuals is large when the organism is ingested without food 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).

1.18.3 Isolation and Identification

The enrichment media used for *vibrios* exploit their greater tolerance for alkaline conditions. In alkaline peptone water (pH 8.6 –9.0) the incubation period must be limited to 8 h to prevent overgrowth of the *vibrios* by other organisms. Tellurite/bile salt broth (pH 9.0 –9.2) is a more selective enrichment medium and can be incubated overnight. The most commonly used selective and differential agar used for *vibrios* is thiosulfate/citrate/bile salt/sucrose agar (TCBS). The medium was originally designed for the isolation of *V. parahaemolyticus* but other enteropathogenic *vibrios* grow well on it, with the exception of *V. hollisae. V. parahaemolyticus, V. mimicus, and V. vulnificus* can be distinguished from *V. cholerae* on TCBS by their inability to ferment sucrose which results in the production of green colonies. *V. cholerae* produces yellow colonies. Individual species can then be differentiated on the basis of further biochemical tests (Adams & Moss, 2008).

1.18.4 Association with Foods

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

1.19 *Escherichia coli*

E. coli is an almost universal inhabitant of the gut of humans and other warm-blooded animals where it is the predominant facultative anaerobe though only a minor component of the total microflora. Strains of *E. coli* were first recognized as a cause of gastroenteritis by workers in England investigating summer diarrhoea in infants in the early 1940s. Until 1982, strains producing diarrhoea were classified into three types based on their virulence properties: enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), and enterotoxigenic *E. coli* (ETEC). They are not very common causes of food-borne illness in developed countries, but an important cause of childhood diarrhoea in less developed countries (Adams & Moss, 2008).

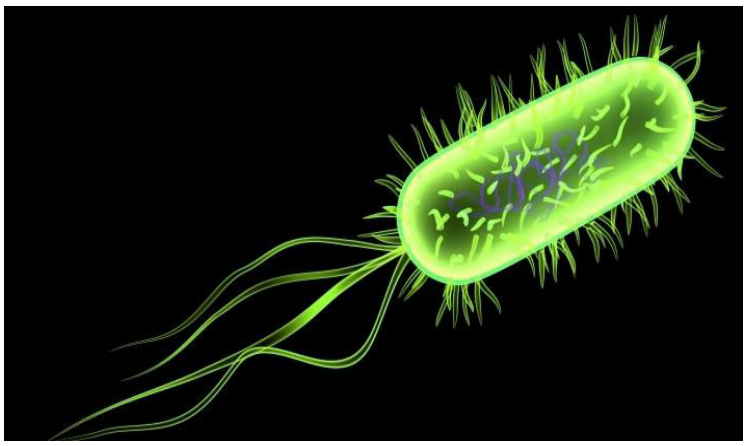

Fig 1.5: ***Escherichia spp***

1.19.1 Characteristics

Escherichia is the type genus of the Enterobacteriaceae family and *E. coli* is the type species of the genus. It is a catalase-positive, oxidase-negative, fermentative, short, Gram-negative, non-sporing rod. Genetically, E. coli is very closely related to the genus *Shigella,* although characteristically it ferments the sugar lactose and is otherwise far more active biochemically than *Shigella* spp. Late lactose fermenting, non-motile, biochemically inert strains of *E. coli* can however be difficult to distinguish from *Shigella. E. coli* can be differentiated from other members of the Enterobacteriaceae on the basis of a number of sugar-fermentation and other biochemical tests (Adams & Moss, 2008).

1.19.2 Pathogenesis and Clinical Features

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

Enterotoxigenic *E. coli* (ETEC). Illness caused by ETEC usually occurs between 12 and 36 h after ingestion of the organism. Symptoms can range from a mild afebrile diarrhoea to a severe choleralike syndrome of watery stools without blood or mucus, stomach pains and vomiting. The illness is usually self-limiting, persisting for 2–3 days, although in developing countries it is a common cause of infantile diarrhoea where it can cause serious dehydration.

Enteroinvasive *E. coli* (EIEC). Infection by EIEC results in the classical symptoms of an invasive bacillary dysentery normally associated with *Shigella.* Like *Shigella,* EIEC invades and multiplies within the epithelial cells of the colon causing ulceration and inflammation, though EIEC strains do not produce Shiga toxin. Clinical features are fever, severe abdominal pains, malaise and often a watery diarrhoea which precedes the passage of stools 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.

Enteropathogenic *E. coli* (EPEC). When the properties of ETEC and EIEC were established it was noted that these strains were rarely of the same serotypes first associated with *E. coli* diarrhoea in the 1950s. Symptoms of EPEC infection, malaise, vomiting and diarrhoea with stools containing mucus but rarely blood, appear 12–36 h after ingestion of the organism. In infants, the illness is more severe than many other diarrhoeal infections and can persist for longer than two weeks in some cases.

Enterohaemorrhagic *E. coli* (EHEC). EHEC, sometimes also known as Verotoxin-producing *E. coli* (VTEC), was first described in Canada where in some areas it rivals Campylobacter and Salmonella as the most frequent cause of diarrhoea. *E. coli* O157:H7 is the most common EHEC serotype reported, although others do occur. EHEC has attracted attention not only because foodborne transmission is more common than with other diarrhoeagenic *E. coli,* but because the illness it causes can range from a non-bloody diarrhoea, through haemorrhagic colitis, to the life threatening conditions haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP) (Adams & Moss, 2008).

1.19.3 Isolation and Identification

Selective techniques for *E. coli* mostly exploit the organism's tolerance of bile and other surfactive compounds, a consequence of its natural habitat, the gut. Aniline dyes and the ability of many strains to grow at temperatures around 44°C are also used as selective agents. The first selective and differential medium was that originally devised by MacConkey in 1905. It has been variously modified since but it is essential characteristics have remained unchanged. Bile salts (and sometimes the aniline dye, crystal violet) act as inhibitors of Gram-positive and some

fastidious Gram-negative bacteria. Lactose is included as a fermentable carbohydrate with a pH indicator, usually neutral red. Strong acid producers like *Escherichia, Klebsiella*, and *Enterobacter* produce pink colonies; non-lactose fermenters such as *Salmonella, Proteus,* and *Edwardsiella,* with rare exceptions produce colourless colonies (Adams & Moss, 2008).

1.19.4 Association with Foods

Faecal contamination of water supplies and contaminated food handlers have been most frequently implicated in outbreaks caused by EPEC, EIEC and ETEC. A number of foods have been involved, including a coffee substitute in Romania in 1961, vegetables, potato salad, and sushi. In the United States, mould-ripened soft cheeses have been responsible for outbreaks in 1971, associated with EIEC in which more than 387 people were affected, and in 1983, caused by ETEC (ST). *E. coli* would not be expected to survive well in a fermented dairy product with a pH below 5 but, where contamination is associated with mould-ripening, the local increase in pH as a result of lactate utilization and amine production by the mould would allow the organism to grow. Outbreaks caused by EHEC serotype O157:H7 have mostly involved undercooked ground meat products and occasionally raw milk. Cattle seem to be an important reservoir of infection and O157:H7 has been isolated from 0.9–8.2% of healthy cattle in the UK (Adams & Moss, 2008).

1.20 *Klebsiella pneumoniae*

Bacteria belonging to the genus *Klebsiella* frequently cause human nosocomial infections. In particular, the medically most important *Klebsiella* species, *Klebsiella pneumoniae,* accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections. The principal pathogenic reservoirs for transmission of *Klebsiella* are the gastrointestinal tract and the hands of hospital personnel. Because of their ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks (Podschun & Ullmann, 1998).

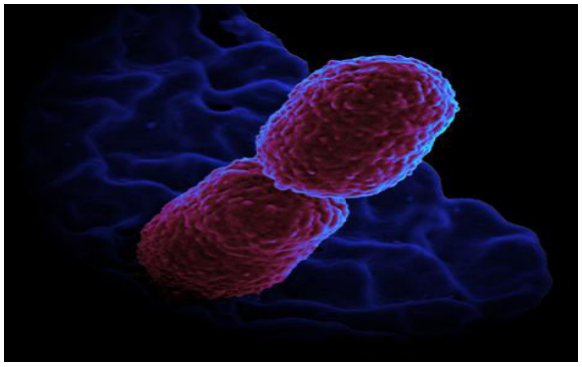

Fig 1.6: ***Klebsiella pneumonia***

1.20.1 Characteristics

Klebsiella pneumoniae 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).

1.20.2 Pathogenesis and Clinical Features

Nosocomial *Klebsiella* infections most commonly involve the urinary and respiratory tracts. Since these two body sites differ considerably with respect to the host defense mechanisms, it should be expected that the pattern of virulence factors found in UTI-causing strains of *Klebsiella* will differ from that observed in strains isolated from pulmonary sources of patients with pneumonia.

Typical *Klebsiella pneumoniae* is an opportunistic pathogen, which mostly affects those with weakened immune systems and tends to cause nosocomial infections. A subset of hypervirulent *K. pneumoniae* serotypes with elevated production of capsule polysaccharide can affect previously healthy persons and cause life-threatening community-acquired infections, such as pyogenic liver abscess, meningitis, necrotizing fasciitis, endophthalmitis and severe pneumonia. *K. pneumoniae* utilizes a variety of virulence factors, especially capsule polysaccharide, lipopolysaccharide, fimbriae, outer membrane proteins and determinants for iron acquisition and nitrogen source utilization, for survival and immune evasion during infection (Li et al., 2014).

1.20.3 Isolation and Identification

Klebsiella species are usually identified and differentiated according to their biochemical reactions. The genus is defined as containing gram-negative, non-motile, usually encapsulated rod-shaped bacteria of the family *Enterobacteriaceae,* which produce lysine decarboxylase but not ornithine decarboxylase and are generally positive in the Voges-Proskauer test (Podschun & Ullmann, 1998).

1.20.4 Association with Foods

Klebsiella pneumoniae (K. pneumoniae) is one of the most important members of *Klebsiella* genus in Enterobacteriacae family, which is responsible for pneumonia (the destructive lung inflammation disease). Vegetables are known as source of contamination with K. pneumonia. Raw vegetables are usually consumed in salads and other dishes (Puspanadan et al., 2012).

1.21 Foodborne Illness Outbreaks in USA:

Food borne diseases are a key reason of illness and death in the United States. Each year in the United States, 31 pathogens natively developed, among them about 9.4 million is foodborne. About 5.5 million (59%) foodborne illnesses were caused by viruses, 3.6 million (39%) by bacteria, and 0.2 million (2%) by parasites. The pathogens that caused the most illnesses were norovirus (5.5 million, 58%), nontyphoidal Salmonella spp. (1.0 million, 11%), C. perfringens (1.0 million, 10%), and Campylobacter spp. (0.8 million, 9%). The 31 pathogens caused 228,744 (90%) hospitalizations annually, of which 55,961 (90%) were caused by contaminated food eaten in the United States. These 31 pathogens caused 2,612 deaths (90%), of which 1,351 (90%) were caused by contaminated food eaten in the United States. Of these, 64% were caused by bacteria, 25% by parasites, and 12% by viruses. The leading causes of death were nontyphoidal Salmonella spp. (28%), T. gondii (24%), L. monocytogenes (19%), and norovirus (11%) (Scallan et al., 2011).

1.22 Foodborne Illness Outbreaks in Australia:

From 1995 through 2000, there were 214 outbreaks of gastroenteritis of foodborne origin resulting in 8,124 cases. Victoria and New South Wales recorded the highest number of outbreaks during the six-year period, the average number of outbreaks per million population by jurisdiction for the six-year period was highest in the Northern Territory, South Australia and Victoria. There were six multi-state outbreaks resulting in 945 cases. Outbreaks were more frequently reported in the warmer months of October through March predominantly due to the higher incidence of *Salmonella* outbreaks in these months. One hundred and seventy-four (81%) outbreaks had a known aetiology and these outbreaks accounted for 79 per cent of illnesse. The median number of cases for foodborne outbreaks was 17. There were 20 deaths associated with the outbreaks, equating to a fatality rate of 0.3 per cent. (Dalton.at,al, 2004)

1.24 Food Borne Illness Outbreaks in Korea and Japan

The average prevalence of reported food borne illness from 1981 to 1995 was 2.44 per 100,000 population in Korea, and 28.01 in Japan. The mean case fatality rate in Korea was 0.74% and in Japan, 0.03%. When both prevalence and case fatality rates in Korea and Japan were compared during the same period, the prevalence in Japan was much higher than that in Korea. However, the case fatality rate of patients in Korea was much higher than that in Japan. Comparison study indicates that Food borne illness outbreaks in Korea most frequently involved homemade foods (47% of the total cases); in Japan, restaurants accounted for 31.3%.

Food borne illness cases of bacterial origin in Korea were 59.3% of the total and included *Salmonella* spp. (20.7%), *Vibrio* (17.4%), *Staphylococcus* (9.7%), pathogenic *Escherichia coli* (2.4%), and other species (9.1%); in Japan, 72.8% of the total cases and the majority of the bacterial food borne illness were caused by *Vibrio* (32.3%), *Staphylococcus* (15.9%), *Salmonella* (14.2%), pathogenic *E. coli* (3.0%), and other species (7.2%) (Won-Chang et al., 2001).

1.25 Street Foods Condition in Dhaka city

In Dhaka streets, food vending is everywhere; however there is a lacking of information regarding food borne diseases related to street-vended foods. 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. 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, Shigella spp, Salmonella and Vibrio spp) in different street vended foods collected from different private universities.

4.1 Bacteriological Subculture

4.1.1 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.

4.1.1.1 Sample Category

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

4.1.2 Sample Processing

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

4.1.3 Enrichment of the Organisms

4.1.3.1 Enrichment of *E. coli* and *Klebsiella spp*

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

4.1.3.2 Enrichment of *Salmonella spp* and *Shigella spp*

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

4.1.3.3 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 1.7: Enrichment of the Organisms

4.1.4 Selective Growth of the Organisms

4.1.4.1 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.

4.1.4.2 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.

4.1.4.3 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.

4.1.5 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. 1.8: Autoclave and Hot air Oven

Fig. 1.9: Laminar Air Flow Cabinet

4.1.6 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.

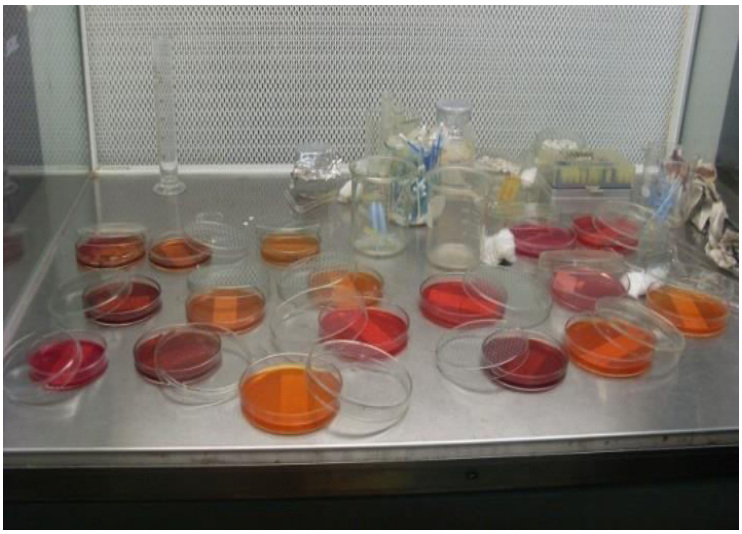

Fig. 2.1: Petri dishes preparation

4.1.7 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. 2.2: 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

4.1.8 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

Broth

- Enrichment Broth
- Trypticase Soy Broth (TSB)
- 0.3% yeast extract (YE)
- BPW (Buffered Peptone Water) broth
- APW (Alkaline Peptone Water) broth

Equipment

- Inoculating loop
- Spirit burner
- Hand gloves
- Mortar and pestle
- Incubator
- Measuring Cylinder (100ml)
- Distilled water
- Analytical balance
- Media preparation bottle

4.2 Biochemical Tests

4.2.1 Kliglar Iron Agar Test (KIA Test)

4.2.1.1 Test Tube Preparation for KIA Test

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

4.2.1.2 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. 2.3: Preparation of test tubes for KIA test

4.2.2 MIO Test

4.2.2.1 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 iodole test.

4.2.2.2 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. 2.4: Preparation of test tubes for MIO test

4.2.3 Citrate Test

4.2.3.1 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).

4.2.3.2 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. 2.5: Preparation of test tubes for Citrate test

4.2.4 Urease Test

4.2.4.1 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.

4.2.4.2 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. 2.6: Preparation of test tubes for Urease test

4.2.5 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.

4.2.6 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 Reagent
- ❖ 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 in purple color	Change in color
SCA (Simmon's Citrate Agar)		Blue	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
KIA	H2S	Black color	No black color
	Gas production	Bubble production	No bubble in test tube

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

Cell counting and serial dilutions

4.2. 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. It is desirable 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 succeeding 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.

4.3. Materials Required:

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

4.4. 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.

4.5. 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}

4.6. Mixing the dilutions into agar plates

1. Nutrient agar was prepared by autoclaving.
2. The bottle of molten agar was placed in a 50°C water bath and the agar was allowed to cool to 50°C.
3. Four empty sterile agar plates (Petri dishes) were marked 10^{-1} to 10^{-4} on the base of the plate NOT the lid. Other required details such as initials, sample type, date and culture conditions to the base of the plates were added.
4. Agar bottle from the 50°C water bath was removed and the outside of the bottle was wiped with paper toweling to remove water. Working quickly to avoid cooling of the agar to 42°C (this is the temperature at which it sets). About 15 mL of molten agar was poured into agar plates. The agar should be approximately 7 mm thick.
5. One mL of each of the dilutions was pipette into the base of correctly labeled plates using a separate pipette to avoid carryover errors.
6. Each plate was gently swirled to mix the 1 mL of diluted sample into the 15 mL of agar.
7. The plate was left without moving for at least 13 minutes to allow the agar to set
8. When the agar was set, the plate was incubated as appropriate

4.5. 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.

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.

Table 4.5.1: Colony counting of various samples

Sample Name	**Dilution 1**	**Dilution 2**	**Dilution 3**	**Dilution 4**
Plan Cake	Uncountable	15	Uncountable	37
Dim Chop	33	Uncountable	Uncountable	Uncountable
Alur Chop	Uncountable	Uncountable	35	19
Kabab	21	43	Uncountable	uncountable
Chaltar Achar	9	23	30	Uncountable
Butter Ban	Uncountable	40	Uncountable	29

For plan cake 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:

37 colonies on plate 4 x dilution factor of 10,000 = 370,000 cells/ml.

For dim chop 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:

33 colonies on plate 1 x dilution factor of 10 = 330 cells/ml.

For alur chop 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 = 35000 cells/ml.

For kabab plate 2 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:

43 colonies on plate 2 x dilution factor of 100 = 4300 cells/ml.

For chaltar achar 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:

30 colonies on plate 3 x dilution factor of 1000 = 30000 cells/ml.

For butter ban plate 2 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 2 x dilution factor of 100 = 4000 cells/ml.

Table 4.5.2: Number of colonies per ml of sample

Sample Name	**Plain Cake**	**Dim Chop**	**Alur Chop**	**Kabab**	**Chaltar Achar**	**Butter Bun**
Colony forming unit (CFU) (cells/ml)	370,000	330	35,000	4,300	30,000	4,000

4.6 Bacteriological Colony Morphology

Table 4.6.1: Bacterial colony morphology isolated from different street vended food samples

Name of The University	Sample	Agar Plates				
		MacConkey	TBX	BGA	XLD	TCBS
ULAB	Beguni 3	No Growth	No Growth	No Growth	No Growth	Yellow
	Alur Chop	No Growth	No Growth	No Growth	No Growth	Yellow
	Boroi Achar	No Growth	No Growth	No Growth	No Growth	No Growth
UIU	Velpuri 1	No Growth	No Growth	No Growth	No Growth	Blue
	Alur Chop 2	Mucoid Pink	No Growth	No Growth	No Growth	No Growth
	Fuchka 2	Mucoid Pink	No Growth	No Growth	No Growth	No Growth
EWU	Fuchka 1	Flat Dot Pink	No Growth	No Growth	No Growth	No Growth
	Alur Chop 3	Mucoid Pink	No Growth	No Growth	No Growth	No Growth
	Danish	No Growth	No Growth	No Growth	No Growth	No Growth
BRAC	Fuchka 2	No Growth	Blue	No Growth	No Growth	No Growth
	Beguni 2	No Growth	No Growth	No Growth	No Growth	Yellow
	Velpuri 3	Colorless	No Growth	No Growth	No Growth	No Growth
GTC	Plain Cake	Mucoid Pink	No Growth	No Growth	No Growth	No Growth
	Velpuri 3	No Growth	No Growth	No Growth	No Growth	Green
	Velpuri 4	Mucoid Pink	No Growth	No Growth	No Growth	No Growth

Table 4.6.1 shows bacterial colony morphology isolated from different street food samples. 15 food samples were collected from the area around five different private universities in Dhaka city. In total 13 samples show growth of different pathogenic or nonpathogenic microorganisms. Among them 5 samples show positive growth of our suspected organisms (*E.coli, Klebsiella spp., Vibrio spp.*) and 2 samples show no growth in these agar media.

Table 4.6.2: Bacterial colony morphology isolated from different street vended food samples

Name of The University	Sample	Agar Plates				
		MacConkey	TBX	BGA	XLD	TCBS
AUST	Fuchka 2	No Growth	No Growth	No Growth	No Growth	Yellow
	Velpuri 5	Mucoid Pink	No Growth	No Growth	No Growth	No Growth
	Alur Chop 3	No Growth	No Growth	No Growth	No Growth	Yellow
Stamford University	Alur Chop 5	Mucoid Pink	No Growth	No Growth	No Growth	No Growth
	Velpuri 1	Mucoid Pink	No Growth	No Growth	No Growth	No Growth
	Fuchka 1	Mucoid Pink	No Growth	No Growth	No Growth	No Growth
BIU	Beguni 1	Mucoid Pink	No Growth	No Growth	No Growth	No Growth
	Velpuri 1	No Growth	No Growth	No Growth	No Growth	Green
	Alur Chop 5	No Growth	No Growth	No Growth	No Growth	Yellow
UAP	Beguni 1	Mucoid Pink	No Growth	No Growth	No Growth	No Growth
	Velpuri 3	Mucoid Pink	No Growth	No Growth	No Growth	No Growth
	Velpuri 2	No Growth	No Growth	No Growth	No Growth	Green
Green University	Fuchka 1	No Growth	No Growth	No Growth	No Growth	Yellow
	Fuchka 1	No Growth	No Growth	No Growth	No Growth	No Growth
	Alur Chop 4	No Growth	No Growth	No Growth	No Growth	Yellow

Table 4.6.2 shows bacterial colony morphology isolated from different street food samples. 15 food samples were collected from the area around five different private universities in Dhaka city. In total 13 samples show growth of different pathogenic or nonpathogenic microorganisms. Among them 5 samples show positive growth of our suspected organisms (*E.coli, Klebsiella spp., Vibrio spp*.) and 2 samples show no growth in these agar media.

Fig 2.7: Bacterial Colony on Agar Plates

Table 4.6.3: Number of food samples with growth of suspected organisms determined by colony morphology (n=30)

Name of The University	No. of samples with +ve growth by *E.coli*	No. of samples with +ve growth by *Klebsiella spp*	No. of samples with +ve growth by *Vibrio spp*
ULAB	0	0	2
UIU	0	2	0
EWU	2	0	0
BRAC	0	1	1
GTC	0	2	1
AUST	0	1	2
Stamford University	0	3	0
BIU	1	0	1
UAP	0	2	0
Green University	1	0	2

Table 4.6.3 shows the number of food samples contaminated with the targeted organisms. In total 25 samples were suspected to be contaminated with either *E.coli* or *Klebsiella spp.*, 11 samples were suspected to be contaminated with *Vibrio spp.*

4.7 Suspected Organisms from Biochemical Tests

Table 4.7.1: Identification of the suspected organism (*Klebsiella spp.*) from different biochemical tests

Samples	Plates	Colony Morphology	M	I	O	Citrate	Urease	Oxidase	KIA			Suspected Microoragani-sms
									Slunt/ Butt	Gas	H2S	
Fuchka 2	TBX	Blue	-	+	-	-	-	-	A/A	-	+	*Klebsiella pneumonia*
Alur Chop 5	MacConkey	Mucoid Pink	-	+	-	+	-	-	A/A	-	+	
Plain Cake	MacConkey	Mucoid Pink	-	+	-	+	-	-	A/A	-	+	
Beguni 1	MacConkey	Mucoid Pink	-	+	-	+	-	-	A/A	-	+	
Velpuri 1	TBX	Blue	-	+	-	+	-	-	A/A	-	+	

Table 4.7.1 shows identification of the suspected organism (*Klebsiella spp.*) from different biochemical test. In total 8 (27%) food samples were identified to be contaminated with our suspected organism *Klebsiella spp.* from these biochemical tests.

Table 4.7.2: Identification of the suspected organisms (*E.coli, Vibrio*) from different biochemical tests

Samples	Plates	Colony Morphology	M	I	O	Citrate	Urease	Oxidase	KIA			Suspected Microorganisms
									Slunt/ Butt	Gas	H2S	
Fuchka 1	TCBS	Yellow	+	+	-	+	-	-	K/A	-	+	*Vibrio*
Fuchka 1	MacConkey	Flat Dot Pink	+	+	-	+	-	-	A/A	-	+	*E.coli*
Fuchka 1	MacConkey	Flat Pink	-	+	-	+	-	-	A/A	-	+	
Fuchka 2	TCBS	Yellow	+	+	-	+	-	-	K/A	-	+	*Vibrio*
Beguni 3	TCBS	Yellow	+	+	-	+	-	-	K/A	-	+	

Table 4.7.2 shows identification of the suspected organisms (*E.coli, Vibrio*) from different biochemical tests. In total 2 (7%) food samples were identified to be contaminated with *E.coli* and 3 (10%) food samples were identified to be contaminated with *Vibrio spp.* from these biochemical tests.

Table 4.7.3: Presence of suspected organisms in no of food samples from different university

Name of The University	*E.coli*	*Klebsiella spp.*	*Vibrio spp.*	*Shigella spp.*	*Salmonella spp.*
ULAB	0	0	1	0	0
UIU	0	1	0	0	0
EWU	1	0	0	0	0
BRAC	0	1	0	0	0
GTC	0	1	0	0	0
AUST	0	0	1	0	0
Stamford	0	1	0	0	0
BIU	1	0	0	0	0
UAP	0	1	0	0	0
GU	0	0	0	0	0

Table 4.7.3 shows presence of suspected organisms in no of food samples from different university. . In total 10 (34%) food samples were identified to be contaminated with *E.*coli, Klebsiella *spp.* and *Vibrio spp.* except *Shigella spp.*and *Salmonella spp.*

Table 4.7.4: Incidence of food borne pathogens in various street vended food samples

Pathogens	Food Categories			
	Fried Items (n=12)	**Spicy Items (n=17)**	**Baked items (n=1)**	**Total (n=30)**
E.coli	2(17%)	Nd	Nd	2(7%)
Klebsiella spp.	2(17%)	2(12%)	1(100%)	5(17%)
Vibrio spp.	1(8%)	2(12%)	Nd	3(10%)
Shigella spp.	Nd	Nd	Nd	Nd
Salmonella spp.	Nd	Nd	Nd	Nd

Table shows incidence of food borne pathogens in various street vended food samples. Among 12 fried items, 2(17%) samples were suspected to contain *E.coli*, again 2(17%) were to contain *Klebsiella spp.* and 1(8%) were to contain *Vibrio spp.*

Among 17 spicy items 2(12%) samples were suspected to contain *Klebsiella spp.*, again 2(12%) were to contain *Vibrio spp.* among 1 baked items, only 1(100%) samples were suspected to contain *Klebsiella spp.*

5.1 Discussion:

There is a growing demand for street vended food products that is not enough information about the microbiological quality of these products in Dhaka city, Bangladesh. This study was accompanied to find out the enteric bacteria specially *E.coli, Klebsiella spp., Vibio spp., Shigella spp. and Salmonella spp.* In this study, 30 different food samples were collected from 10 private universities in Dhaka city, Bangladesh. Five agar media MacConkey, Tryptone Bile X-glucoronide (TBX) agar, Thiosulfate Citrate Bile Salt-sucrose (TCBS) agar, Brilliant Green Agar (BGA) and Xylose-Lysine Desoxycholate agar (XLD) were used to detect the existence of certain microorganisms in food items. MacConkey and TBX agar are used for the identification and isolation of *E. coli* and *Klebsiella.* MacConkey agar is highly selective for gram negative bacteria. Those who ferment lactose give pink colonies and those who are non-fermenting give colorless colony on plate. On TBX agar *E.coli* gives blue green colored colony. For these agars, samples are enhanced in yeast extract and TSB (trypticase soy broth) broth and then inoculated on plate. TCBS Agar is highly selective for *Vibrio* species isolation. They generally give yellow colony on the plate. In this study, most of the colonies on TCBS were yellow and brownish-yellow. Some green colonies were also found. After biochemical tests, we can say that yellow colonies were may be of Vibrio species. Before inoculation, specimen is enriched in APW (alkaline peptone water) broth. XLD and BGA are used for isolation of *Salmonella* and *Shigella* species from food specimen. *Salmonella* gives red colonies and some with black centers. *Shigella* species gives red colonies. Here samples are enriched in BPW (buffered peptone water) broth before inoculation. A study revealed that 95(72%) of the food samples had pathogenic bacterial contaminations in Jigjiga City, Eastern Ethiopia. Three different bacterial species were isolated: *E. coli* 68 (51.5%), *S. aureus* 85 (64.4%) and 26 (19.7%) *Salmonella species*. The highest prevalence of *S. aureus* 23/33 (69%) was seen in 'Sambusa'; the highest incidence of *E. coli* 24/33 (73.5%) was observed in 'Pasta', while the highest *Salmonella* incidence was observed in 'Ades'. This study revealed that there is a reasonable gap on food safety knowledge among street food venders. The microbial profile was also higher compared to standards set by the World Health Organization (Nguyen et al., 2014).

A Study was conducted on 37 street vended food samples which were examined for bacterial identification. The isolates were identified as *Escherichia coli* (37.5%), *Pseudomonas aeruginosa* (3.57%), *Staphylococcus aureus* (14.20%), *Salmonella sp.* (5.36%), *Klebsiella sp.*

(10.71%), *Shigella sp.* (19.64%) and *Enterobacter sp.* (8.93%) respectively. (Sharma & Mazumdar, 2014).

A study was conducted on 60 food samples in some minimally and fully processed ready-to-eat foods in Kano metropolis, Nigeria, *Escherichia coli* recorded the highest frequency of occurrence of 24 (46.6%), followed by *V. cholerae* with 15 (25.0%) while *Salmonella typhi* recorded the least occurrence rate of 6 (10.0%). Overall, the fully processed foods were observed to be less contaminated with *enteropathogenic* bacteria than the minimally processed foods. The results indicated that most of the ready – to – eat food samples examined in this study did not meet bacteriological quality standards (Bukar et al., 2010).

A study has been done to analyze the microbiological quality of salads served along with street foods of Hyderabad. A total of 163 salad samples, 53 of carrot and 110 of onion samples, were collected from four different zones of Hyderabad. About 74% and 56% had *Staphylococcus aureus* in carrots and onions, respectively. Fifty-eight percent of carrots and forty-five percent of onions samples contained *Salmonella*, 68% of carrots and 24% of onions had *Yersinia* (Sabbithi et al., 2014).

A study was conducted in Amravati, India. Forty water sample of panipuri were aseptically collected from eleven locations of Amravati City. Analysis of the food samples revealed that 93% of panipuri water samples had high loads of bacterial pathogens such as Escherichia coli (41%), Staphylococcus aureus (31%), Klebsiella spp. (20%), Pseudomonas spp. (5%) and yeast (3%). It is suggested that regular monitoring of the quality of street foods must be practiced to avoid any food-borne infection in future (Tambekar et al., 2011).

6.1 Conclusion:

In this study the street food vendors from the selected areas indicated that more viable microbial counts than the foods standardized at home. The gram negative microorganisms were present in significant numbers in street foods. So this study has undoubtedly revealed that some of the most popular types of foods that were vended on the streets of Dhaka City do not meet the required acceptable quality and safety levels. Measures need to be taken to ensure that street vendor food ingredients should be produced and stored hygienically at appropriate temperatures and well protected from flies, dust, wind, and all sources of contamination. Utensils should be washed using detergents and clean hot water. Health education of should be provided to the vendors and implementation of hygienic protocols enforced more vigorously.

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