PREVALENCE OF MICROBIAL HAZARDS IN STREET FOOD AND RESTAURANT SALAD OF DHAKA CITY

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
Bachelor of Science in Microbiology

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled. “Prevalence of Microbial Hazards in Street Food and Restaurant Salad of Dhaka City” Submitted by [Pavel Islam (Student ID:19326031)] of Fall, 2024 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology on 22-02-23

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Ethics Statement

The study strictly adhered to the principles and standards of the Helsinki declarations. However, because this study did not include any human or animal experimentation, ethical clearance was not required. Before collecting data, the street food vendors provided informed consent, and the respondents' privacy and data confidentiality were maintained strictly. This survey was done as part of the BFSA project-2023, and partial data that I obtained was used in this study with the project director's approval.

Abstract

Food Safety remains major concern for consumers and street foods are often related to the food poisoning. This study was conducted to determine the prevalence of pathogenic microorganisms associated with the street food and the mixed salad of restaurants of Dhaka city. To evaluate the microbial hazard, a total of 113 street food samples were examined. Four types of street foods including aloevera sharbat (10 samples), sugarcane juice (10 samples), fuchka (10 samples), and sandwiches (10 samples) and mixed salad (3 samples) of restaurant and their associated utensils, including, preparation mug, serving glass, plate and spoon swabs were collected and analyzed for microbial quality (aerobic plate count (APC), coliform count, yeast and mold count) and safety parameters including presence of *E.coli*, *Salmonella*, *Vibrio cholera*e, and *Listeria* spp. Enrichment was used to detect the presence of *Salmonella*, *Vibrio cholera*e, and *Listeria* spp. Analytical Profile Index (API 20E) were performed for the confirmation of presumptively assumptive bacteria. The findings revealed that the street food samples and their utensils including mug/jug, glass, serving glass, plate, and spoon, were severely contaminated with various microorganisms. The samples were heavily contaminated with APC coliform, fecal coliform, yeast and mold, indicating the poor quality of the street food and the presence of *E. coli* was found in 90.6% samples indicating the presence of fecal materials in the street foods. On an average, coliform was the most widespread microorganisms followed by fecal coliform with a slight different range, while comparing five types of street food samples. *E. coli* prevalence was found in 90.6% samples, while *Vibrio cholera, Salmonella spp.* and *Listeria spp*. was detected in 5-10% of the samples. This study highlighted the prevalence of pathogenic microbes in street food which is human heal concern. Therefore, interventions including training and awareness to the vendors and consumers and accessibility to safe water of the street vendors should be ensured to improve the safety of street food.

Keywords: Prevalence, street food vendors, aloevera sharbat, sugarcane juice, and mixed salad,

Dedication (Optional)

To my parents & Mubashshira

Thank you for being the anchors of my life.

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Pavel Islam February,2024

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List of Acronyms

API	Analytical profile index
ATCC	American Type Culture Collection
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BSL	Biosafety Level
CAGR	Compound annual growth rate
CFU	Colony forming unit
FIBC	Flexi Intermediate Bulk Container
GDP	Gross Domestic Product
LSA	Listeria Selective Agar
SMAC	Sorbitol MacConkey Agar
TABC	Total aerobic bacterial count

TCC	Total coliform count
TFCC	Total Fecal coliform count
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
UV	Ultra violate
XLD	Xylose Lysine Deoxycholate
g	gram
l	liter
ml	milliliter
pH	Negative logarithm of Hydrogen ion concentration
spp.	Species
E. coli	Escherichia coli
°C	Degree centigrade
et al.	and others

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The street food sector contributes significantly to the socioeconomic well-being of metropolitan dwellers. It meets the nutritional demands of lower- and middle-class customers by providing nutritious and affordable food options. Due to its appealing flavors and easy accessibility, street food has become very popular and is a favorite option for a wide variety of people. Street foods not only meets the dietary needs of city dwellers, but it also makes an important contribution to the overall vibrancy and accessibility of urban food options (Ackah *et al*,2011; Morales & Cross, 2007; Muzaffar *et al*., 2009). In many developing nations, street food provides an important portion of people with a means of livelihood and nutrition for low-income people in cities. food and drinks that are ready to eat and can be enjoyed on the streets without extra work are known as street meals (Rane, 2011). Usually, hawkers and sellers sell these foods in the streets or other comparable public areas. While street food vendors may have some benefits many agree that they often face challenges such as poverty, low literacy rates, and lack of having enough knowledge regarding essential aspects such as safe handling of food, environmental concerns, proper hygiene and sanitation procedures, food presentation, serving etiquette, hand hygiene ,ingredient sourcing, and the utilization of potable water. Consequently, it is believed that consuming street food creates a significant risk to the health of the general public (Bhowmik, 2010). Microbial foodborne diseases indicate an important threat to health associated with street food consumption (Biswas *et al.,* 2010). Moreover, the multidrug resistance of foodborne bacteria made the public health situation concerning food safety even worse. (Ali *et al.,* 2012). Every year, foodborne diseases impact over 30 million persons in Bangladesh (*The State of Food Insecurity in the World 2012,* n.d.). The majority of street food in Bangladesh is cooked by hand and sold to customers at various transport terminals, on the side of the road, or by nomadic vendors (Al Mamun *et al.,* 2013). Research was done in Dhaka City Corporation to determine the cleanliness and sanitation practices of street food sellers as well as the socioeconomic situations of the vendors. The results of the research showed that twenty-five percent of street food sellers lack formal education and are uneducated, unable to write their names. Due to the inexpensive investment needed for the street food industry, a large number of vendors (88%) turned out to be business owners. They usually work without restrooms for 13–18 hours every day. a variety of the locations

researched, nearly all the vendor's businesses (68%) were situated on the walkway, and 18% and 30% of the vending carts were positioned close to sewage and municipal drains, respectively. Microbiological studies of various food items, potable water, and samples from hand swabs indicated the presence of a huge number of pathogens, coliform bacteria, and aerobic bacteria (FAO,2010). Studying the epidemiology diseases caused by food is essential for maintaining public health. This knowledge makes it easier to prevent and control these diseases, make sure that resources are allocated effectively, monitor and assess food safety protocols, create new standards, and determine whether treatments are cost-effective. The study aims to assess the microbiological risk associated with street food-related foodborne illnesses, examining the nature of the issue, surveillance methods, risk factors, and mitigation strategies *(*Khairuzzaman *et al.,* 2014).

1.2 Literature review

1.2.1 what is street food

The term "street food sector" denotes the industry providing food and beverages prepared for consumption and available for purchase through vendors, primarily in street and similar public places (FAO, 1990). Street food has great both social and economic importance (FAO, 2009). It makes it difficult to tell apart local businesses from handmade small enterprises, in city areas (Canet, 1997). Its importance in the city's development and the operation of the urban economy indicates the habits of living and surviving in African cities. In the continent and other major Third-World cities, the sale and consumption of food from the outside is known by several names: "street food," "popular catering," and "Roadside food (“The State of Food and Agriculture 2007,” 2007). It has been found that the term of "eating out" varies based on the circumstance. Eating out includes four main aspects: socializing, emotional connection, tradition, and hygiene or cleanliness maintenance (Warde,2000).

& Martens, 2000) . The concept of "eating out" relates to any food item leading to in a charge at an outside food service (Soula *et al*., 2020). Street foods and dining are merged in out-of-the-house hospitality (T, 2018). The informal food business, often known as street food, is one of the links in a food chain that, primarily in cities, plays an essential role in satisfying the demands of all socio-professional kinds of food (Ohiokpehai, 2003). Homemade cuisine is what's referred to be street food, whereas stale food is sold or enjoyed outside of houses (Albuquerque *et al*., 2019).

1.2.2 popular street food

Street vended foods are becoming popular among urban people as they are inexpensive, convenient, and attractive (WHO, 1996). One of the popular street foods in Bangladesh is fuchka, which has a mix of names like panipuri, gol gappa,. It's somewhat sweet, slightly sour, and a little spicy (Springer, 2022). sugarcane juice is also commonly used as a delicious drink in both urban and rural areas (Begum, 2015). Aloe vera juice which is a blend of gel and water) is widely drunk and has been proven to promote heart health. This sharbat is a favorite street meal in Bangladesh (Saha, n.d.).

1.2.3 Preparation of street food

The World Health Organization (WHO) has defined Street vended as foods and beverages prepared and sold by vendors in public places for immediate consumption or later consumption without further processing or preparation." (WHO, 1996).

1.2.3.1 Aloevera Sharbat

1. i)Ingredients:
    a. Aloe Vera leaf- 1
    b. Lemon juice- two tablespoon
    c. Honey- as per taste
    d. Powdered jaggery- 1 teaspoon
    e. Cold Water- three-fourth serving glass/mason jar
    f. Cumin seeds (jeera)- half teaspoon
    g. Dry red chill- half
    h. Black salt- as per taste
    i. Star Thorn-1 teaspoon
    j. Sesame-1 teaspoon
    k. Black cumin-1 teaspoon
    l. Psyllium Husk-1 teaspoon
    m. Gold leaf-1 teaspoon
    n. Seed of the hollyhock1 teaspoon

ii)Method

Cut and discard the gel from the aloe vera leaf, then mix it with lemon juice, honey, and jaggery. Add cold water and mix again. Grind jeera and red chili coarsely, then add the juice to a glass or mason jar with spice mix, black salt, and chaat masala (Saha, n.d.).

1.2.3.2 Fuchka

Fuchka spheres are crispy hollow spheres filled with mashed potatoes, chickpeas, onions, cucumber, lime, coriander, and green chilies. Garnishes include boiled eggs and tamarind water sauce for a sweet, tangy, and spicy taste (Springer, 2022).

i) For the Fuchka Shell:

a) 1 ½ Cup Whole Wheat Flour
b) 2 Tablespoons Rice Flour
c) 1 Tablespoon Semolina (Shuji)
d) 2 Teaspoons Star Thorn (Talmakhna Seeds)
e) 3 Cups Vegetable Oil or Soybean Oil
f) 2 Cups Water

In a large mixing bowl, combine whole wheat flour, rice flour, semolina, and star thorn for the Fuchka shell. Knead the ingredients for 5 minutes, ensuring the dough is neither too hard nor too soft. Store the dough for half an hour. Roll the dough into a thin layer, then separate a small round part from the dough using a sharp bowl or cookie cutter. Preheat vegetable oil in a large wok and deep fry the raw Fuchka shells until they are puffed and float on the oil. Remove the shells using a strainer.

ii) For the Fuchka Puree:

a) 1 Cup Chickpeas (Dabli Lentil)
b) 2 Teaspoons Ground Red Chilli
c) 1 Teaspoon Turmeric Powder
d) 1 Teaspoon Salt
e) 2 Fresh Potatoes
f) 1 ½ Teaspoon Chaat Masala Seasoning
g) 1 teaspoon Chilli Flex

h) ½ Cup Chopped Green Chilli
i) 1 Tablespoon Chopped Coriander leaves
j) 1 Large Egg
k) ½ Cup Diced Onion
l) ½ Cup Tamarind Chutney/ Sauce

Mix boiled daal, potatoes, Chaat Masala Seasoning, Chilli Flex, green chili, coriander leaves, onion, ground red chili, salt, and tamarind chutney in a bowl. Add shredded boiled eggs for optional. Blend tamarind pulp, chili flakes, ground cumin, Chaat masala seasoning, lime juice, green pepper, sugar, salt, and water.

iii)For the Chutney/Sauce:

a) ½ Cup Tamarind Pulp
b) 1 Teaspoon Chilli Flex
c) 1 Teaspoon Ground Roasted Cumin (Jeera Gura)
d) 1 Teaspoon Chaat Masala Seasoning
e) 1 tablespoon Lime Juice
f) 1 tablespoon Finely Chopped Green Pepper
g) 2 Tablespoons Granulated Sugar
h) 1 Teaspoon Salt

iv) Method

Mix tamarind pulp, chili flakes, cumin, Chaat masala seasoning, lime juice, green pepper, sugar, salt, and water. Blend well, then fill a small bowl with the chutney. Create a hole in Fuchka balls by poking them with a thumb. Fill them with puree, arrange on a plate with chutney, and garnish with cucumber, eggs, coriander, and onion (Hossain, 2023).

1.2.3.3. Sugarcane Juice

i) Ingredients:

a) Sugarcane (medium-sized)
b) Water
c) Ice cubes

d) Strainer or muslin cloth

ii) Method

Wash sugarcanes, peel hard outer layer, and blend with ginger. Add water and grind well. Extract sugarcane juice and strain through a cloth or strainer. Add lemon juice, black salt, ice cubes, and serve chilled. Add lemon juice and ice cubes for a refreshing drink.

1.2.3.4 Sandwich

i) Ingredients:

a) Sliced sandwich bread
b) Condiments (salt, mustard)
c) sandwich sliced meat
d) cheese
e) Lettuce
f) Tomatoes
g) Pickles
h) Knives (1 for slicing, and 1 for spreading condiments)
i) Plate
j) Paper towels

ii) Method

Cleanse the vegetables and pat dry before assembling them into a sandwich. Cut up tomatoes and any other veggies that require slicing. Place the supplies on a sanitized surface and grab two slices of bread. Spread the condiment evenly over one side of the sliced bread after filling a condiment jar to the recommended quantity. Blend condiments to explore a variety of flavors. Make sure the meat(s) reaches the bread's edge when placing them on a single piece of bread that has the condiment on the side. Slice the meat in half if it's round, then align the straight edges with opposing sides of the bread. Add enough cheese to cover the entire sandwich and place it on top of the meat. Top with desired toppings (lettuce, pickles, and tomato slices). Top the toppings with the second slice of bread, coated with the condiment. Cut the sandwich into forms like triangles or rectangles that will make handling it simpler. If desired, cut it into ornamental forms like hearts or stars. Present and savor your sandwich. (Instructables, 2017).

1.2.3.5 Mix Salad

i) Ingredients:

a) 2 large tomatoes, diced
b) 1 large onion, cut into thin strips
c) 4 green Cucumber, chopped
d) 1 bunch of cilantro leaves, minced into tiny strips
e) 2 limes, juiced
f) salt to taste

ii) Method

Combine tomatoes, onion, green Cucumber, and cilantro in a bowl. Add lime juice, and rice vinegar, and season with kosher salt. Chill for 45-60 minutes, then stir before serving. Adjust the amount of vinegar based on corn sweetness and lime acidity (*Mixed Salad With Bangla Touch Recipe From Bangladesh*, n.d.).

1.2.4 Microbial contamination of street food and associated outbreak

Food and drinking water that has been tainted by microorganisms are often considered major channels for the spread of foodborne illnesses worldwide (WHO, 1999). Over the past few decades, foodborne diseases have been causing significant morbidity in both urban and rural areas of Bangladesh *(Haq and Rahman,1991; Henry et al., 1990; Islam et al., 1993: 2008; Luby et al., 2006; Ram et al., 2007; Saha et al., 2009; Sheikh et al., 2002).* Poor hygiene during food preparation and a lack of knowledge about food safety are common causes of foodborne infections (WHO, 1999). A major worry is that the food sold by street vendors microbiological quality. Street food customers are in some degree of danger of developing foodborne infections due to the microbial contamination of the food they purchase from street vendors (INFOSAN, 2010).

Because foodborne infections are thought to spread globally through street vending of food and drink (Mensahet al., 2002; Mosupye and von Holy, 2000; Muleta and Ashenafi, 2001;Murindamombe et al., 2005). Regular consumers of these specific items from the street, as well as youngsters attending school, are more vulnerable to the majority of foodborne illnesses (WHO, 1996). The outbreak of diarrhea in Dhaka in 2018 and surrounding areas was investigated by a study focusing on the primary pathogen, Vibrio cholerae, which had a high isolation rate of 27%. The study found that the epidemic was primarily caused by V. cholerae,

which was associated with severe dehydration and vomiting among those receiving care at the Dhaka Hospital. During the outbreak, the isolation rate of V. cholerae rose to 27%, a trend similar to previous diarrhea epidemics in Bangladesh caused by floods. The type of V. cholerae that caused the outbreak were similar to those resulting in cholera in the time frame for comparison. The study suggested that host-mediated amplification of circulating strains of V. cholerae might account for these epidemics. Patients during the epidemic were more likely to present with severe dehydration and vomiting, primarily due to V. cholerae. This research offers valuable insights into the epidemiology and management of diarrhea outbreaks, aiding in better preparedness for future incidents (Hasan et al., 2021).

In the year, 2021 A major diarrhea epidemic in the Barisal division 2021 resulted in eight deaths and hundreds of hospital admissions across six districts. The diarrhea crisis has impacted 18 out of 40 upazilas across 6 districts of the Barisal division. The district of Bhola had 8,090 hospitalizations from the start of the year and Wednesday, making it the area hardest hit by the issue. Just in the past month, the total amount was 4,252. Water contamination has been detected by Barisal district health experts as the origin of this disease. Two research groups from the Institute of Epidemiology, Disease Control and Research (IEDCR), according to Dr. Shyamal Mandal, detected E. Coli bacteria in the stool of patients suffering from diarrhea. The E. Coli bacteria is the source of acute diarrhea and cholera-like symptoms (Dhali, 2021).

In the year, 2022 The diarrhea epidemic that began in Dhaka has now expanded to Kurigram, Rajshahi, and surrounding districts in Bangladesh. Patients suffering from diarrhea have become more numerous in Rajshahi.41 kids were hospitalized with diarrhea on Monday at Rajshahi Medical College Hospital (RMCH). From last Thursday to Sunday, around 140 youngsters were admitted to the hospital. According to hospital experts, many individuals are drinking juice on the streets to relieve their thirst due to the heat. Typically, polluted water is used for making the juice in an unhygienic environment.

According to the Hospital, the number of diarrhea patients is growing as temperatures rise. "Though it is mainly a water-borne disease, people are getting diarrhea due to eating different types of street food in this weather," he went on to say. According to icddr,b figures, 8,437 individuals were hospitalized to the specialist hospital for diarrhea treatment between March 21 and Sunday, averaging 1,200 per day. On March 23, 1,272 patients, the highest number, were hospitalized. Diarrhea epidemics are typical in Bangladesh on both sides of the monsoon season, but they are unusual this early in the year, he noted.

"The summer is often when diseases develop. "We need to look into why the arrival time has changed," Dr Baharul Alam added. Jatrabari, Shanir Akhra, and Kadamtali are the locations from which the majority of the patients come from in Dhaka (Abdullah, 2022).

1.2.5 Bacterial parameters

a. Total Aerobic Bacterial Count: Total aerobic bacterial counts (TABC) indicate the shelf life of food products. If the TABC of a food product is high, it indicates that the product's shelf life is short and that food is spoiling (Aziz,2020).

b. Total Coliform Count: Total coliform counts or TCC indicate the hygiene status of the food processing area (Aziz,2020).

c. Total fecal coliform count: Fecal coliform is a subgroup of coliform or TC that includes coliform species found only in the gastrointestinal tract and feces of warm-blooded animals, including humans. They are now known as "thermotolerant coliforms" because they can live and ferment lactose at temperatures of up to 440 degrees Celsius. They can be discovered in warm-blooded animals' digestive tracts and feces (Dahal, 2023).

d. Escherichia coli: Escherichia coli detects whether the product is fecally contaminated. Escherichia coli (E. coli) is commonly found in human digestive organs and warm-blooded animals. A few strains, such as Shiga toxin-producing E. coli (STEC), can cause real foodborne illness (World Health Organization: WHO, 2018). It is mostly transmitted to humans by the consumption of infected foods, and one strain can cause severe food poisoning (*E Coli Enteritis: MedlinePlus Medical Encyclopedia*, n.d.).

e. Salmonella spp.: The total amount of Salmonella spp. shows the pathogenicity of the food product. Salmonella spp. cause salmonella food poisoning. When food or drink contaminated with tainted feces is consumed, human illness occurs. Infection with gastrointestinal salmonella usually affects the small intestine (Gabbey, 2019).

f. Listeria spp.: Listeria also suggests food pathogenicity, similar to salmonella (Aziz,2020).

g. Total Yeast and Mold: Both yeasts and molds contribute to food deterioration and breakdown. They may infiltrate and grow any type of food at any time; they invade crops in fields before harvesting and during storage, such as grains, nuts, beans, and fruits. They also thrive on refined diets and food mixes (Nutrition, 2022)

h. Vibrio Spp.: Cholera is a highly infectious disease spread through the usage of contaminated food or water (Azman *et al.,* 2013). Cholera transmission is strongly connected to a not having access to potable water and sanitation services (World Health Organization: WHO & World Health Organization: WHO, 2023).

1.2.6 Survival of microorganisms in street food.

Microbes can contaminate food at any point in the manufacturing process, including processing, transporting, storing, displaying, preparing, and serving it for consumption. The main problems include air pollution, exposure to household and other animals, especially mice and insects, inadequate infrastructure, the lack of drinkable water, improper hygiene procedures by food handlers, and food storage at temperatures that encourage microbial development (Amare *et al.* 2019, Abebe *et al*. 2020).

Street food microbiological infection can be affected by high ambient temperatures. In Quetta, Pakistan, the summer months have the highest total aerobic bacterial counts in samples of ready-to-consume street food, with an average temperature of 37°C (Raza *et al.* 2021). The scientists concluded that one possible explanation for why microbial contamination is higher in the summer is an increase in housefly numbers.

Contamination of raw materials may occur from enteric pathogens in untreated sewage utilized as soil fertilizer or in water used to irrigate crops. These diseases can spread rapidly, especially if they are used to make street food in an unsanitary environment. Several street foods around the world contain significant levels of microorganisms along with other foodborne diseases (Birgen *et al.* 2020; Budiarso *et al.* 2021; Ferrari *et al*. 2021; Salamandane *et al*. 2021).

1.2.7 Isolation, identification, and confirmation of microorganisms

Microorganisms can be found on all inanimate surfaces, making them a constant source of potential contamination in laboratories. Experimental performance is dependent on a scientist's capacity to sterilize work surfaces and tools while avoiding contact with non-sterile surfaces using sterile instruments and solutions. The processes for a few plating strategies often used in the lab to separate, distribute, or count microorganisms, such as bacteria and phages, are described here. All five approaches involve aseptic techniques or processes to preserve the sterility of experimental materials. (1) Streak plating, (2) Pour plating, (3) Counting viable bacterial colonies using spread-plating, followed by four soft agar overlays and five replica plating steps. If there are strains of microorganisms in the laboratory bank that are not harmful,

these techniques can be used there. BSL-2 organisms must undergo these procedures in a biosafety cabinet. To find out how biohazards are classified, as well as the required safety precautions and containment facilities for the specific microorganism in question, consult the most recent editions of the Infectious Substances Material Protection Data Sheets (MSDS) and Biosafety in Microbiological and Biomedical Laboratories (BMBL) publications. Bacterial strains and phage stocks can be obtained from (ATCC) by researchers, companies, and collections under the management of particular organizations, including the American Type Culture Collection (Sanders, E. R., 2012). It is best to experiment with non-pathogenic strains while examining different plating methods. Following the instructions in this protocol, students should be able to perform plating processes without polluting the medium. Carry out plating processes without contaminating the media.

- Isolate single colonies of bacteria by the process of streak-plating.
- To assess the concentration of bacteria, use pour-plating and spread-plating approaches.
- When dealing with phages, perform soft agar overlays.
- Use the replica-plating technique to move bacterial cells from one plate to another.
- Select the appropriate plating process, and provide an experimental assignment.

1.2.7.1 Analytical profile index or API

Analytical profile index or API is a fast biochemical test that is performed on the strip for identification and confirmation of bacteria. Different types of API tests are used for different bacteria such as- API 20E is used to identify the *Enterobacteriaceae* group, API 20NE is used to identify the non-enterobacteriaceae group, and API Listeria is used for *Listeria* spp.etc.

1.2.8 Sociodemographic Characteristics of Street Food Vendors

The official sector of Bangladesh's economy does not include street food sellers. They are often referred to as working in the informal sector, which is defined by occasional, unstable, and minor revenue generation.

The number of street food sellers, the size of their businesses, and the sustainability of their endeavors are accordingly not routinely documented. Street selling may be the second most important type of work for Bangladesh's urban poor, behind pushing rickshaws. It is especially significant among young and middle-aged males who moved to Dhaka over the

past 10 or five years (Baker,2007). In Dhaka, there are around 300,000 street vendors and 750,000 rickshaw pullers who make their living (Islam,2005). Dhaka has one of the most significant amounts of street vendors in the world; in Asia, the other cities with similar numbers are Mumbai (about 200,000), Delhi (around 200,000), Calcutta (around 150,000), and Bangkok (around 100,000) (Bhowmik *et al.* 2012).

The precise number of Dhaka's street food vendors is hard to estimate. On the other hand, Benjamin (Benjamin,2011) carried out a three-year (2007–2010) study of street food sellers in Dhaka. There are approximately 90,000 to 100,000 street food sellers selling prepared foods, and about 418,000 individuals, or 2.9 percent of Dhaka's total population relies on the income these vendors brings in, based on the survey and official labor information. According to these statistics, every supplier serves an average of 84 consumers per day. It also indicates that over eight million individuals, or fifty-five percent of Dhaka's population, consume street food on a regular basis (Goswami,2016). Selling street food is a common, a prominently observable social activity that is both economically efficient and deeply ingrained in both city economies and city life. The significance of the street food system in Dhaka is undeniable (Cross *et al*.2011, Rane 2011, Alter 2005).

Below is an introduction to the sellers' socioeconomic backgrounds to help you get familiar with the street food vendors: Street food vendors are both men and women, married or bachelor. They range in age from 20 to 60, with most of them falling in the 30 to 40-year-old age enclosed.

(i) A large number of street food sellers and their families come from rural origins, either moving to cities later in life or staying in rural areas and traveling daily to the city for employment reasons.

(ii) The street food vendors have relatively low levels of education, with most of them having educations ranging from grades 5 to 8.

(iii) The unstable financial state of their families' places restrictions on a lot of street food businesses.

The street food sellers' occupation background shows that, before establishing street food businesses, they engaged in several irregular, low-paid, urban-based occupations involving a lot of physical effort. Their survival did not come from their involvement in such activities. As a result, the vendors migrated to various places of work. Street food vendors are small independent business owners who do not rely on government agencies or other institutional frameworks to support them. Their businesses only grow according to their abilities and the

support that their surrounding social networks—such as their families and other close friends—provide. Both the vendors and their family members rely on the profits from their commercial businesses for their daily needs. As a consequence, food vendor sellers' businesses have not only provided them and their dependent families an alternative to survival, but they in addition reduced the chance that they would eventually become an economic and social burden on the government. A significant amount of the population gets calories fulfilled through street food. The food that can be purchased on the streets is immediately available and affordable price. However, sometimes, it is provided right to the client's front door. Therefore, street food not only fulfills the need for food, especially for those from low-income families but also provides to the needs of busy consumers who shortage the time to prepare meals for themselves or head to other restaurants where the food is likely more costly and the service takes more time (Khairuzzaman *et al.,* 2014).

1.2.9 Working Conditions and Occupational Hazards of Street Food Vendors

Due to their work, street sellers are subject to face particular types of livelihood hazards. in a legal, physical, and sociological context. For most street vendors, the biggest and greatest constant concern is that local government officials may take them off the streets forcibly or capture their goods. Elections, major events, and efforts that improve the appearance of old city functions are often linked with a higher risk of shifting. Street sellers are less efficient in unstable institutional systems with irregular and unexpected standards, just as formal business owners (Bhowmik *et al*. 2012, Goswami *et al.* 2016). There are extra regular occupational dangers for street vendors. Every day, a lot of people have to carry and transport huge quantities of products from their workplace to their sales location. The actual settings in which they function often absence essential facilities including solid waste disposal, clean running water, and restrooms. The insufficient installation related to equipment designed for fire safety and the insufficient management of traffic in business areas subject street vendors to physical harm. They are also vulnerable to severe weather conditions and a significant amount of air pollutants. Young children who must join their parents to sell products on the streets face the most from these health risks. The government in question should pay particular attention to the issues that are faced by street food vendors, as their population and the number of consumers they provide are both growing.

Many street vendors also often encounter potential risks to their income and earnings. Street sellers occasionally face financial danger due to harassment from government officials, which

includes removals, product seizures, and demands for payment (Brown,2010). Considering the conditions in which they work and the diverse array of occupational risks they encounter, street vendors' legal rights could work as mediators. Such as a seller in a regulated market with an established structure may be more likely to have a permit or approval, which would reduce their vulnerability to certain threats. Similarly, a street vendor who functions as a staff member and sells a certain sort of goods—like newspapers—may be safer since they are more secure by the law. For this purpose, obtaining some sort of legal standing is an essential requirement for street business associations in several places *(*Khairuzzaman *et al.,* 2014).

1.2.10 Growing Demand of Street Food

Bangladesh's urban population is fast growing. The population of Dhaka, the nation's capital, almost tripled from 5.3 to 9.3 million within the past ten years (Faruque *et al.* 2010). Because of the fact that a lot of urban residents spent most of their days outside and have little time or money for consuming food, this pattern has increased demand for ready-to-eat, affordable foods. Street food vendors have become a significant industry as a consequence of rapid urbanization; in Dhaka alone, a few 200,000 people make their living from this industry (Rane,2011; Zain,2002). The primary reasons why street food is becoming increasingly famous are its reasonable price, availability, and simplicity. Due to their immediate as well as indirect engagement with street food vendors, women play a crucial role in the business.

Furthermore, a majority of the street vendors are heads of homes led by women (Ackah *et al.* 2011; Goswami,2016). The variety among street food vendors can be seen in the kinds of food they prepare and sell, the size of their procedures, the way they work, the locations where they make and sell food, the kinds of consumers they provide, among other characteristics. Ingredients for street cuisine are largely undocumented and particular to a certain place. It is hard to present a menu of every street food that is eaten during the globe because there are so many varieties. Street foods like chola boot (chickpeas), bhelpuri(puffed rice with potatoes), and samucha (deep-fried dough stuffed with vegetables and/or meat) are prominent in Bangladesh, as are beverages like lassi(yogurt and water) and sugarcane juice. Other frequently consumed snacks include singhara (flour wraps stuffed with vegetables, spices, and occasionally liver), ghugni(boiled and mashed white peas with spices), and other cakes (Tabashsum *et al*., 2013).

1.2.11 Consumers of Street Food

There is not much information on street food habits and how they influence food choices. According to consumer studies conducted by FAO in 2006 (Fao,*2006*) and other researchers, the majority of people who consume street food in most nations are laborers from the informal sector like other vendors, hustlers, and low wage workers. Housewives, office professionals, and children and students were other significant client characteristics (*The State of Food Insecurity in the World 2010,* n.d.). The research also found that street meals were consumed by people of all economic levels and that they accounted for a sizable amount of daily household food budgets—between 25 and 47 percent in Bogor and Chonburi, Thailand, particularly (Zain *et al*. 2002).

Street foods are consumed in different ways across several nations. Although in Bangladesh, for example, they were considered supplemental and only a small portion of people bought them on a regular basis, in other nations they were an everyday part of the diet and were bought daily (Biswas *et al.* 2010;Barro *et al.,* 2006). It was found that a few consumer groups—students, homeless people, and independent wage laborers—purchase practically all of their food from vendors (Zain *et al*. 2002). While evaluating the cost of meals from larger restaurants and fast-food outlets to that of street food, street food is typically cheaper. Furthermore, because of the advantages of scale, street food may be prepared for less money than the same food cooked at home, especially in cities where fuel and supplies might be expensive *(*Khairuzzaman *et al.,* 2014).

1.2.12 Safety of Street Foods

One of the main concerns of food control officials relate to the hygiene aspects of street food vendors. Many times, vendor exhibits are basic buildings without running water, restrooms, or laundry facilities. Increasing awareness through partnerships with municipal authorities, food sellers, government agencies, consumer groups, standard-setting bodies, and some nonprofit organizations can lead to better street food safety. In particular situations, vendors are eager to take part in efforts that offer the bare minimum of facilities so they can operate in hygienic environments. In fact, in a study of street food vendors in Lusaka and Harare, the vendors said they would be happy to pay for utilities like power as well as running water, but would prefer that the water stations, trash cans, and laundry facilities be provided by the city government

(Muinde *et al.* 2005). To improve business circumstances and encourage vendors and their families to live better lives, it is therefore suggested that local authorities, vendors, and policymakers form a strong cooperation.

1.2.12.1. Microbiological Safety.

Climate change, the globalization of the food market, and shifting consumer tastes for fresh and quick meals have created new obstacles in the fight against food-borne diseases(Feglo & Sakyi, 2012). Food-borne diseases occur from consuming polluted meals and food items, as food is biological in nature and may stimulate the growth of germs (Sheth *et al.,* 2005). Human food-borne diseases are linked to around 250 distinct kinds of viruses, bacteria, parasites, poisons, metals, and prions (Tambekar *et al.,* 2009). While viruses account for over 50% of foodborne illnesses, bacterial agents are often the cause of hospitalizations and fatalities linked to foodborne diseases. From mild gastroenteritis to potentially fatal neurological, hepatic, and kidney syndromes, infections can range in seriousness. and can be brought on by both the disease-causing microbe's toxin or the body's response to this infection (Schelin *et al.,* 2011).. The most common cause for fatal and deadly foodborne diseases is bacteria spread through food. Out of the numerous thousands of unique bacterial species, Salmonella, Clostridium, Campylobacter, Vibrio, Staphylococcus, Bacillus, and Enteropathogenic Escherichia coli species account for about 90% of food-poisoning incidents (*FSHN033/FS099: General Overview of the Causative Agents of Foodborne Illness*, n.d.). For example, Salmonella was the most widespread cause of bacterial foodborne disease in the United States and France in the last decade of the 20th century, resulting in 5,700–10,200 cases; Campylobacter (2,600–3,500 cases) and Listeria (304 cases) were the next most common causes (Vaillant *et al.,* 2005). Listeria, Enterobacter, and Aeromonas species were the most widespread kinds of bacteria found in ready-to-eat meals in South Africa (Nyenje *et al.,* 2012). However, Bangladesh and other developing countries do not have access to any such databases. As a result, our laboratory has been performing a number of tests since October 2012 to evaluate the microbiological quality of Bangladeshi street meals (Tabashsum *et al.,* 2013). The most common foodborne pathogens, such as Salmonella spp., Escherichia coli O157, O111, O26, and other E. coli, other coliforms, Listeria spp., and Staphylococcus spp., were analyzed in over 100 street food samples of 20 different kinds, including singara, jhal-muri, chatpati, chetoipitha, chola/Bengal gram, jilapi, jar drinking water, pickles, amra, tehari, vegetable rolls, sugarcane juice, raw cucumber slices, milk, other juices, beverages, baked goods, and other foods. (Khairuzzaman

et al., 2014) Moreover, at least seven antibiotics have been found to be resistant to the pathogenic organisms that were identified. These studies proved that food offered on Dhaka's streets pose a microbiological risk to the public's health (Tabashsum *et al.,* 2013). Furthermore, (Al Mamun *et al.,* 2013.) indicated that out of several street-vendered food items, 54% of the samples of sliced fruits, 59% of the samples of jhalmuri, 29% of the samples of chotpotis, 53% of the samples of vajavuji, and 100% of the samples of sharbat had inadequate microbiological quality. This study also revealed that the food being sold on the school premises was of low microbiological quality, posing a health risk to Dhaka city's schoolchildren (Ackah.,2011;Al mamun *et al,*2013).

1.2.12.2. Chemical Safety.

Street meals have been associated with pollutants including pesticide residues and nonfood grade substances like dyes and preservatives. Unlawful coloring chemicals, including textile colors, and pesticide residues were discovered during a chemical investigation of street food in Bogor (Rane,2011). The use of salt, spices, nitrates, and sugar appropriately plays an important role in preventing food degradation; presently, the need to maintain low costs can result in the procurement of affordable items from unapproved vendors that include prohibited chemical alterations. Coloring agents and other agents may also be applied to cover up the poor quality of inexpensive materials. Food may be poisoned with heavy metals and pesticide residues because of the environment in which street meals are served. The sources of these pollutants might be the raw materials, tools, or transportation techniques utilized. The absence of suitable storage facilities could also contribute to the matter (Sheth *et al.,* 2005). Street food vendors obtain their pots and other items from both formal and informal manufacturers/retailers, according to Ghanaian research. Lead, cadmium, arsenic, mercury, and copper were detected in several of the street food samples at greater concentrations than in typical meal samples, indicating potential tool leakage (Donkor *et al.,* 2009). Further tests indicated that lead from the pots purchased from unauthorized producers might contaminate the meals. The scrap metal used to make these pots may originate from a variety of sources, including abandoned vehicles, automobile batteries, and commercial machinery—all of which are clearly unsuitable for use with food. It's essential to prevent their ongoing use. Vendor interviews additionally revealed that a portion of their cutlery is obtained from unknown sources (Barro, 2006). This was clarified through the fact that pots and utensils are typically taken by the police during their raids of these vendors' equipment. The vendors use customized pots because they are worried

about losing their expensive pots, which puts consumers at risk of heavy metal poisoning in their meals. To reduce the exposure of consumers to pesticide residues and heavy metals from food served on the street, more study has to be done (Seth,2005).

1.2.12.3. Personal Hygiene.

Consuming prepared meals and ingredients from street or market sellers presents a danger to the public's health, particularly because of inadequate hygiene levels. Most of the time, the vendors lack adequate restrooms, and some of them begin working without taking a bath. For the sake of their goods, some vendors spend the night in places where they hawking (Muinde *et al*. 2005). Unclean hands and packing materials like leaves, old newspapers, and reusable plastic bags can potentially contaminate foods and ingredients gradually (Roberts, 2008). However, a lot of vendors are aware of the requirement to dress appropriately and neatly. A few of the female merchants had aprons and helmets on. The majority of vendors understand the significance of having hygienic clothing and utensils after certain awareness-raising activities (Barro, 2006). Yet they are unable to maintain proper hygiene since there are no water supplies close to their places of employment and inadequate drainage systems. Furthermore, some food workers wash their hands in the exact same bucket that is used to clean utensils, raising the risk of feces infecting food. However, the majority of food vendors operate their businesses without health qualifications or approvals, which creates additional issues and requires in-depth instruction in food handling and preparation methods (Faruque *et al.* 2010). Instead of using liquid soap, which could be more effective at cleaning their utensils, food vendors utilize affordable bar soap, which makes for ineffective cleaning. In addition, washed plates are often stored in an unclean area, plastic bowl, or paper box, leading to recontamination of the plates.

1.2.12.4. Environmental Hygiene.

Insufficient space around food vending outlets is facilitated because of inadequate waste disposal facilities. As a result, there is a higher chance of food contamination and a rise in insect populations. A lot of the time, the town or municipal plans do not involve vending places, consequently services like garbage collection are not provided. Local government officials frequently have to deal with the dilemma that accepting illicit businesses as clients will mean supporting them. Additionally, these vendors are not entitled to the service since their activities

are unlawful and they do not make contributions to the maintenance of the public's facilities or the supply of public services.

This results in a further decline in the area's clean facilities condition where the food is sold (Teplitski *et al.*, 2009). Poor sanitary conditions in the area where food is vended also contribute to poor food transportation and storage conditions. In certain areas, street food vendors buy their veggies, maize meal, and other seasonings from authorized stores, so there's less worry about the raw ingredients' safety. Nevertheless, the majority of the merchants lack permanent stands where they may keep their raw supplies on available. generally, they keep their products overnight at home and move them—often with inadequate coverings—to their locations for business the next day. Food is therefore more likely to get contaminated when being transported (Benjamin 2011, Goswami, 2016).

1.2.13 Significance of this research work

This research work played a very significant part in creating efficient preservation techniques and understanding the consequences of bacterial load. Dhaka city's street food-based microbial infections pose a significant risk to human health. Due to the unexpected microbial contamination from the vendors, consumers of Aloevera Sharbat, Sugarcane Juice, Fuchka, and Sandwich, Mixed salad from restaurants were affected by Contamination by food-borne pathogenic bacteria while serving the food. Consequently, raising concerns about public health and increasing risks related to food safety. Then we did an investigation and research and mitigated the microbial contamination along the quality and safety standards of Aloevera Sharbat, Sugarcane Juice, Fuchka, and Sandwich restaurant salads. Thus, research-based practices, educational initiatives, and legal frameworks may all be addressed.

Chapter 2

Materials and methods

2.1 Outline of the research study

The working strategy for sample collection, sample processing, isolation, biochemical test & confirmation of the microorganisms was as follows.

1. Preparation for the sampling and experiment: Media preparation, normal saline along with a swab stick sterilization

↓

2. Street food samples were collected from different areas of Dhaka city.

↓

3. Sample processing: Serial dilution and spread plate method was performed on different selective and non-selective petri plates.

↓

4. All petri plates were incubated for 24 hours at 37 ° C except DRBC. DRBC were incubated at 30 ° C. One set of SMAC agar was incubated at 44 °C to check the presence of fecal coliform.

↓

5. Samples were enriched by adding TSB and incubate at 37 ° C.

↓

6. After incubation Colonies were counted. If the bacterial growth was absent streaked on selective plate from enriched samples and incubate at 37 ° C.

↓

7. API 20E (Analytical Profile Index) was performed for the detection of the isolates.

↓

8. Isolates were preserved at -80 °C by preparing stock culture.

2.2 Preparation & sterilization of culture media & materials

Different selective and non-selective culture media were used in this experiment, such as

Table 2.1: Name of the culture media

Media Name	Targeted Microorganism/purpose
Tryptic Soya Agar	Total aerobic bacteria
Sorbitol MacConkey (SMAC) Agar	Total coliform and fecal coliform bacteria
DRBC (Dichloran Rose Bangle Chloramphenicol) Agar	Total Yeast and mold count
Chromocult Coliform Agar	*Escherichia coli*
Xylose Lysine Deoxycholate (XLD) Agar	*Salmonella* spp.
Tryptone Bile X-glucuronide (TBX) Agar	*Escherichia coli*
Thiosulfate citrate bile salts sucrose (TCBS) agar	*Vibrio* spp.
Listeria Selective Agar	*Listeria* spp.
Tryptic Soy Broth	Sub-culture and stock culture

The media were prepared as follows:

1. Each media was measured in Electric balance and dissolved in distilled water as per directions

of manufacturers.

2. For autoclaving all the growth media and special solutions, 121 °C temperature & 15 psi

pressure was used except Thiosulfate citrate bile salts sucrose (TCBS) agar and XLD agar. For preparing Thiosulfate citrate bile salts sucrose (TCBS) agar and XLD Agar 99 °C temperature was used for sterilizing in the Microwave oven.

3. Then fresh agar plates were prepared at room temperature inside the laminar airflow (Class-2

Al biological safety cabinet),

4. Then kept in the incubator for 18-24 hours. The fresh media plates were stored at 4 °C until used, which was rapped with paraffin film. The media dried at room temperature under aseptic conditions before inoculating the microorganism. For the Preservation and maintenance of different materials, i.e., heat sensible chemicals and reagents and Solutions, etc., a refrigerator was used at 4 °C temperature. The light-sensitive reagents are stored in a light-resistant Duran wrapped well with aluminum foils.

5. Autoclave below things for sample collection and sample processing:

-9 ml saline in Falcon tubes for the dilution of samples.

-5ml saline along with cotton swab. For sample collection.

-500ml Durans for collecting samples.

-Micropipette tips for 1ml pipette and 100µL.

-Glycerol for stock culture,

-Measuring cylinder for solid samples.

Normal saline autoclave for solid sample preparation

6. Ultraviolet (UV) for sterilizing the biosafety cabinet along with spreader, racks, 9 mL saline falcon, discard beaker,

7. Cleaning the sample container box with sanitizer.

2.3 Calibration of pH meter

The pH meter is the device used to test pH. The chemistry of the electrode varies with time, changing the pH values, hence pH meters need to be calibrated. Depending on how the instruments are configured, there is an advised time range of one week to one month for pH meter measurements. Whenever a new electrode is used or the battery is changed, calibration is also required. Measurements and calibration of pH meters Transfer around 25 milliliters of each buffer solution into individual 50-milliliter beakers. Select the appropriate "CAL" button. To get rid of extra water droplets, lightly shake the electrode after cleaning it with distilled water. The electrode should be kept properly in the pH 4.0 buffer solution. Adjust the pH meter's measurement to 4.0 by pressing the "RUN/ENTER" or appropriate button. Insert the

electrode into the buffer solution with a pH of 7.0, select the right "RUN/ENTER" button, and adjust the pH to 7.0. When the pH value reaches a steady value, wait to record it. The calibration results will appear on the device as soon as its reading stabilizes. After a two-point calibration, you have the option of initiating testing or proceeding with a three-point calibration using a pH 10.0 buffer solution. To test the pH of samples, press the appropriate button or "M" for measurement mode.

2.4 Sample collection

The swab stick collected was dunked separately in a sterile falcon tube with 5 ml of sterile sodium chloride (0.85%) solution. Generally, 0.85% Sodium Chloride or normal saline works as a transport medium (Zaman et al., 2018). Swab samples were collected in sterile falcons, and then kept in a box containing ice packs until delivery to the Laboratory within a maximum of one hour of collection. Food samples were processed in the laboratory immediately upon receipt. However, if an analysis was on hold due to the late arrival of samples, those samples were refrigerated at 0–4 °C until examination but not longer than 12 hours.

Table 2.2: Descriptions of the Aloevera Sharbat sampling.

Sl. No.	Location and Id	Sampling time and date	Sl. No.	Location and Id	Sampling time and date
1	Bongo Bazar, Bangshal (ALV-1)	04.09.23 10:50 AM	6	Gausia Market, New Market (ALV-6)	27.08.23 7.20 AM
2	In between Patuatuli Road and Wise Ghat Road, Islampur, Kotwali (ALV-2)	04.10.23 8:10 AM	7	In front of Milon Auditorium, Council Road, Demra (ALV-7)	18.09.23 5:15 PM
3	Besides of Sena Kalyan Bhaban, Toyenbi Circular Road, Motijheel (ALV-3)	26.09.23 7:40 AM	8	Firmgate, beside of Govt. Science College (ALV-8)	10.09.23 7:05 AM
4	Shahjalal Avenue, Ajampur Bus Stand, Uttara East (ALV-4)	30.09.23 6:30 PM	9	Mohammadpur Townhall Bazar (ALV-9)	18.09.23 7:30 PM
5	Mohakhali TB Gate (ALV-5)	12.09.23 6:30 AM	10	Majar Road, Darussalam (ALV-10)	16.09.23 5:30 PM

Table 2.3: Descriptions of the Sugarcane samples

Sl. No.	Location and Id	Sampling time and date	Sl. No.	Location and Id	Sampling time and date
1	Abdullahpur Bus Stand, Uttara (SC-1)	16.10.23 12:15 PM	6	In front of Haji M. A Gafur Square Market, Staff Square Market, Demra (SC-6)	17.10.23 3:35 PM
2	Amtoli Mor, Banani (SC-2)	16.10.23 1:50 PM	7	Besides of Baytun Noor Jame Mosque & Infront of Joy Motors, Kajla, Jartabari (SC-7)	17.10.23 3:50 PM
3	Opposite of Mohakhali Bus Terminal (SC-3)	16.10.23 2:15 PM	8	Infront of Shanto CNG Re-Fueling Station, South Mugda (SC-8)	17.10.23 4:25 PM
4	Besides the gate of the Deputy Police Commissioner's office, Mirpur-2 (SC-4)	16.10.23 4:30 PM	9	Opposite of Paltan Model Police Station (SC-9)	17.10.23 4:55 PM
5	Infront of Trade Consortium CNG LPG Diesel Station, Gabtoli Mazar Road Mor (SC-5)	16.10.23 5:00 PM	10	Opposite of Ananda Bazar, Nimtoli, Bangsal (SC-10)	18.10.23 11:15 AM

Table 2.4: Descriptions of the Sandwich samples

Sl. No.	Location and Id	Sampling time and date	Sl. No.	Location and Id	Sampling time and date
1	Sandwich 1 (Mokarram Bhaban, Kashem's Tea Stall, Dhaka University)	18.07.23 08.30 AM	6	Sandwich 06 (Opposite of Kallyanpur bus stand)	13.11.23 10.47 AM
2	Sandwich 02 (Infront of Bangabandhu national stadium gate-1, Paltan)	13.07.23 09.30 AM	7	Sandwich 07 (10 College Street, Science lab, Newmarket)	13.11.23 11.10 AM
3	sandwich 03 (Road-14, Sector-1, Uttara)	13.11.23 09.47 AM	8	sandwich 08 (Beside of sub-registrar office, Demra)	13.11.23 12.20 PM
4	sandwich 04 (Mohakhali bus terminal)	13.11.23 08.10 AM	9	sandwich 09(Beside of Babu Bazar Jumma mosque, Islampur road)	13.11.23 01.15 AM
5	sandwich 05 (Opposite of sub registry office, Tejgaon)	13.11.23 07.45 AM	10	sandwich 10(Taltola bus stant, Aggargaon)	13.11.23 3.10 AM

Table 2.5: Descriptions of the Mix Salad samples

Sl. No.	Location and Id	Sampling time and date	Further Information
1	Sample-1(Hotel Dhanshiri, Topkhana Road, Press Club)	13.11.23 12.15 PM	Carried to lab by poly. Item: Cucumber, green chillie, lemon
2	Sample-2(Lovely Hotel, Nilkhet)	07.08.23 12.50 PM	Carried to lab by maintaining cooling temperature. Item: Cucumber
3	Sample-3(Mitali hotel, Chankharpool)	14.08.23 01.00 PM	Carried to lab by maintaining cooling temperature. Item: Onion, green chillie, lemon

Table 2.6: Descriptions of the Fuchka samples

Sl. No.	Location and Id	Sampling time and date	Sl. No.	Location and Id	Sampling time and date
1	Dhanmondi 8A, Rabindra Sarobor (FC-1)	23.10.23 5:05 PM	6	Hatirjheel (FC-6)	24.10.23 5:30 PM
2	Mirpur Central puja mandap, Mirpur 2 (FC-2)	23.10.23 6:10 PM	7	Baily road beside of Artisan (FC-7)	24.10.23 6:00 PM
3	Sher e bangla nagar, infront of the gate of womens polytechnic institute, Agargaon (FC-3)	23.10.23 7:15 PM	8	Infront of the gate of puja mandap, Deilla East Demra (FC-8)	25.10.23 3:50 PM
4	Uttara sector 11, Road-06 (FC-4	24.10.23 3:15 PM	9	Opposite of Shahi Mosque, Laxmibazar (FC-9)	25.10.23 5:00 PM
5	Infront of the gate of DNCC market Banani (FC-5)	24.10.23 4:50 PM	10	Beside of balur field, Lalbagh (FC-10)	25.10.23 7:00 PM

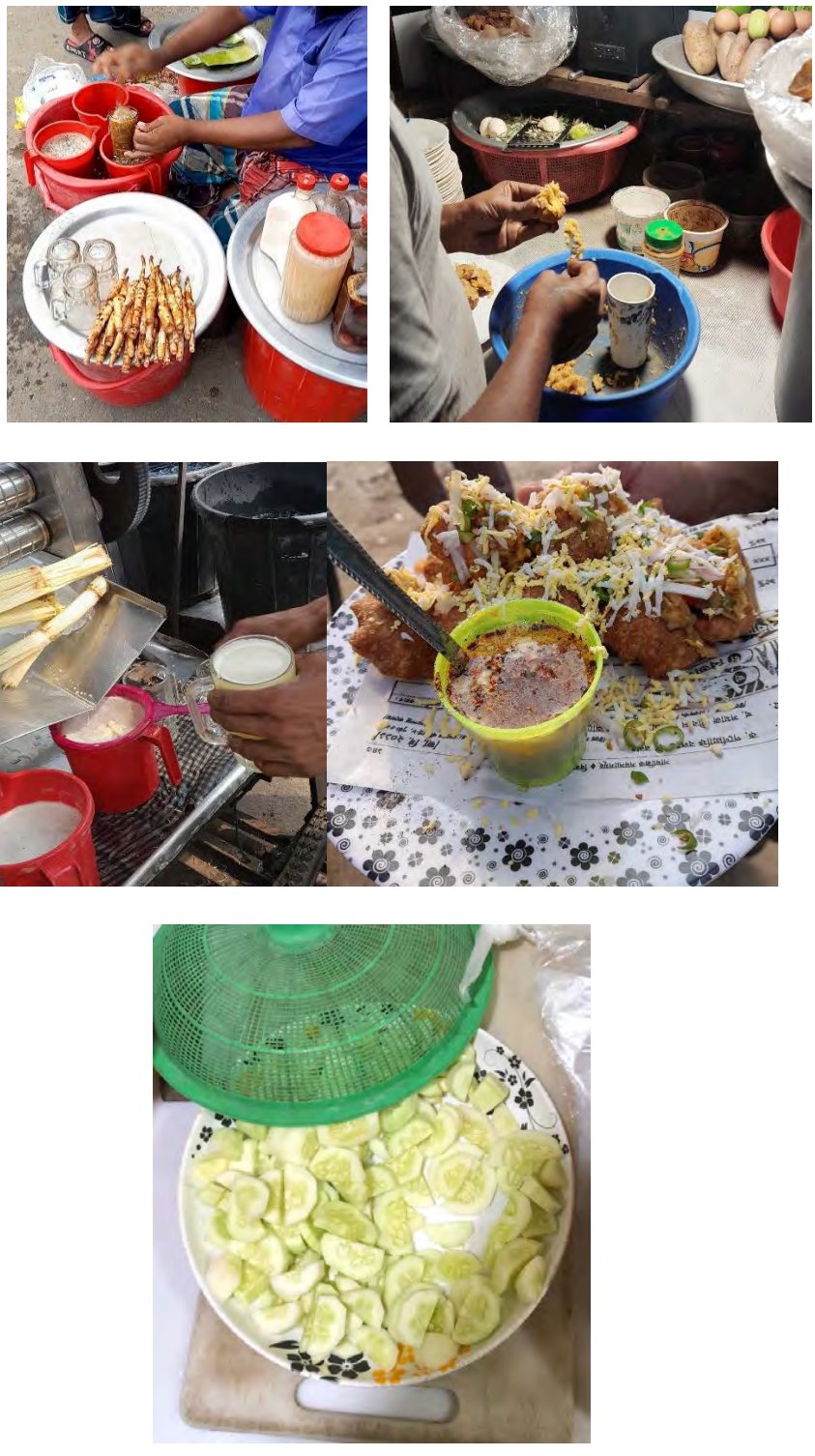
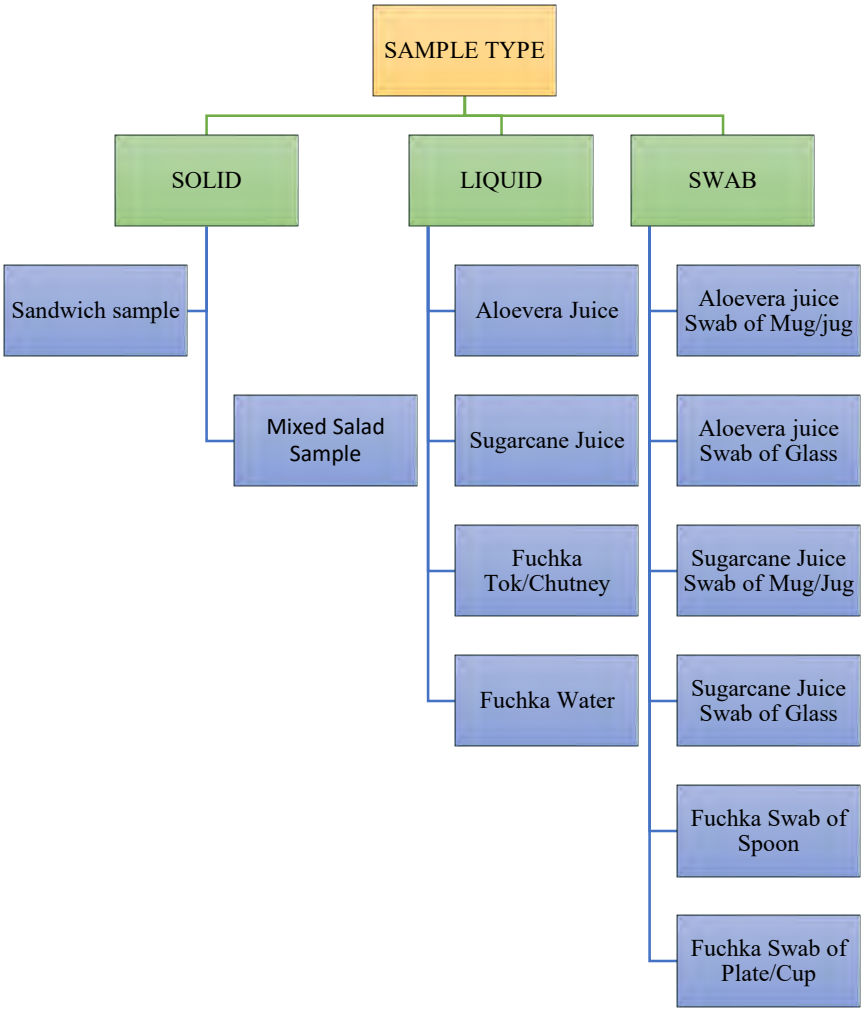


Figure 2.1: Preparation of the street food

2.4.1 Types of the samples



2.5 Sample processing

At first, we collected the sample of Aloevera(ALV), Sugarcane Juice (FC), Fuchka tok or chutney, and Fuchka water sample along with the swab sample of consumers' clean glass/Spoon and Mug/Jug/plate/, Then plates were labeled according to Media name,(TSA/SMAC/DRBC/TBX/TCBC/XLD/LSA) sample name(ALV/SC), ID(A/B/C), and date for spreading the samples.

2.5.1 Serial dilution

A ten-fold dilution decreases the viral suspension or solution's concentration by a factor of 10, or to a tenth of its initial amount. Ten-fold serial dilutions are a sequence of ten-fold dilutions (*Estimation Method for Serial Dilution Experiments*, 2014) In the ten-fold serial dilutions, 9 times greater sterile normal saline was used.

In the Liquid Sample, at the first label the falcon. then take the sample bottle and mixed it properly by shaking it suitably, then took 1 ml sample using a sterile 1000 µl micropipette and added it into 9 ml sterile normal saline after that, Vortexing the sample at 230rpm for appropriate mixing of the dilution. Carefully mix the solution by vortexing it. Conduct the next dilution. 1 ml of the solution from tube 1 will be added to 9 ml of dilution liquid in tube 1:10 for the second serial dilution. Then again vortex the sample. Repeat the process for 2 more times. Finally, we have the third dilution of 1:100 and the Fourth dilution factor, 1:1000 respectively. In this process, in the end, we have the 10^4 times serial dilution for the liquid sample.

For, the swab sample, we took the 5 ml swab cotton falcon, and mixed it properly by using vortex at 230RPM for it suitably, then took 1 ml sample using a sterile 1000 µl micropipette and added it into 9 ml sterile normal saline after that, Vortexing the sample at 230rpm for appropriate mixing of the dilution. Carefully mix the solution by vortexing it. Conduct the next dilution. 1 ml of the solution from tube 1 will be added to 9 ml of dilution liquid in tube 1:10 for the second serial dilution. Then again vortex the sample. Repeat the process Finally, we have the third dilution of 1:100, respectively. In this process, in the end, we have the 10^3 times serial dilution for the swab sample.

For the solid sample, In the Liquid Sample, at the first label of the Stomacher bag (lateral strainer bag), then we added 9 times greater sterile normal saline water. Here for the samples, we used 90 ml of Normal Saline with 10 ml of solid sample added into a stomacher bag. Then, the Stomacher sample circulated at 230 rpm for 30 seconds. then took 1 ml sample from the robust strainer strip side, which allowed us to avoid all the debris from the samples using a sterile 1000 µl micropipette and adding it into 9 ml sterile normal saline after that, Vortexing the sample at 230rpm for appropriate mixing of the dilution. Carefully mix the solution by vortexing it. Conduct the next dilution. 1 ml of the solution from tube 1 will be added to 9 ml of dilution liquid in tube 1:10 for the second serial dilution. Then again vortex the sample.

Repeat the process for 2 more times. Finally, we have the third dilution of 1:100 and the Fourth dilution factor, 1:1000 respectively. In this process, in the end, we have the 10^4 times serial dilution for the liquid sample.

Then, take 15/20 minutes to adjust the bacteria to the condition. Considering that bacteria use a technique known as binary fission to multiply rapidly.

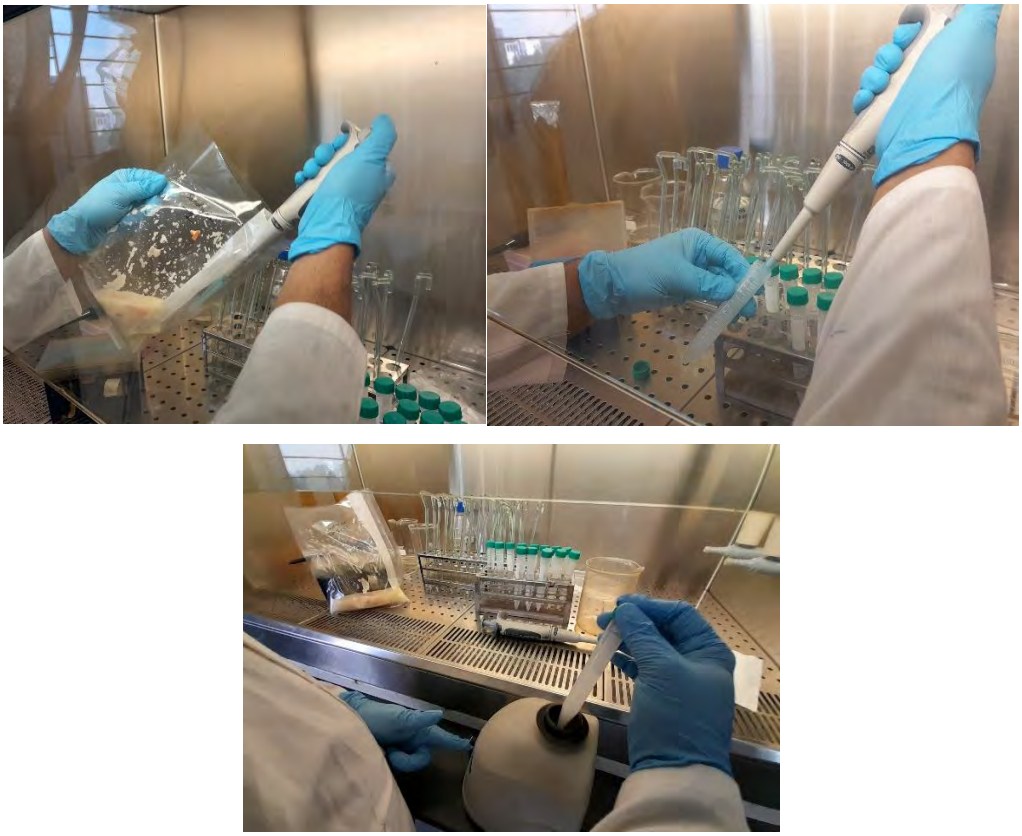


Figure 2.2: Serial dilution from sample

2.6 Microbiological analysis

2.6.1 Spread plate method

All bacterial colonies were grown on Selective and Non-Selective Media. Here we used 10^3 to 10^4 serial dilutions for the growth of TABC in TSA plates for samples (Aloevera Sharbat, Sugarcane, Fuchka water, Fuchka Tok/Chutney). We used sample of 10^1 to 10^2 serial dilution for selectice media. Then, from appropriate dilutions, the sample was placed into the fresh agar media for the surface spreading technique to be followed. Both selective and non-selective agar media are used in the detection of the microorganisms. Tryptic Soy Agar or (TSA) medium

was used for the total aerobic bacteria. Different selective medium such as Sorbitol MacConkey (SMAC) agar was used for coliform & Fecal Coliform and Tryptone Bile X-glucuronide (TBX) Agar was used for *E. coli*, Xylose Lysine Deoxycholate (XLD) agar was used for *Salmonella* spp., Thiosulfate citrate bile salts sucrose (TCBS) agar was used for *Vibrio* spp., Listeria selective Agar (LSA) was used for *Listeria* spp., and Dichloran Rose-Bengal Chloramphenicol (DRBC) Agar was used for yeast & mold was used.

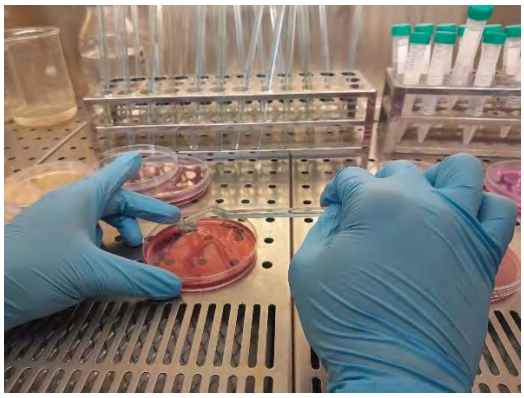


Figure 2.3: Spreading of sample

2.6.2 Observation & colony counting

These media were observed accordingly, and incubated at 37 ℃ for 24 hours for non-selective and selective (Except Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar) incubated at 30℃ for 72hour to 108Hour and Fecal Coliforms Incubated in 44℃ for 24 hours) based on their characteristics and appearance on different media. After incubation of the spread-plated media at the expected temperature. the total numbers of isolated colonies were recorded. For the growth of yeast& mold DRBC agar medium was incubated at 30 °C for 3-5 days to detect the fungal growth.

$$\left(\frac{CFU}{milli\ litter\ of\ sample}\right) = \frac{(No.of colonies)}{(Inoculum\ size(ml) \times dilution\ Factor)}$$

2.6.3 Enrichment & Single Colony Isolation

2.6.3.1 Enrichment

Sample enrichment has been performed to favor the growth of a single microorganism over others. In general, this was achieved by adding nutrients or environmental conditions that enable organisms to expand. For the detection and identification of microorganisms with a range of nutritional needs, enrichment methods have been used to lift a limited number of desired organisms to detectable levels. For enrichment, Tryptic soy broth (TSB) was added to the sample.

In the Liquid Sample, (Aloevera Sharbat, Sugarcane Juice, Fuchka water, Fuchka Tok/Chutney) 5mL Tsb(Tryptic Soya Broth) was added in 10^1 Sample dilution(1 ml Sample with 9 ml Normal saline) for enrichment After the spread plating process was completed.

For, the swab sample (Aloevera juice Swab of Mug/jug, Aloevera juice Swab of Glass, Sugarcane Juice Swab of Mug/Jug, Sugarcane Juice swab of Glass, Fuchka Swab of Spoon, Fuchka Swab of Plate/Cup) 5mL Tsb(Tryptic Soya Broth) was added for the enrichment of the sample with 3mL Saline dilution along with Cotton swab.

For the solid sample (Sandwich sample, Mixed Salad Sample) 10 ml TSB was added in the stomacher bag with the solid sample.

Then, After the incubation of 24 hours, the incubation was done for Nutrient samples. After that, the streak plate method was done on the selective media. The plates were incubated for 24-48 hours at 37 °C (Except DRBC; for DRBC incubation temperature is 30 °C), and the plates showed the actual presence and absence of pathogens on all selective plates.

2.6.3.2 Pure colony isolation

A bacteriological loop was heated until the entire wire blazed, to make sure the heated loop was cold, contact it with a sterile area of the agar after removing the cultured plate. To select a tiny sample, simply touch the edge of the initial colony that was chosen. After that, the streak plate technique was used. colonies are generated after 24-48 hours of incubation at 37 °C. (Except DRBC; for DRBC incubation temperature is 30 °C and Fecal Coliforms are Incubated in 44℃ for 24 hours),

2.7 Confirmation of presumptively assumptive bacteria

2.7.1 API 20E

API 20E is a rapid detection system of analytical profile index standardized system. It combines 20 biochemical tests that offer widespread capabilities. It is used in identifying the species of the *Enterobacteriaceae* group The API 20E strip comprises 20 micro-tubes containing dried-out substrates for the exhibit of enzymatic action or the maturation of sugars

Chapter 3

Results

3.1 Measurment of pH

pH is the volume of acidity and alkalinity of a water form. The pH scale is a mathematical representation of the hydrogen ion concentration, ranging from 0.0 to 14.0 pH units. Higher pH values are regarded as basic, whereas lower pH values are regarded as acidic. The neutral pH is 7.0. The concentration of hydrogen ions increases tenfold with each pH unit. Water with a pH of 4.0, for instance, contains ten times the amount of hydrogen ions than water with a pH of 5.0. In a similar vein, pH 3.0 has 100 times as many hydrogen ions as pH 5.0. Therefore, a small change in pH can result in a big difference in the quality of the water. (Sonkar & Kumar, 2022) In-room Temperature, we took pH for the Sample(Aloevera Sharbat, Sugarcane Juice, Fuchka water, Fuchka Tok/Chutney).

Table 3.1: Aloevera Sharbat Juice sample pH Measurement

Sl. No.	Location	pH Level	Sl. No.	Location	pH Level
1	Bongo Bazar, Bangshal (ALV-1)	4.79	6	Gausia Market, New Market (ALV-6)	5.40
2	In between Patuatuli Road and Wise Ghat Road, Islampur, Kotwali (ALV-2)	5.47	7	Infront of Milon Auditorium, Council Road, Demra (ALV-7)	5.17
3	Besides of Sena Kalyan Bhaban, Toyenbi Circular Road, Motijheel (ALV-3)	5.71	8	Firmgate, beside of Govt. Science College (ALV-8)	5.51
4	Shahjalal Avenue, Ajampur Bus Stand, Uttara East (ALV-4)	5.70	9	Mohammadpur Townhall Bazar (ALV-9)	5.23

5	Mohakhali TB Gate (ALV-5)	5.71	10	Majar Road, Darussalam (ALV-10)	6.15

Table 3.2: Fuchka Tok/Chutney and Cleaning water pH Measurement

Sample Locations (Fuchka Sample)	Fuchka Tok/Chutney pH level	Fuchka Water pH level	Sample Locations (Fuchka Sample)	Fuchka Tok/Chutney pH level	Fuchka Water pH level
Dhanmondi 8A, Rabindra Sarobor (FC-1)	3.00	7.32	Hatirjheel (FC-6)	3.03	7.75
Mirpur Central puja mandap, Mirpur 2 (FC-2)	2.00	7.82	Baily road beside of Artisan (FC-7)	3.35	7.57
Sher e bangla nagar, infront of the gate of womens polytechnic institute, Agargaon (FC-3)	3.00	7.34	Infront of the gate of puja mandap, Deilla East Demra (FC-8)	3.53	7.75
Uttara sector 11, Road-06 (FC-4)	3.03	7.62	Opposite of Shahi Mosque, Laxmibazar (FC-9)	2.98	7.65
Infront of the gate of DNCC market Banani (FC-5)	3.03	7.53	Beside of balur field, Lalbagh (FC-10)	3.58	7.62

Table 3.3: Sugarcane Juice sample pH Measurement

Sample Locations: Sugarcane		Sample Locations: Sugarcane	

	pH level		pH level
Abdullahpur Bus Stand, Uttara (SC-1)	3.82	Infront of Haji M. A Gafur Square Market, Staff Square Market, Demra (SC-6)	5.11
Amtoli Mor, Banani (SC-2)	4.11	Besides of Baytun Noor Jame Mosque & Infront of Joy Motors, Kajla, Jartabari (SC-7)	5.03
Opposite of Mohakhali Bus Terminal (SC-3)	4.47	Infront of Shanto CNG Re-Fueling Station, South Mugda (SC-8)	5.11
Besides of the gate of the Deputy Police Commissioner's office, Mirpur-2 (SC-4)	4.34	Opposite of Paltan Model Police Station (SC-9)	3.88
Infront of Trade Consortium CNG LPG Disel Station, Gabtoli mazar Road Mor (SC-5)	4.32	Opposite of Ananda Bazar, Nimtoli, Bangsal (SC-10)	5.24

3.2 Microbial analysis of the Street food samples

3.2.1 Cultural characteristics of microorganisms

The tested samples were analyzed for microbiological tests and isolates were picked up from the 6 types of selective agar media. Colony size, margin, elevation, surface structure, color etc. were studied in the colonies found in different selective microbiological media. (Figure 3.1)

Table 3.4: Colony characteristics of microbes on different selective media

Selective Medium (agar)	**Full Name of the media**	**Cultural Characteristics**				**Presumptive Bacteria Isolates**
		Color	**Size**	**Form**	**Elevation**	
SMAC	Sorbitol MacConkey Agar	Pink to red	Medium	Circular	Convex	*Coliform*

TBX	Tryptone Bile Glucuronic Agar	Medium Blue	Small	Circular	Convex	*Escherichia coli*
TCBS	Thiosulfate Citrate Bile Salt	Yellow or green	Small	Comma-shaped	Curved	*Vibrio* spp.
LSA	Listeria Selective Agar	blue	Small	Circular	Convex	*Listeria* spp.
XLD	Xylose lysine deoxycholate (XLD) agar	Black centered colonies	small	regular	flat	*Salmonella*
DRBC	Dichloran Rose-Bengal Chloramphenicol	Pink color	Large	gel	Flat	Yeast and Mould

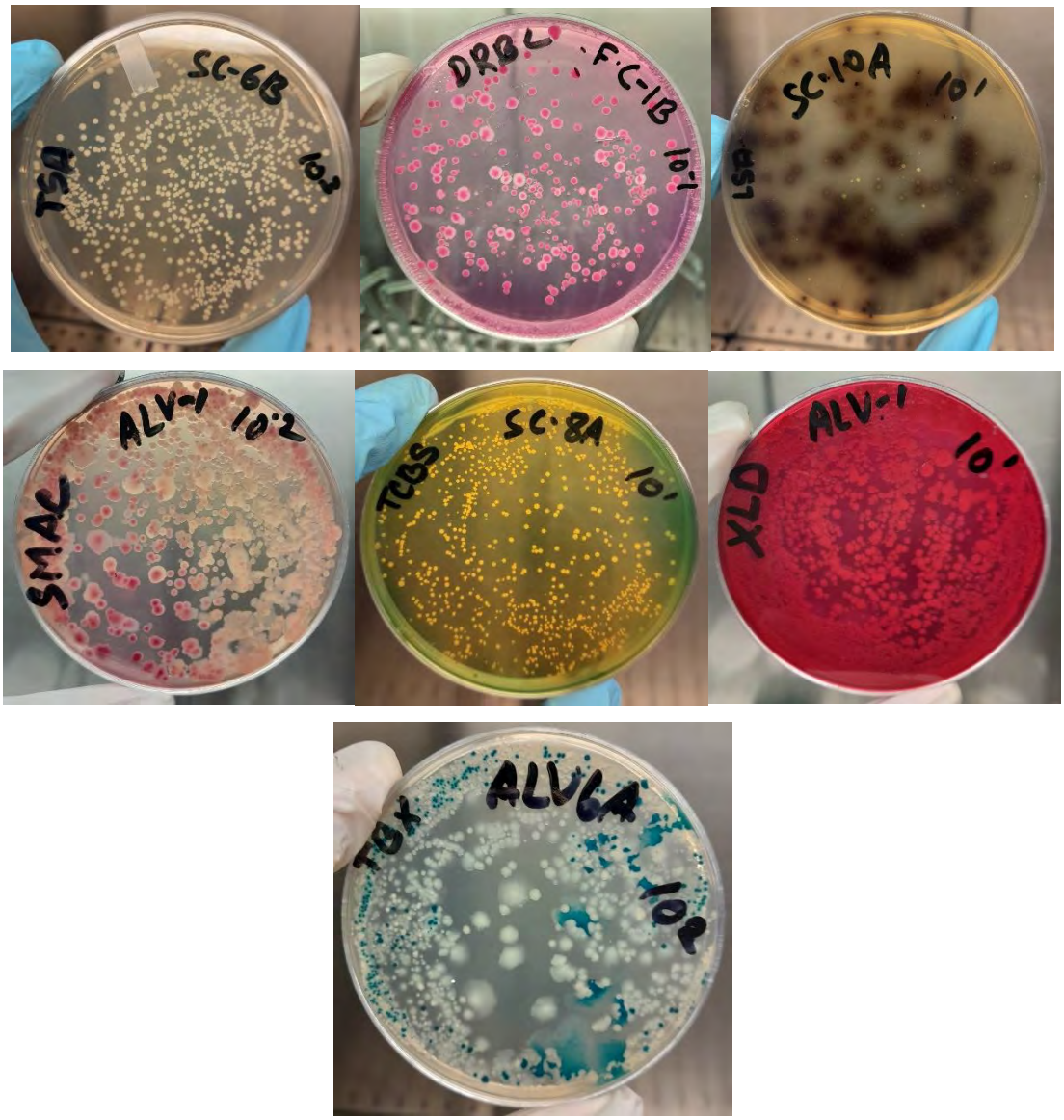


Figure 3.1: Colonies on Media (TSA agar, Sorbitol MacConkey Agar(SMAC), Tryptone Bile Glucuronic Agar(TBX), Thiosulfate Citrate Bile Salt(TCBS),Listeria Selective Agar (LSA),Xylose lysine deoxycholate (XLD) Agar, Dichloran Rose-Bengal Chloramphenicol Agar (DRBC).)

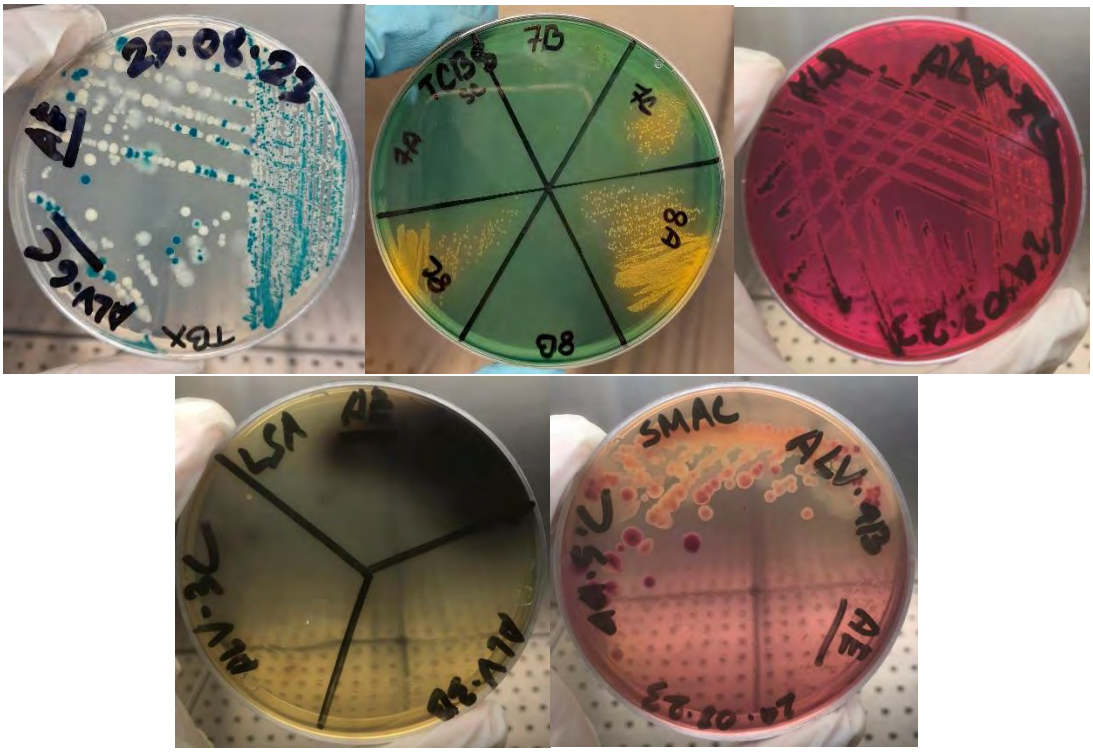


Figure 3.2: Colonies After Enrichment (Sorbitol MacConkey Agar (SMAC), Tryptone Bile Glucuronic Agar(TBX), Thiosulfate Citrate Bile Salt(TCBS), Listeria Selective Agar (LSA),Xylose lysine deoxycholate (XLD) Agar,)

3.3 Microbiological count of sample in various selective and non-selective differential media

3.3.1 Aloevera Sharbat Juice, ALV-A:

A total of eight microbial parameters were observed from ten different Aloevera Sharbat Juice samples of Dhaka City collected from different crowded places in the city. Around 2.20 and 2.93 log cfu/ml was the range of the total aerobic bacterial count. The range for total coliform count was found to be 2.02 to 2.92 log cfu/ml. The range of the total fecal coliform count was 2.02 to 2.74 log cfu/ml. A range of 2.99 to 2.05 log cfu/ml was noted for the overall fungal count. The range of Escherichia coli data was 2.01–2.45 log cfu/ml. Although Salmonella spp. was recognized, no growth was seen in any of the samples. The count of Vibrio cholerae varied between 2.29 and 2.61 log cfu/ml. The range of the listeria count was 2.05 to 2.96 log cfu/ml. (Figure 3.3a) Then, we performed the enrichment of the results to indicate the presence and count of four species of bacteria, including E. coli, Salmonella spp., Vibrio spp., and Listeria spp. It compares the outcomes for every species of bacterium and sample location before and after enrichment. After enrichment, E. coli is found everywhere except in front of Milon

Auditorium and Shahjalal Avenue. Firmgate is the only space where Salmonella spp. are absent after enrichment. after enrichment, Vibrio spp. have been found in several places, Only missing in in front of Milon Auditorium (ALV-07) and Bongo Bazar(ALV-01). after enrichment, no Listeria spp. is Found

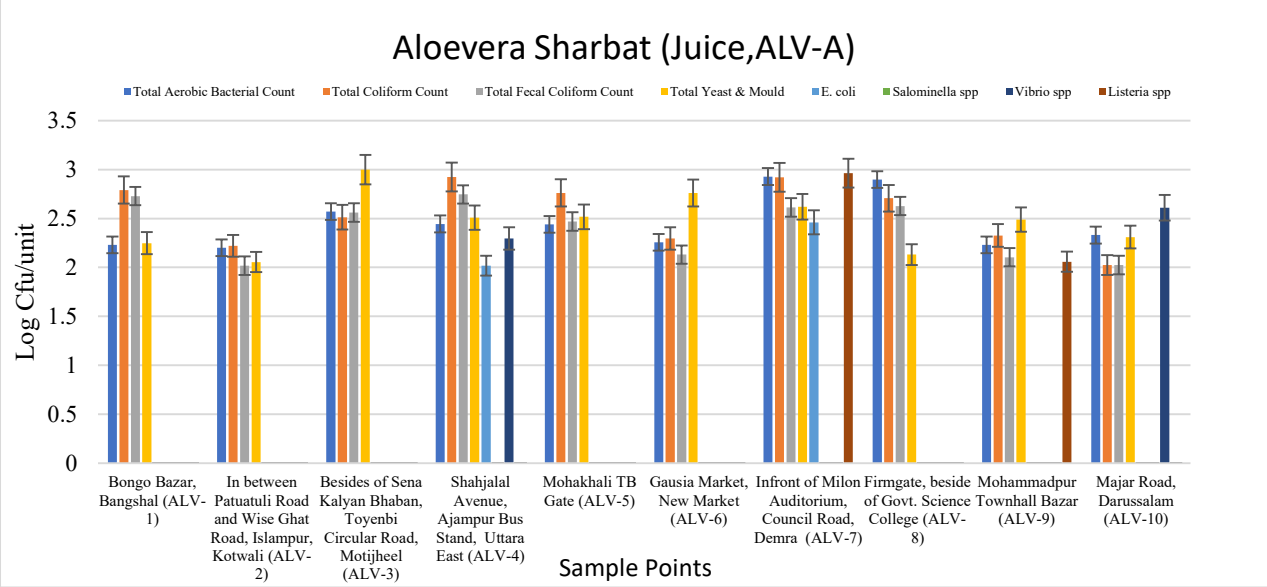


Figure 3.3a: Microbiological colony count of the samples (Aloevera Sharbat Juice, ALV-A)

log cfu/ml

3.3.2 Aloevera Sharbat Glass, ALV-B:

A total of eight microbial parameters were observed from ten different Aloevera Sharbat Glass samples of Dhaka City collected from different crowded places in the city. Total Aerobic Bacterial Count has a maximum value of 2.91 log cfu/ml at the intersection of Patuatuli Road and Wise Ghat Road in Islampur, Kotwali (ALV-2), and a minimum value of 2.01 at the Milon Auditorium, Council Road, Demra (ALV-7). The highest values for both Total Coliform Count and Total Fecal Coliform Count were found in Shahjalal Avenue (ALV-4), with counts of 2.96 log cfu/ml and 2.35 log cfu/ml , respectively; Bongo Bazar (ALV-1) and two other places had the lowest counts for Fecal Coliform, with a count of 0. At Mohammadpur Townhall Bazar (ALV-9), the maximum value for Total Yeast & Mould is 2.79, while the lowest value is 0 at three different sites. As indicated with the zero counts for these, none of the samples contain any *E. Coli, Salmonella* spp., *Vibrio spp*., or *Listeria* spp. (Figure 3.3b)

The enrichment test was conducted to determine the presence and count of six parameters (TFCC, TYM, E. coli, Salmonella spp., Vibrio spp., and Listeria spp.) in the Aloevera Sample

(Glass, ALV-B), The majority of the samples had E. Coli before they were enriched, except Mohammadpur Townhall Bazar. Only Gausia Market indicates the presence of E. coli during enrichment; the remaining samples are either absent of the bacteria or were not studied. the exception of Vibrio spp. at Patuatuli Road and Wise Ghat Road, Islampur, Kotwali Road, Mazar Road, Gabtoli, and Bongo Bazar, Bangshal were absent in front of Milon Auditorium, Council Road, Demra and Salmonella spp. at Firmgate, beside of Govt. Science College, these bacteria were present after enrichment. No listeria was detected after enrichment.

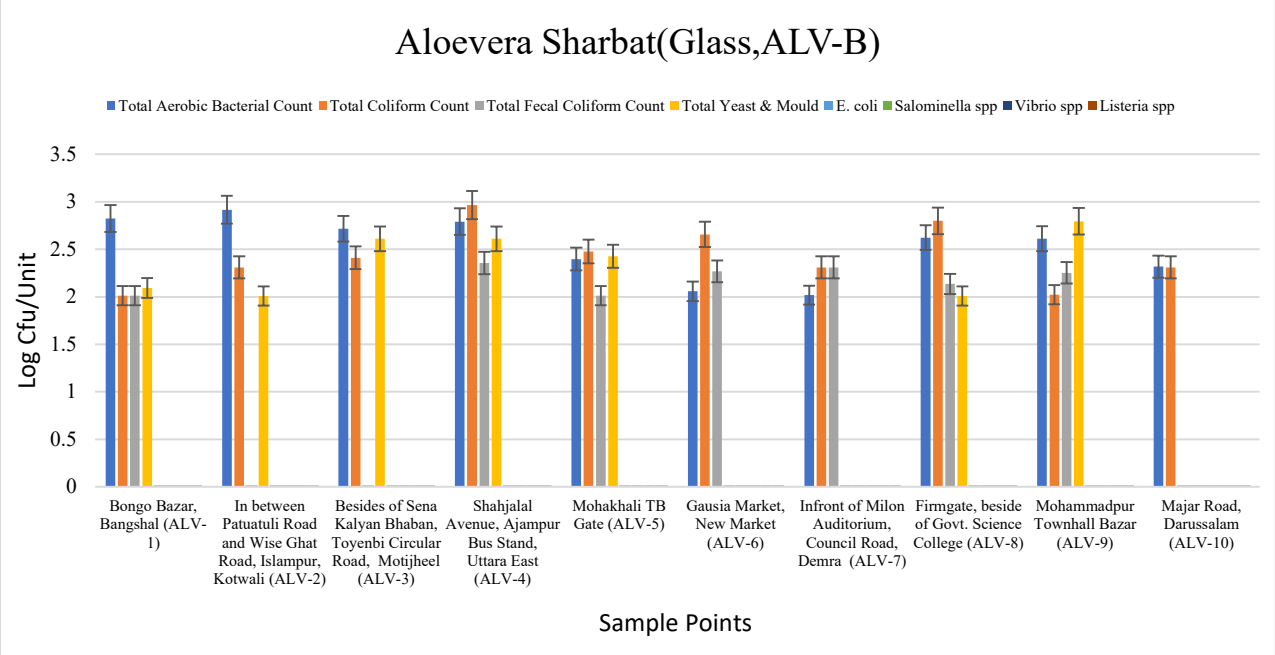


Figure 3.3b: Microbiological colony count of the samples (Aloevera Sharbat Glass, ALV-B)

log cfu/ml

3.3.3 Aloevera Sharbat Mug/Jug, ALV-C:

A total of eight microbial parameters were observed from ten different Aloevera Sharbat mug/jug samples of Dhaka City collected from different crowded places in the city. The total aerobic bacterial count ranged from 2.22 to 2.99 log cfu/ml. In the case of Total Coliform Count the range observed from 2.02 to 2.9 log cfu/ml. Total fecal coliform count ranged from 2.02 to 2.9 log cfu/ml. The total fungal count was observed from 2.2 to 2.70 log cfu/ml. *Escherichia coli* was recorded from 2.00 log cfu/ml. *Salmonella spp., Listeria*. *Vibrio cholerae*. was observed but no growth was detected in any sample. (Figure 3.3c) After enrichment, the sample contains TFCC in Firmgate, and All samples that had E. coli, except the front of Milon Auditorium, Council Road, Demra (ALV-7). The total number of mould and yeast was found in Shahjalal Avenue, Ajampur Bus Stand, Uttara East (ALV-4) after enrichment, no sample

contained Salmonella spp. After enrichment, *Vibrio spp*. was detected in six samples and missing in four. In addition to Sena Kalyan Bhaban, Toyenbi Circular Road, Motijheel (ALV-3), Shahjalal Avenue, Ajampur Bus Stand, Uttara East (ALV-4), Bongo Bazar, Bangshal (ALV-1), Mohakhali TB Gate (ALV-5), and Mohammadpur Townhall Bazar (ALV-9) were the samples that had *Vibrio spp.* present. None of the samples contained *Listeria spp.*

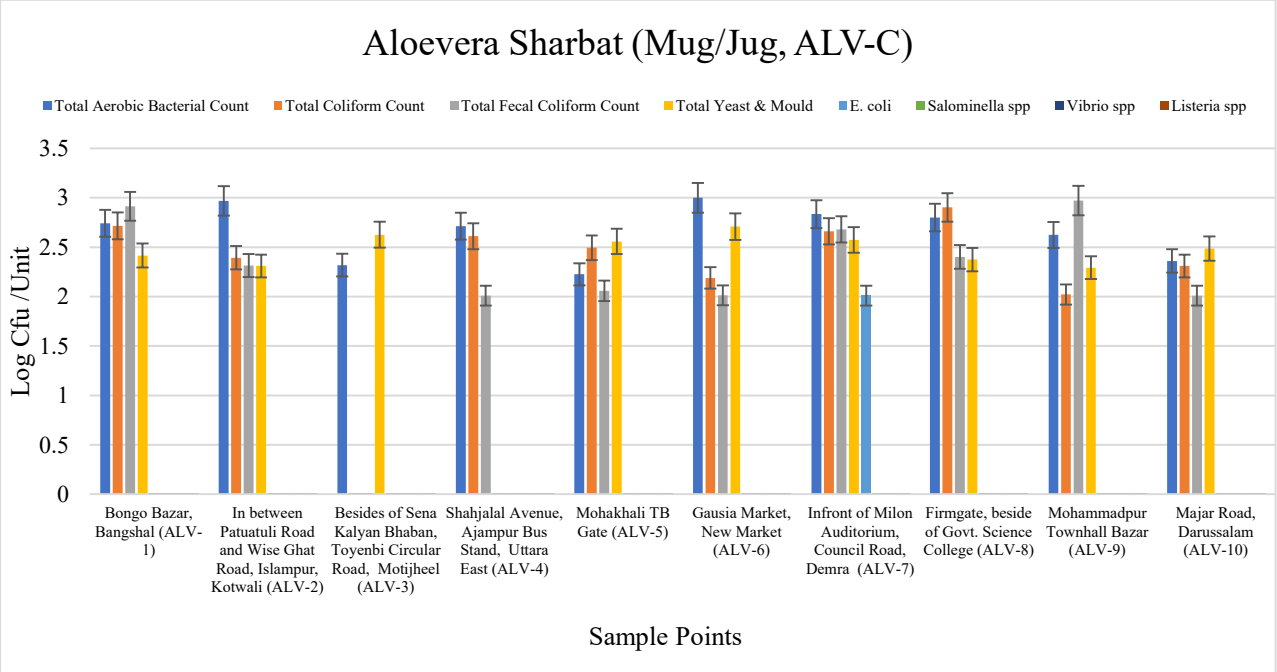


Figure 3.3c: Microbiological colony count of the samples (Aloevera Sharbat Mug/Jug, ALV-C)

log cfu/ml

3.3.4 Sugarcane Juice, SC-A:

A total of eight microbial parameters were observed from ten different Sugarcane Juice samples of Dhaka City collected from different crowded places in the city. The total aerobic bacterial count ranged from 2.07 to 2.93 log cfu/ml. highest value of total aerobic bacterial count was found in SC-7, while the lowest value was found in SC-3. In the case of Total Coliform Count the range observed from 2.01 to 2.9 log cfu/ml. the highest value of total coliform count was found in SC-8, while the lowest value was 2.017033339, found in SC-2. The total fecal coliform count ranged from 2.1 to 2.9 log cfu/ml. highest value of total fecal coliform count was found in SC-1, while the lowest value was found in SC-3 and SC-8. The total fungal count was observed from 2.36 to 2.99 log cfu/ml. the highest value of total yeast and mould was found in SC-7, while the lowest value was found in SC-6. *Escherichia coli* was recorded from

2.0 to 2.88 log cfu/ml. highest value of E. coli was found in SC-3. *Salmonella spp.* count ranged from 2.05 to 2.70 log cfu/ml. highest value of Salmonella spp was found in SC-6. *Vibrio cholerae*. count ranged from 2.24 to 2.91 log cfu/ml. highest value of Vibrio spp was found in SC-4. *Listeria*. count ranged from 2.22 to 2.91 log cfu/ml. highest value of Listeria spp was found in SC-6. (Figure 3.4a). Only one location showed a change in *E.coli* after enrichment SC-8 had less than 10 E.coli before enrichment but became positive after enrichment, *Salmonella spp.* No change was detected. SC-6 had less than 10 *Vibrio spp.* before enrichment, but became positive after enrichment, No change in *Listeria spp.* after enrichment

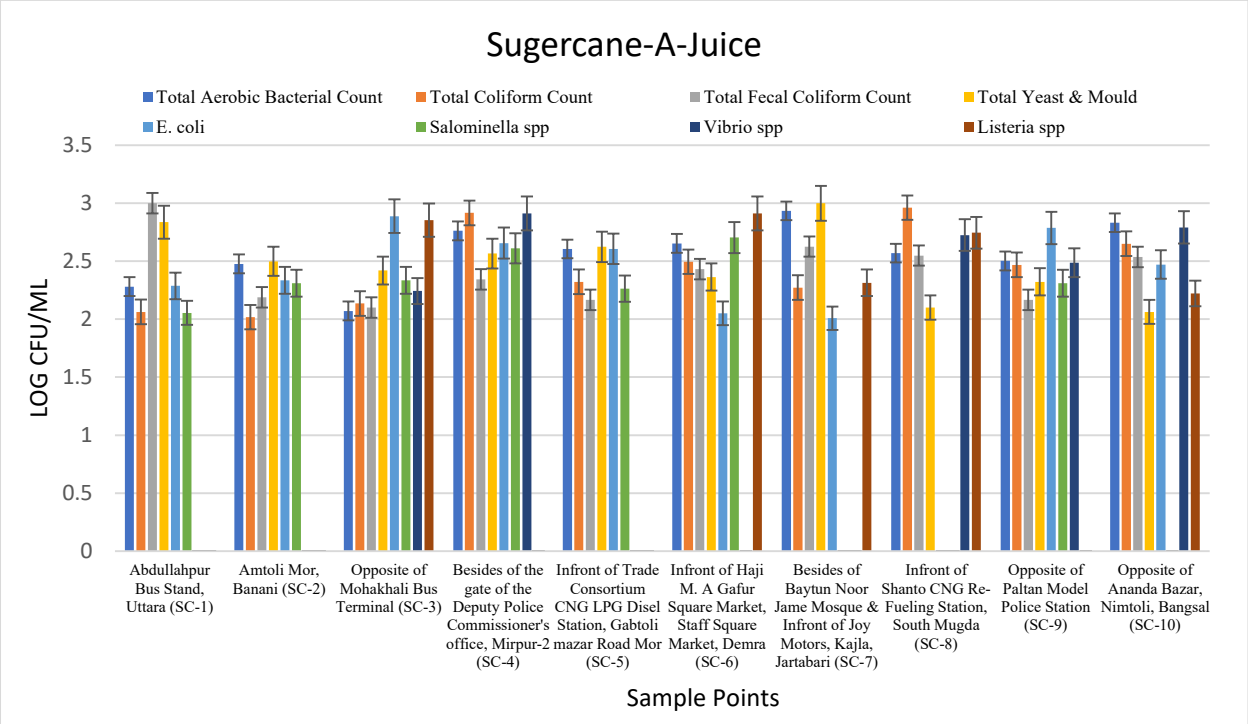


Figure 3.4a: Microbiological colony count of the the samples (Aloevera Sharbat Mug, ALV-C) log cfu/ml.

3.3.5 Sugarcane Juice Glass, SC-B:

A total of eight microbial parameters were observed from ten different Sugarcane Juice Glass samples of Dhaka City collected from different crowded places in the city. The total aerobic bacterial count ranged from 2.19 to 3.00 log cfu/ml. highest value is at Abdullahpur Bus Stand, Uttara (SC-1) and while the lowest value is at Besides of the gate of the Deputy Police Commissioner's office, Mirpur-2 (SC-4). In the case Total Coliform Count the range observed from 2.19 to 2.79 log cfu/ml. The highest value is at Infront of Trade Consortium CNG LPG Diesel Station, Gabtoli Mazar Road Mor (SC-5), while the lowest value is at Abdullahpur Bus

Stand, Uttara (SC-1) and Opposite of Ananda Bazar, Nimtoli, Bangsal (SC-10). Total fecal coliform count ranged from 2.01 to 2.92 log cfu/ml. The total fungal count was observed from 2.05 to 2.98 log cfu/ml. *Escherichia coli* was Absent. *Salmonella spp.* was observed, Present only at Opposite of Mohakhali Bus Terminal (SC-3) with a value of 2.00 Vibrio spp are absent in all locations. *Listeria spp* are absent in all locations. (Figure 3.4b) After enrichment, TFCC is present in SC-10. After enrichment, *E. coli* is only absent in SC-04.SC-07, SC-09. After enrichment, *Vibrio spp.* was detected in seven samples and missing in three.SC-01, SC-08,SC-09 were missing. Three locations. shows the presence *Salmonella spp* of after enrichment.SC-01.SC-06.SC-10. Only two locations showed a presence in *Listeria spp.*after enrichment SC-07, SC-10

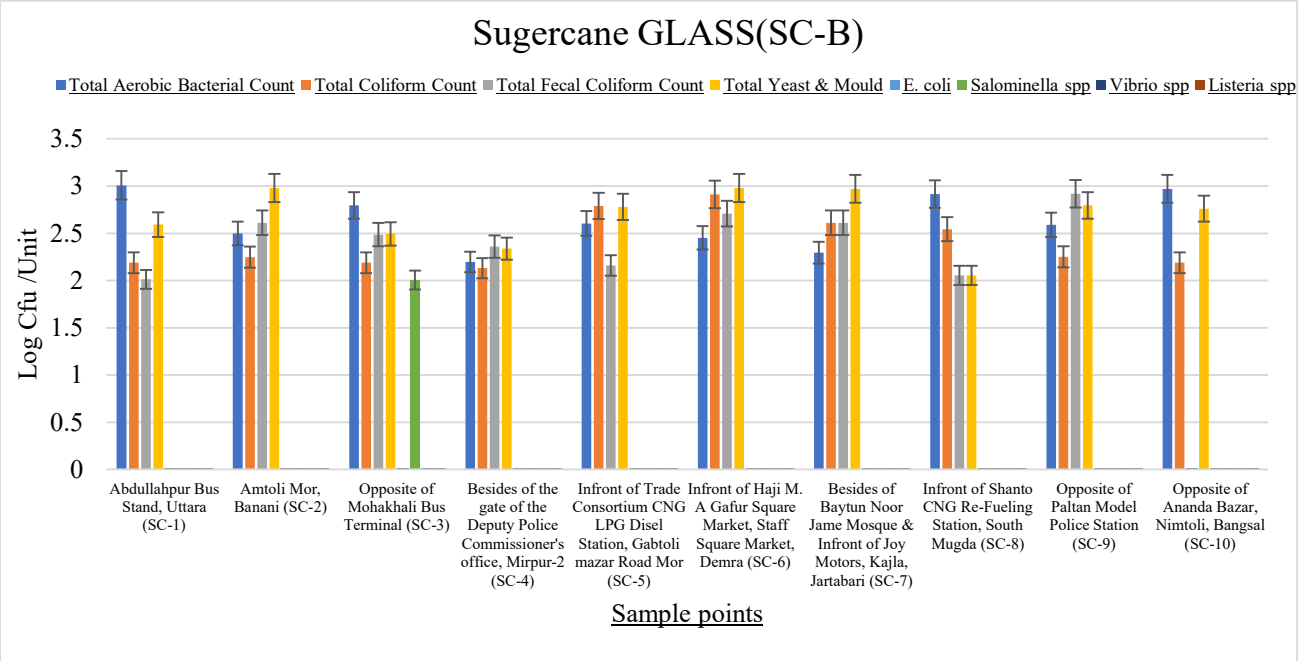


Figure 3.4b: Microbiological colony count of the samples(Sugarcane Glass, Sc-B) log cfu/ml.

3.3.6 Sugarcane Juice Mug/Jug, SC-C:

A total of eight microbial parameters were observed from ten different Sugarcane Juice Mug or Jug samples of Dhaka City collected from different crowded places in the city. The total aerobic bacterial count ranged from 2.02 to 3.00 log cfu/ml. highest total aerobic bacterial count was found in SC-7 while the lowest was found in SC-10 In the case Total Coliform Count the range was observed from 2.00 to 2.91 log cfu/ml. highest total coliform count was found in SC-7 while the lowest was found in SC-9.

Total fecal coliform count ranged from 2.0 to 2.48 log cfu/ml. highest total fecal coliform count was found in SC-1.The total fungal count was observed from 2.01 to 2.98 log cfu/ml. The highest total yeast and mould count was found in SC-7 while the lowest was found in SC-9. *E. coli* was detected only in SC-2 (2.0043 log CFU/ml), while the other samples were free of *E. coli. Salmonella spp, Vibrio spp, Listeria spp*. were not detected in any of the samples, except for *Listeria spp* in SC-4 (2.0086 log CFU/ml). (Figure 3.4c) After enrichment tests, *E.coli* was not found in Sc-02, SC-07. After enrichment, *Salmonella spp.* was absent at (SC-2), (SC-7) and SC-03, SC-08. After enrichment *Vibrio spp*. Was present only in six locations, Missing in four locations. SC-01, SC-04,SC-05,SC-10. After enrichment, *Listeria spp.* was present at Three locations, SC-01,SC-05, SC-06

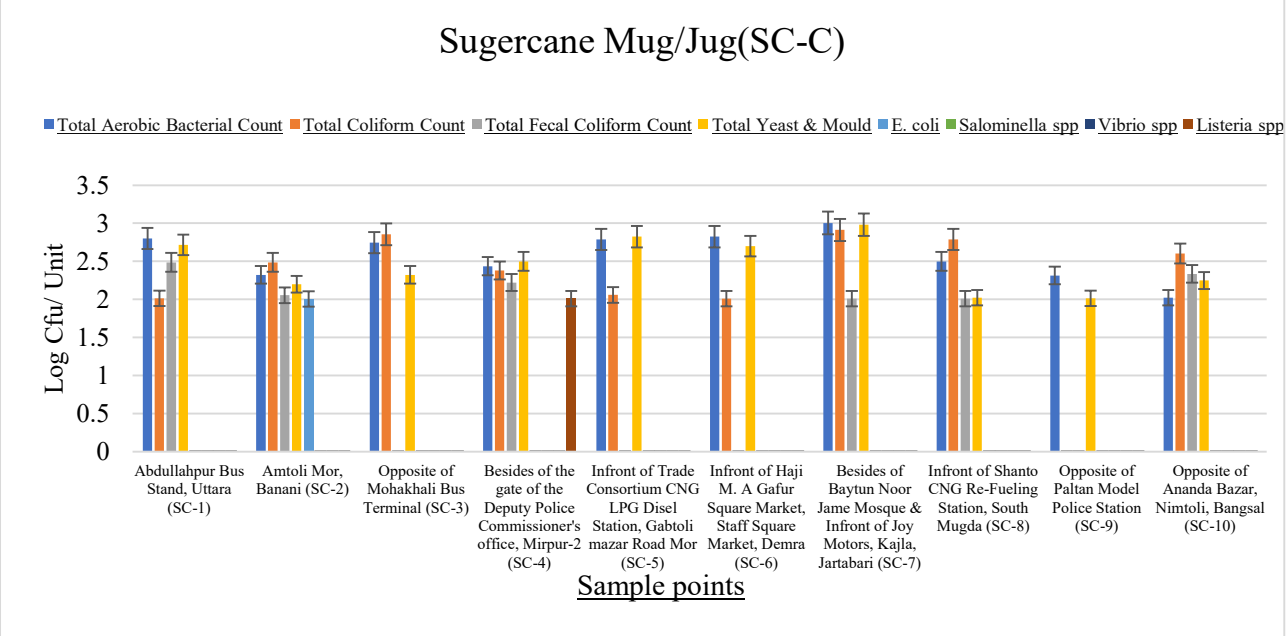


Figure 3.4c: Microbiological colony count of the samples(Sugarcane Mug or Jug, Sc-A) log cfu/ml.

3.3.7 Sandwich:

A total of eight microbial parameters were observed from ten different Sandwich samples of Dhaka City collected from different crowded places in the city. Total Bacterial Aerobic Count ranged from 3.77 to 7.92 log cfu/unit. Total Coliform Count Sandwich range from2.60 to 5.05 log cfu/unit. Total Fecal Coliform Count ranged from 2 to 5.23 log cfu/unit. Total Yeast & Mould ranged from 4.08 to 5.69 log cfu/unit. Total count of *E. coli* ranged from 2 to 5.08 log cfu/unit. The *Salmonella* spp ranged from 2 to 4.04 log cfu/unit. *Vibrio* spp ranged from 4.47 to 5.30 log cfu/unit. *Listeria* spp has no growth in all samples. (Figure 3.5)

Following enrichment, *E. coli* was detected in Uttara but not detected or tested in other places. *Salmonella* spp Following enrichment, it was missing from certain locations but present at Paltan and College Street. In *Vibrio spp* After enrichment, they were found in Demra, College Street, Uttara, and the Mohakhali bus station, but they were not examined anywhere. *Listeria spp* In every area, Before and After enrichment, *Listeria spp*. were missing.

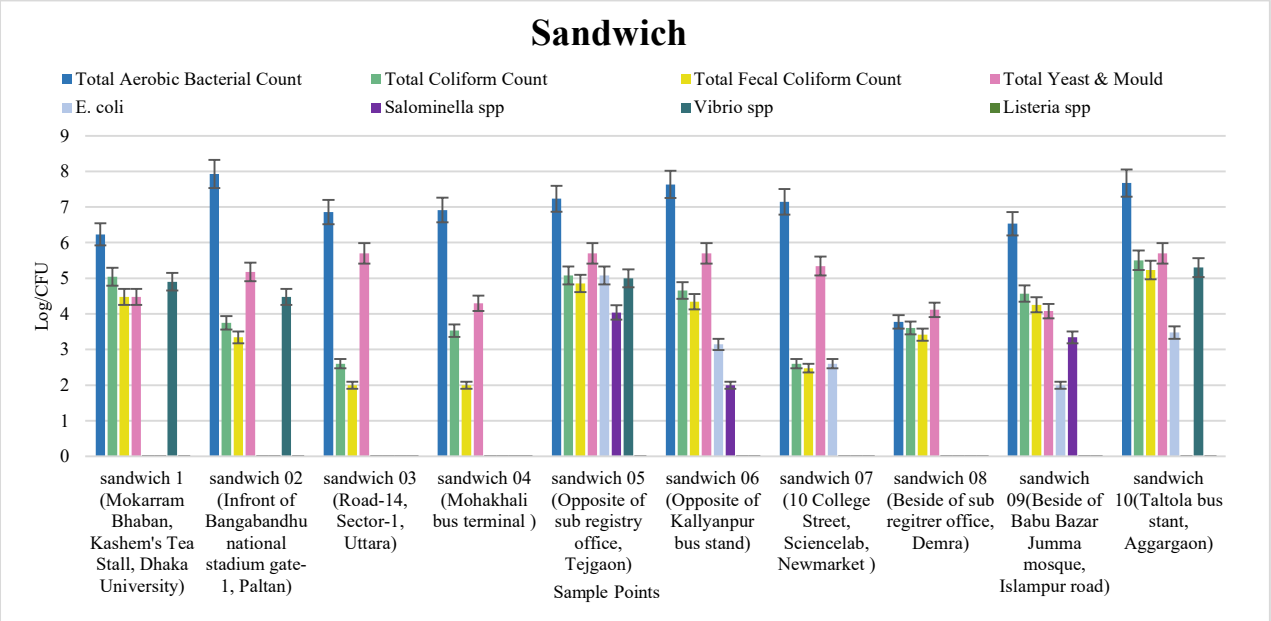


Figure 3.5: Microbiological colony count of the samples (sandwich) log cfu/ml.

3.3.8 Mixed salad:

A total of eight microbial parameters were observed from three different mixed salad samples of Dhaka City collected from different crowded Restaurants in the city. Total Aerobic Bacterial Count The highest value, 5.23, was found in Sample 2 (Lovely Hotel, Nilkhet). Sample-1 (Hotel Dhanshiri, Topkhana Road, Press Club) had the lowest value of 0. In Total Coliform Count All samples have a maximum and a minimum of zero. Total Fecal Coliform Count For all samples, the maximum and lowest values are zero. Total Yeast and Mould The highest value, 3.672097858, was measured in Sample-3 (Mitali Hotel, Chankharpool). Sample-1 (Hotel Dhanshiri, Topkhana Road, Press Club) had the lowest value of zero. E. coli, Salmonella spp. Vibrio cholera for all samples, the maximum and lowest values are zero. Listeria spp 2.69, the highest value, is located in Sample 3 (Mitali Hotel, Chankharpool). Samples 1 and 2 contain the lowest value, 0. (Figure 3.6) Three samples are calculated both before and following enrichment. Following enrichment, no E. Coli, Vibrio spp., or Listeria spp. were found in Sample 1, which had the lowest level of bacterial contamination. After enrichment, Sample 2

exhibits the greatest level of bacterial contamination, including E. Coli, Vibrio spp., and Listeria spp. After enrichment, Sample 3 contains E. Coli, but no counts of Listeria or Vibrio species were made.

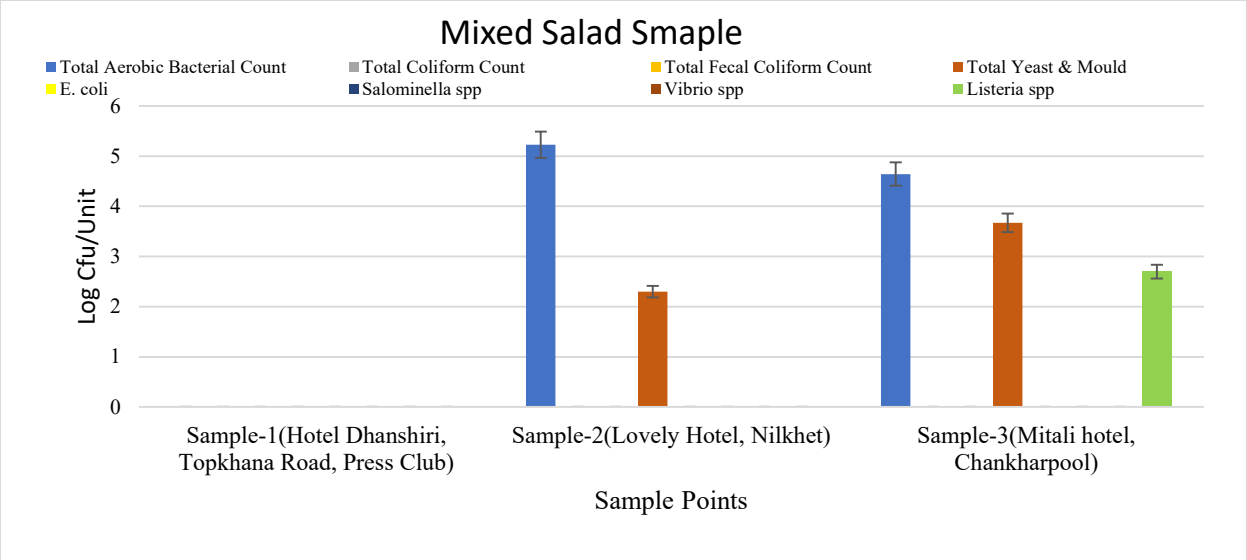


Figure 3.6: Microbiological colony count of the the samples (Mix Salad) log cfu/ml

3.3.9: Fuchka sample (Tok/Chutney):

A total of eight microbial parameters were observed from ten different Fuchka samples (Tok/Chutney) of Dhaka City collected from different crowded places in the city. The total aerobic bacterial count ranged from 2.02 to 5.98 log cfu/ml. highest value is at Dhanmondi 8A, Rabindra Sarobor (FC-1) while the lowest value is at Beside of balur field, Lalbagh (FC-10). In the case of Total Coliform Count the range observed from 2.09 to 2.96 log cfu/ml. highest value is in Infront of the gate of puja mandap, Deilla East Demra (FC-8) while the lowest value is in front of the gate of DNCC market Banani (FC-5). Total fecal coliform count ranged from 2.09 log cfu/ml. The highest value is at Mirpur Central puja mandap, Mirpur 2 (FC-2). The total fungal count was observed from 2.02 to 2.96 log cfu/ml. *Escherichia coli* was recorded from 2.0 to 2.03 log cfu/ml. The highest value is at Mirpur Central puja mandap, Mirpur 2 (FC-2), while the lowest value is at Beside of balur field, Lalbagh (FC-10). *Salmonella spp.* was observed but no growth was detected in any sample. *Vibrio cholerae*. count Not detected. *Listeria*. count ranged from 2.24 to 2.99 log cfu/ml. The highest value is at Uttara sector 11, Road-06 (FC-4) while the lowest value is at Infront of the gate of puja mandap, Deilla East Demra (FC-8), (Figure 3.7a). After enrichment, Total Coliform Count. only FC-5, FC-6, FC-

7, and FC-9 all show the presence of coliforms.After enrichment, In Total Fecal Coliform Count. only FC-2 shows an absence in fecal coliforms. In all other locations TFCC was detected. After enrichment, FC-1, FC-3, FC-5, and FC-9 have growth in *E.coli*.None of the locations have detectable *Salmonella spp.* before or after enrichment. After enrichment, only FC-8 and FC-9 have they show the presence of *Vibrio spp*. After enrichment, only FC-9 have *Listeria Spp*. presence.

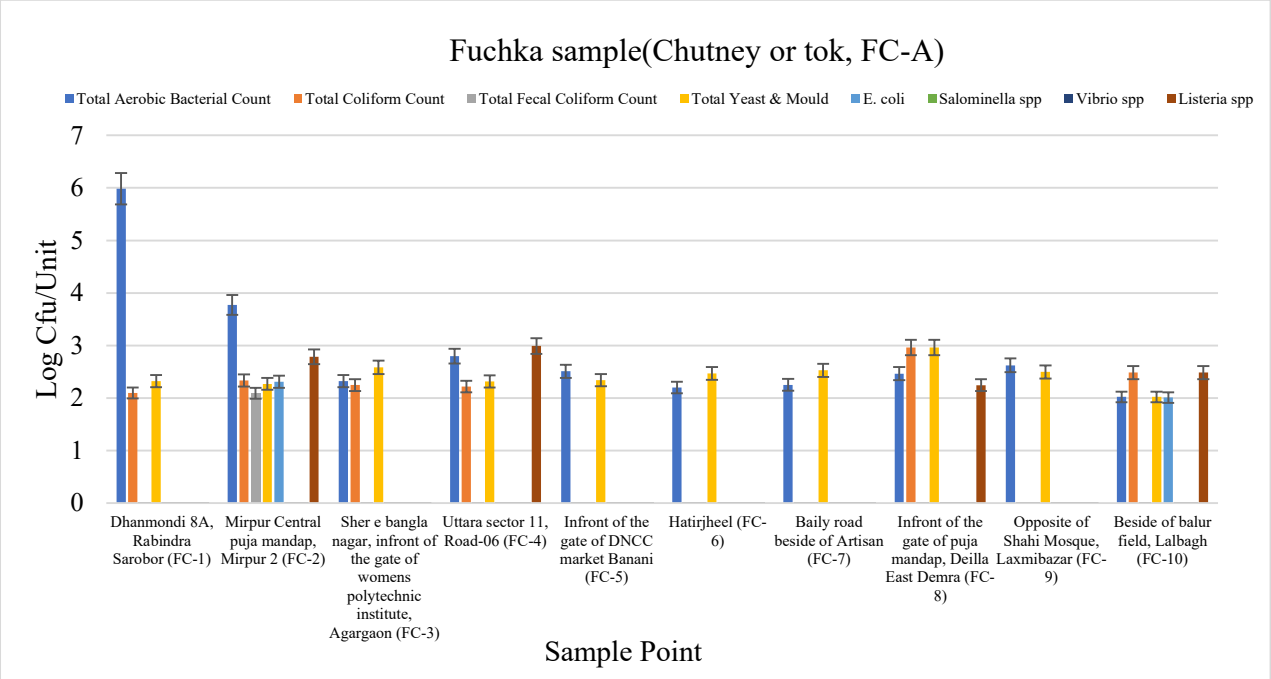


Figure 3.7a: Microbiological colony count of the samples(Fuchka sample, Chutney or tok, FC-A) log cfu/ml.

3.3.10: Fuchka sample (Spoon):

A total of eight microbial parameters were observed from ten different Fuchka Spoon samples of Dhaka City collected from different crowded places in the city. The total aerobic bacterial count ranged from 2.05 to 5.17 log cfu/ml. The highest value is at Dhanmondi 8A, Rabindra Sarobor (FC-1) while the lowest value is at front of the gate of puja mandap, Deilla East Demra (FC-8) In the case of Total Coliform Count the range observed from 2.02 to 2.99 log cfu/ml. Total Coliform Count The highest value is at Mirpur Central puja mandap, Mirpur 2 (FC-2), while the lowest value is at the front of the gate of DNCC market Banani (FC-5). Total fecal coliform count ranged from 2 to 2.58 log cfu/ml. The highest value is at Mirpur Central puja mandap, Mirpur 2 (FC-2) while the lowest value is at Dhanmondi 8A, Rabindra Sarobor (FC-1).The total fungal count was observed from 2.09 to 4 log cfu/ml.The highest value is at

Dhanmondi 8A, Rabindra Sarobor (FC-1). *Escherichia coli* was recorded from 2.30 log cfu/ml. The only observed value is Mirpur Central puja mandap, Mirpur 2 (FC-2). *Salmonella spp.* was observed only growth was detected in 2.7 log cfu/ml. At Mirpur Central puja mandap, Mirpur 2 (FC-2). *Vibrio cholerae*. were observed but no growth on any sample was found. *Listeria*. count ranged from 2.13 to 2.60 log cfu/ml. The highest value is at Uttara sector 11, Road-06 (FC-4) while the lowest value is at Mirpur Central puja mandap, Mirpur 2 (FC-2) (Figure 3.7b). Following enrichment, TCC was present in Hatirjheel (FC-6) and Laxmibazar (FC-09) and absent at Banani (FC-5). TFCC was operational at Sher e Bangla Nagar (FC-3)(FC-3), Banani (FC-5), Hatirjheel (FC-6), and Laxmibazar (FC-9) after enrichment. After Yeast & Mould enrichment, Deilla (FC-8) sample was identified. After enrichment for *E. coli* in the following areas: Hatirjheel (FC-6), Lalbagh (FC-10), Road-06 (FC-4), Uttara sector 11, Banani (FC-5), and Hatirjheel (FC-1). *Salmonella spp.* was detected in Laxmibazar (FC-9) and Lalbagh (FC-10) after enrichment. Following enrichment, *Vibrio spp*. was missing from the remaining locations and present at Dhanmondi (FC-1), Mirpur (FC-2), Baily road (FC-7), Laxmibazar (FC-9), and Lalbagh (FC-10). Following findings of enrichment for *Listeria spp,* the following areas showed the presence of the bacteria: Hatirjheel (FC-6), Baily Road (FC-7), Shahi Mosque's location (FC-9), and Laxmibazar (FC-9).

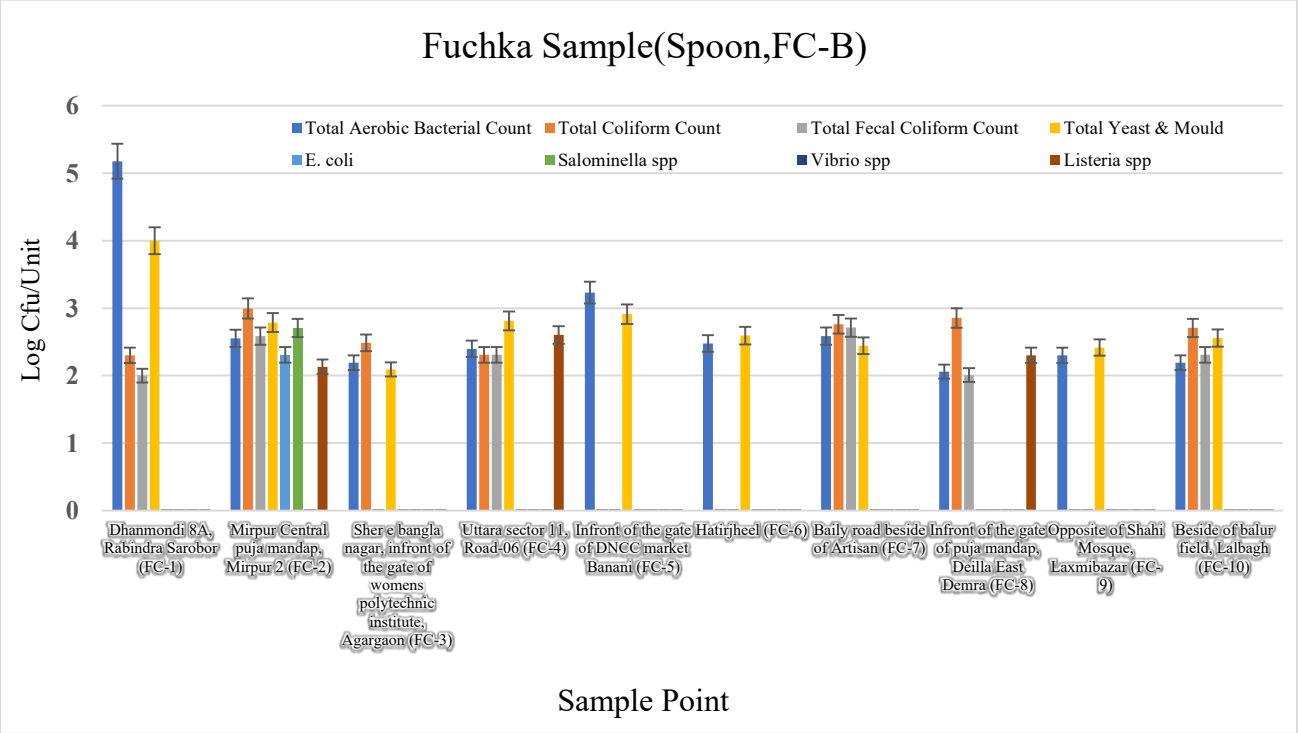


Figure 3.7b: Microbiological colony count of the samples(Fuchka sample, Spoon, FC-B) log cfu/ml.

3.3.11: Fuchka sample (Plate/Cup):

A total of eight microbial parameters were observed from ten different Fuchka Plate/Cup samples of Dhaka City collected from different crowded places in the city. The total aerobic bacterial count ranged from 2.02 to 2.79 log cfu/ml. highest value is at Uttara sector 11, Road-06 (FC-4) with while the lowest value is at **front of the gate of DNCC market Banani (FC-5). In the case of Total Coliform Count the range observed from 2.01 to 3 log cfu/ml. The highest value is at Uttara sector 11, Road-06 (FC-4)while the lowest value is at Mirpur Central puja mandap, Mirpur 2 (FC-2). Total fecal coliform count ranged from 2.00 to 2.70 log cfu/ml. The highest value is at Mirpur Central puja mandap, Mirpur 2 (FC-2) with while the lowest value is at Dhanmondi 8A, Rabindra Sarobor (FC-1). The total fungal count was observed from 2.00 to 2.96 log cfu/ml. The highest value is at Dhanmondi 8A, Rabindra Sarobor (FC-1) while the lowest value is at the front of the gate of puja mandap, Deilla East Demra (FC-8) with 2.008600172. *Escherichia coli* was recorded 2.00 log cfu/ml. Only at Uttara sector 11, Road-06 (FC-4). *Salmonella spp.* was observed but no growth was detected in any sample. *Vibrio cholerae*. was observed but no growth was detected in any sample. *Listeria spp.* count ranged from 2.15 to 2.30 log cfu/ml. The highest value is at the front of the gate of puja mandap, Deilla East Demra (FC-8) while the lowest value is at Dhanmondi 8A, Rabindra Sarobor (FC-1). (Figure 3.7c).

Only one location (Beside of balur field, FC-10) showed the absence of *E.coli* after enrichment. All other locations had *E. coli* present after enrichment. Three locations (Uttara sector 11, Road-06, FC-4; in front of the gate of DNCC market Banani, FC-5 and Hatirjheel, FC-6) showed the absence of *Salmonella spp.* after enrichment. All other locations had Salmonella sp. present after enrichment. Two locations (in front of the gate of DNCC market Banani, FC-5; and Hatirjheel, FC-6) showed the absence of *Vibrio spp.* after enrichment. All other locations had *Vibrio spp.* present after enrichment. Three locations (Uttara sector 11, Road-06 (FC-4).; Hatirjheel, FC-6; and Baily road beside of Artisan, FC-7) showed the absence of *Listeria spp.* after enrichment. All other locations had *Listeria spp.* Present.

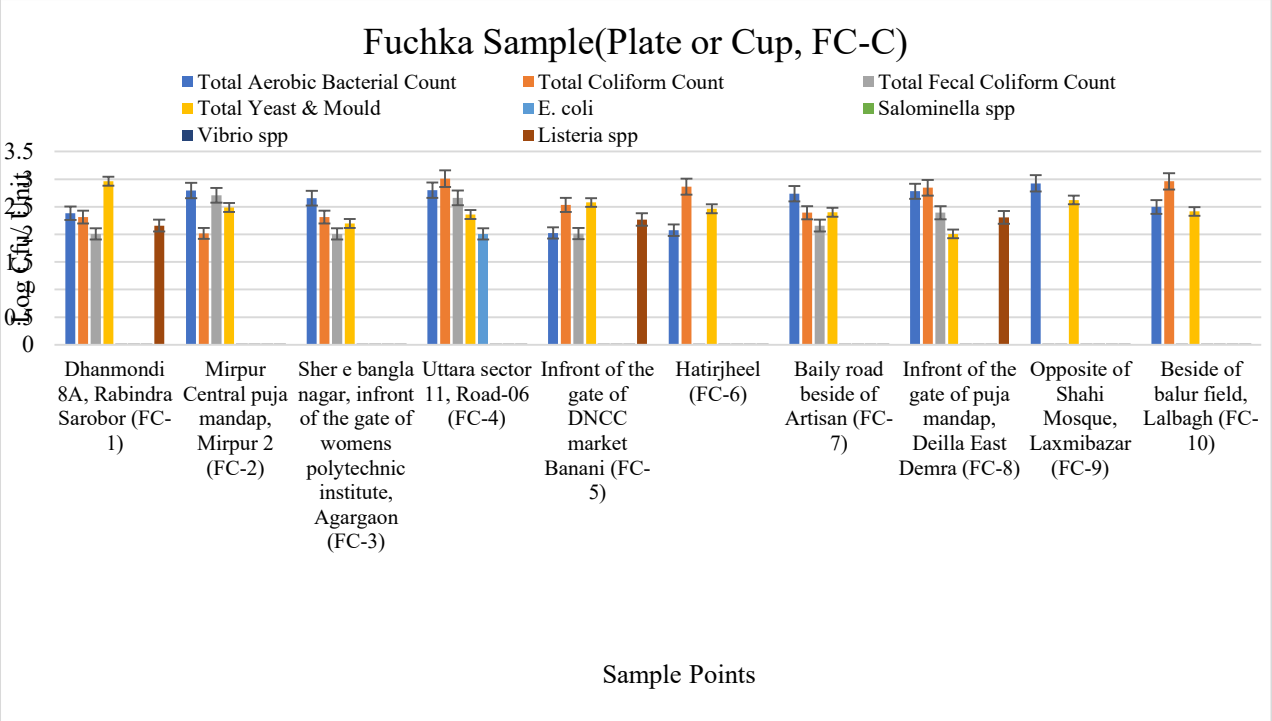


Figure 3.7c: Microbiological colony count of the samples(Fuchka sample, Plate or cup or, FC-C) log cfu/ml.

3.3.12: Fuchka sample (Water):

A total of eight microbial parameters were observed from ten different Fuchka water samples of Dhaka City collected from different crowded places in the city. The total aerobic bacterial count ranged from 2.21 to 2.91 log cfu/ml. The highest value is at Uttara sector 11, Road-06 (FC-4) while the lowest value is at Opposite of Shahi Mosque, Laxmibazar (FC-9). In the case of Total Coliform Count the range observed from 2.004 to 2.68 log cfu/ml. The highest value of Total Coliform Count is at the front of the gate of puja mandap, Deilla East Demra (FC-8) while the lowest value is at the front of the gate of DNCC market Banani (FC-5). Total fecal coliform count ranged from 2.04 to 2.48 log cfu/ml. The highest value of Total Fecal Coliform Count is at Uttara sector 11, Road-06 (FC-4), and Hatirjheel (FC-6) while the lowest value is at Baily Road beside Artisan (FC-7). The total fungal count was observed from 2.12 to 2.9 log cfu/ml. The highest value of total Yeast & Mould in front of the gate of DNCC market is Banani (FC-5) while the lowest value is at Dhanmondi 8A, Rabindra Sarobor (FC-1). *Escherichia coli* was detected at 2.95 log cfu/ml. only at Uttara Sector 11, Road-06 (FC-4). *Salmonella spp.* was observed but no growth was detected in any sample. *Vibrio cholerae*. was observed but no growth was detected in any sample. *Listeria*. count 2.18 log cfu/ml. Only detected in Beside of balur field, Lalbagh (FC-10).(Figure 3.7d) After enrichment, Total Coliform Count was

detected from FC-1, FC-2, FC-3, FC-6, and FC-9 samples. After enrichment, Total fecal coliform count was detected for FC-1, FC-2, FC-3, FC-5, FC-8, FC-9, and FC-10 samples. After total fungal and mould enrichment FC-2, FC-3, FC-4and FC-10, which are growth was detected. *E. coli* is not detected at any of the sites; it is found in FC-7 and FC-8.*Salmonella spp.* Present at FC-1, FC-6, and FC-10; absent at all other locations. *Vibrio spp.* available for FC-4, FC-5, and FC-6, but not present for the remaining samples. *Listeria spp.* Present in FC-1 and FC-10, but missing from all other places.

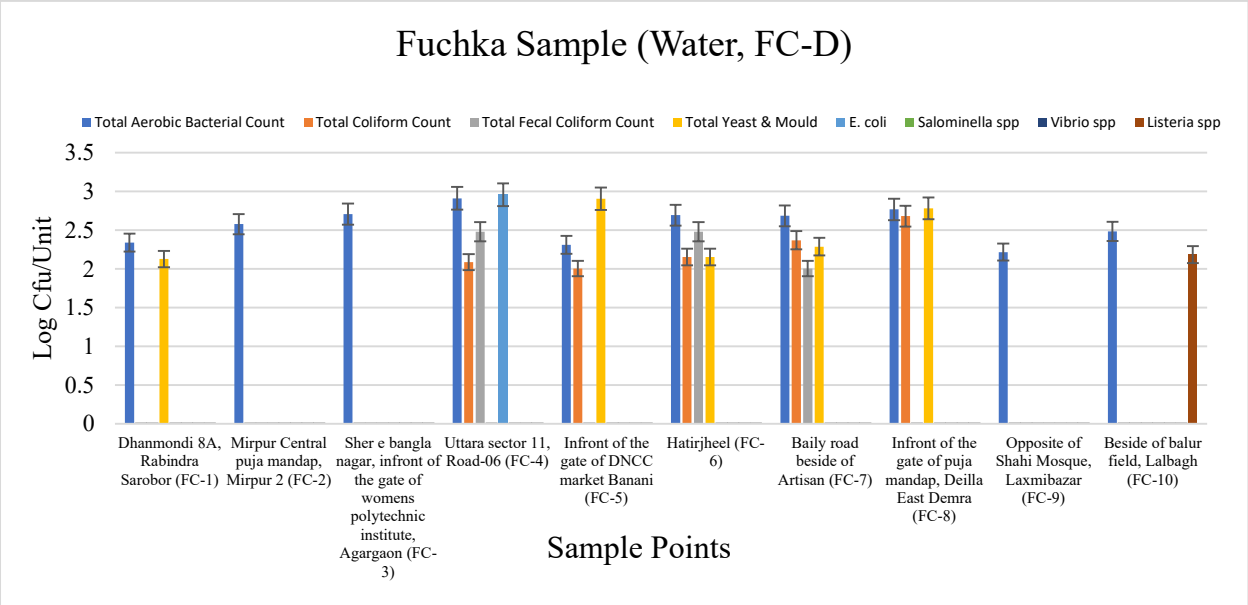


Figure 3.7d: Microbiological colony count of the samples(Fuchka sample, Water, FC-D) log cfu/ml.

3.4 Identification with Analytical Profile Index (API)

3.4.1 API 20E Strep

For confirmation of *Enterobacteriaceae* and other non-fastidious, Gram-negative rods, Analytical Profile Index (API) was performed by using API 20E strips.

Table 3.5: Identification table of API 20E Strep

READING TABLE

TESTS	ACTIVE INGREDIENTS	QTY (mg/cup.)	REACTIONS/ENZYMES	RESULTS	
				NEGATIVE	POSITIVE
ONPG	2-nitrophenyl-ßD-galactopyranoside	0.223	ß-galactosidase (Ortho NitroPhenyl-ßD-Galactopyranosidase)	colorless	yellow (1)
ADH	L-arginine	1.9	Arginine DiHydrolase	yellow	red / orange (2)
LDC	L-lysine	1.9	Lysine DeCarboxylase	yellow	red / orange (2)
ODC	L-ornithine	1.9	Ornithine DeCarboxylase	yellow	red / orange (2)
CIT	trisodium citrate	0.756	CITrate utilization	pale green / yellow	blue-green / blue (3)
H_2S	sodium thiosulfate	0.075	H_2S production	colorless / greyish	black deposit / thin line
URE	urea	0.76	UREase	yellow	red / orange (2)
TDA	L-tryptophane	0.38	Tryptophane DeAminase	TDA / immediate: yellow	TDA / immediate: reddish brown
IND	L-tryptophane	0.19	INDole production	JAMES / immediate: colorless pale green / yellow	JAMES / immediate: pink
VP	sodium pyruvate	1.9	acetoin production (Voges Proskauer)	VP 1 + VP 2 / 10 min: colorless	VP 1 + VP 2 / 10 min: pink / red (5)
GEL	Gelatin (bovine origin)	0.6	GELatinase	no diffusion	diffusion of black pigment
GLU	D-glucose	1.9	fermentation / oxidation (GLUcose) (4)	blue / blue-green	yellow / greyish yellow
MAN	D-mannitol	1.9	fermentation / oxidation (MANnitol) (4)	blue / blue-green	yellow
INO	inositol	1.9	fermentation / oxidation (INOsitol) (4)	blue / blue-green	yellow
SOR	D-sorbitol	1.9	fermentation / oxidation (SORbitol) (4)	blue / blue-green	yellow
RHA	L-rhamnose	1.9	fermentation / oxidation (RHAmnose) (4)	blue / blue-green	yellow
SAC	D-sucrose	1.9	fermentation / oxidation (SACcharose) (4)	blue / blue-green	yellow
MEL	D-melibiose	1.9	fermentation / oxidation (MELibiose) (4)	blue / blue-green	yellow
AMY	amygdalin	0.57	fermentation / oxidation (AMYgdalin) (4)	blue / blue-green	yellow
ARA	L-arabinose	1.9	fermentation / oxidation (ARAbinose) (4)	blue / blue-green	yellow
OX	(see oxidase test package insert)		cytochrome-OXidase	(see oxidase test package insert)	

3.4.2. API 20 Strep Results:

Figure 3.8a: Analytical Profile Index (API) of *Escherichia coli* (99.8 % confirmed)

食品総合研究所 - 茨城県つくば市

API 20 E V5.0 印刷 テキスト保存 新規検索

検体番号

日付 23/08/18

コメント

UNACCEPTABLE PROFILE

ストリップ	API 20 E V5.0
プロファイル	7044554
注釈	

菌種名	% ID	T	非典型反応
Escherichia coli 1			ADH 1%

次候補	% ID	T	非典型反応
Aeromonas hydrophila/caviae/sobria 2			CIT 80%
			RHA 1%

Figure 3.8b: Analytical Profile Index (API) of *Escherichia coli*

食品総合研究所 - 茨城県つくば市

API 20 E V5.0　印刷　テキスト保存　新規検索

検体番号

日付 23/08/18

コメント

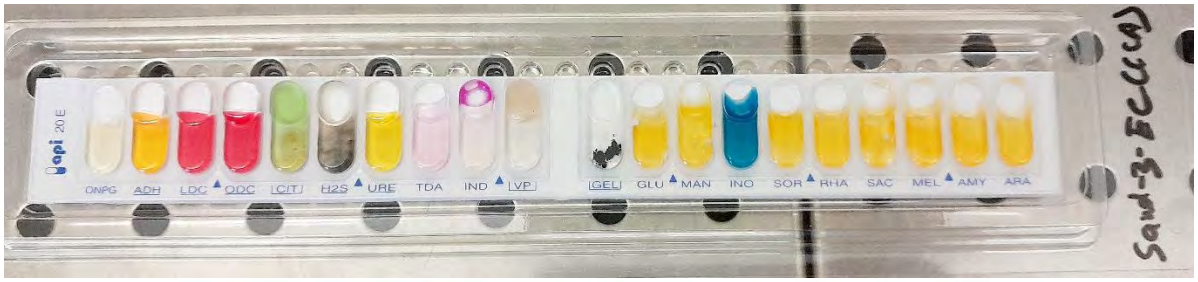
UNACCEPTABLE PROFILE

ストリップ	API 20 E V5.0
プロファイル	7757773
注釈	

菌種名	% ID	T	非典型反
Raoultella ornithinolytica			ADH 0
Serratia odorifera 1			ADH 0

次候補	% ID	T	非典型反
Serratia marcescens			ADH 0
			RHA 1

Figure 3.8c: Analytical Profile Index (API) of *Raoultella ornithinolytica* and *Serratia odorifera*



API 20 E V5.0 印刷 テキスト保存 新規検索

検体番号

日付 23/08/18

コメント

UNACCEPTABLE PROFILE

ストリップ	API 20 E V5.0
プロファイル	7 5 4 4 5 7 3
注釈	

菌種名	% ID	T	
Escherichia coli 1			A
Salmonella enterica ssp arizonae			C

次候補	% ID	T	
Citrobacter braakii			L

Figure 3.8d: Analytical Profile Index (API) of *Escherichia coli* and *Salmonella enterica ssp Arizona*

Chapter 4

Discussion

This research work includes the in vitro derivation of microbial analysis, their pH whether it has effect on consumer. The results indicate varying responses of aloevera sharbat juice pH varied in the study from 4.79 to 6.15, which is quite near to neutral. The pH range for Fuchka tok/Chutney was observed to be 2.00 to 3.58, indicating a significant level of acidity. Subsequent investigation reveals that the fuchka water's pH level ranged from 7.32 to 7.75, which is quite near neutral. The pH range of the sugarcane juice that we measured was 3.82 to 5.24.

The results indicate varying responses of the total aerobic bacterial count ranged between 2.20 and 2.93 log cfu/unit in the ten aloevera sharbat juice samples. A total of 2.02 to 2.92 log cfu/unit of coliform was detected. The overall fecal coliform count ranged from 2.02 to 2.74 log cfu/unit. The Total Aerobic Bacterial Count in 10 Aloevera Sharbat Glass Samples ranges from 2.01 log cfu/unit to 2.91 log cfu/unit at its lowest. Total Fecal Coliform had a count of 0 and Total Coliform Count varied from 2.96 log cfu/unit to 2.35 log cfu/unit. The total aerobic bacterial count in 10 Aloevera Sharbat mug/jug samples varied from 2.22 to 2.99 log cfu/unit. The range for total coliform count was found to be 2.02 to 2.9 log cfu/unit. The range of the total fecal coliform count was 2.02 to 2.9 log cfu/unit. The total aerobic bacterial count in the ten sugarcane juice samples varied from 2.07 to 2.93 log cfu/unit. The range of total coliform counts detected is 2.01 to 2.9 log cfu/unit. The range of the total fecal coliform count was 2.1–2.9 log cfu/unit. The total aerobic bacterial count varied from 2.19 to 3.00 log cfu/unit across 10 Sugarcane Juice Glass samples. The range of 2.19 to 2.79 log cfu/unit was recorded in the total coliform count. The whole count of fecal coliforms varied between 2.01 and 2.92 log cfu/unit The range of the total aerobic bacterial count in 10 Sugarcane Juice Mug or Jug samples was 2.02 to 3.00 log cfu/unit. The range of the total coliform count was found to be 2.00 to 2.91 log cfu/unit. The range of the total fecal coliform count was 2.0–2.48 log cfu/unit. The total bacterial aerobic count (TABC) of 10 Sandwich samples varied from 3.77 to 7.92 log cfu/unit. The range of the total coliform count was 2.60 to 5.505 log cfu/unit. According to our observation The range of the total fecal coliform count was 2.23 to 5.23 log cfu/unit. The total aerobic bacterial count varied from 2 to 5.23 log cfu/unit among 3 mixed salad sample samples. Total Coliform Count: 0 log cfu/unit was recorded in the range. Fecal coliform count overall:

0 log cfu/unit. The total aerobic bacterial count varied from 2.02 to 5.98 log cfu/unit across 10 Fuchka samples tok/chutney. The range of 2.09 to 2.96 log cfu/unit was recorded in the total coliform count. The range of the total fecal coliform count was 2.09 log cfu/unit. The total aerobic bacterial count in 10 Fuchka Spoon samples varied from 2.05 to 5.17 log cfu/unit, whereas the total coliform count showed a range of 2.02 to 2.99 log cfu/unit. The range of the total fecal coliform count was 2 to 2.58 log cfu/unit. Of the ten Fuchka Plate/Cup samples The range of the total aerobic bacterial count per unit was 2.02 to 2.79 log cfu. The range of total coloniform counts detected is 2.01 to 3 log cfu/unit. The range of the total fecal coliform count per unit was 2.00 to 2.70 log cfu. The total aerobic bacterial count ranged from 2.21 to 2.91 log cfu/unit across 10 Fuchka water sample sets. The range of total coloniform counts detected is 2.004 to 2.68 log cfu/unit. The range of the total fecal coliform count per unit was 2.04 to 2.48 log cfu/unit.

The results indicate varying responses of Aloevera Sharbat Juice The total fungal count ranged from 2.99 to 2.05 log cfu/unit. The total yeast and mold content of Aloevera Sharbat Glass varied between 2 and 2.79 log cfu/unit. Mug/jug Aloevera Sharbat There was a noticeable range in the overall fungal count (2.2–2.70 log cfu/unit). The total fungal count in sugarcane juice ranged from 2.36 to 2.99 log cfu/unit. Samples of Sugarcane Juice Glassware Between 2.05 and 2.98 log cfu/unit was the total fungal count found in the ten-sugarcane juice cup or jug samples. Samples of sugarcane juice in mugs or jugs Among three samples of total yeast and mold, the total fungal count varied from 2.01 to 2.98 log cfu/unit. in mixed salad, and the mold count ranged from 2.3 to 3.67 log cfu/unit. Samples of sandwiches Total Mold and Yeast log cfu/unit value varied from 4.07 to 5.69. Among the ten sandwich specimens. Examples of Fuchka (Tok/Chutney) A range of 2.02 to 2.96 log cfu/unit was noted for the overall fungal count. Samples of Fuchka spoons the range of the total fungal count was 2.09 to 4 log cfu/unit. Samples of Fuchka Plate/Cup A range of 2.00 to 2.96 log cfu/unit was noted for the overall fungal count. Samples of Fuchka Plate/Cup There was a 2.12 to 2.9 log cfu/unit variation in the overall fungal count.

According to Bangladesh food safety authority. for ready to drink juice items, Total aerobic colony count for 5 samples should be two for maximum allowance. if it crosses the limit of 100 cfu/ ml than 5 Juice samples considered as the unsatisfactory from a satisfactory level and marginally unacceptable. If all 5 sample has 10000 cfu/ml or more than 10^3 than it is considered and rejected for all parameters. For water samples aerobic colony counts limits till 100cfu/ml. Total coliform limited to absence in per 100ml. sample. moreover, for the fecal

coliform absence in 100ml sample. *E.coli* absence in 100ml sample. For each 5-salad sample Total aerobic bacterial should be two for maximum allowance of units. If it crosses the limit then less than 10 in each 10^5 salad samples considered as the unsatisfactory from a satisfactory level and marginally unacceptable. If all 5 sample has 10^6 or more than 10^6 than it is considered as the rejected for all parameters. Total coliform should be one for maximum allowance it crosses the limit then less than 10^2 for each 5 salad samples considered as the unsatisfactory from a satisfactory level and marginally unacceptable. If all 5 sample has 10^3 or more than 10^3 than it is considered as the rejected for all parameters. Salmonella should be zero for maximum allowance it crosses the limit then less than 10^2 for each 5 salad samples considered as the unsatisfactory from a satisfactory level and marginally unacceptable. If all 5 sample has 10^3 or more than 10^3 than it is considered as the rejected for all parameters.

This test describes the Out of ten, Aloevera Sharbat Juice The *Escherichia coli* values ranged from 2.01 to 2.45 log cfu/unit. in Aloevera Sharbat Glass, No E. Coli is present in any of the samples. Mug/jug Aloevera Sharbat Escherichia coli was only detected in ALV-07 and was measured at 2.00 log cfu/unit. Among 10 samples Sugarcane Juice The range of *Escherichia coli* was 2.0–2.88 log cfu/unit. *Escherichia coli* was absent from 10 samples of sugarcane juice glass. Sugarcane Juice Samples or Mugs Only SC-2 (2.0043 log CFU/unit) out of 10 samples of sugarcane juice mugs or jugs had *E. coli* found in them. The *E. Coli* in sandwich samples varied from 2 to 5.07 log cfu/unit. Of the ten sandwich samples. Among mixed salad Three samples of *E. Coli* showed no signs of growth. Among 10 samples of Fuchka samples (Tok/Chutney) The range of *Escherichia coli* log cfu/unit was 2.0 to 2.03. in 10 Samples of Fuchka spoons, The reported level of *Escherichia coli* was 2.30 log cfu/unit. Samples of Fuchka Plate/Cup The amount of *Escherichia coli* was 2.00 log cfu/unit.Found Only in Road-06, (FC-4) Sector 11 Uttara. among the ten Samples of Fuchka water Particularly at Uttara Sector 11, Road-06 (FC-4), 2.95 log cfu/unit of *Escherichia coli* was found. Among the ten Fuchka water samples.

In 10 samples of Aloevera Sharbat Juice Despite the identification of *Salmonella* spp., none of the samples showed signs of growth. Moreover Between 2.29 and 2.61 log cfu/unit of *Vibrio cholerae* were found.in the 10 samples of Sharbat Glass Aloevera, No samples include *Vibrio* or *Salmonella* types. Among 10 samples of Aloevera Sharbat mug/jug Despite the observation

of *Vibrio cholerae* and *Salmonella* spp., no growth was seen in any of the samples. Sugarcane Juice There were 2.05 to 2.70 log cfu/unit of *Salmonella* spp. There were 2.24 to 2.91 log cfu/unit of *Vibrio cholerae*. In 10 samples of Sugarcane Juice Glass samples *Salmonella* spp. was observed, Present only at Opposite of Mohakhali Bus Terminal (SC-3) with a value of 2.00 log cfu/unit. *Vibrio* spp are absent in all locations. *Salmonella* spp, *Vibrio* spp. were not detected in any of the samples, among 10 samples of sugarcane juice mug or jug samples. Between the ten sandwich samples, *Salominella* spp. varied from 2 to 4.04 log cfu/unit. The range of vibrio spp. was 2 to 5.3, log cfu/unit. mixed salad Among 3 samples *Salmonella* spp. *Vibrio cholera* no growth was detected. Fuchka samples (Tok/Chutney) *Salmonella* spp. was observed but no growth was detected in any sample. Vibrio cholerae. count Not detected. among the 10 samples of fuchka(tok/chutney). Between 10 Samples of Fuchka spoons Only 2.7 log cfu/unit of growth was seen in *Salmonella* spp. In Mirpur 2 (FC-2) at the Mirpur Central puja mandap. Although *Vibrio cholerae* were detected, no growth was discovered in any of the samples. *Salmonella* species are sampled in Fuchka water. Although the observation of *Vibrio cholerae*, no growth was seen in any of the samples. Among 10 samples of Aloevera Sharbat Juice The range of the *listeria* count was 2.05 to 2.96 log cfu/unit.

Furthermore, in our observation Aloevera Sharbat Glass none of the samples contain *Listeria* spp. Aloevera Sharbat mug/jug *Listeria*. was observed but no growth was detected in any sample. In 10 samples of Sugarcane Juice, Listeria. count ranged from 2.22 to 2.91 log cfu/unit. Besides, In Sugarcane Juice Glass samples *Listeria* spp. are absent in all locations.10 samples of Sugarcane Juice Mug or Jug was detected for *Listeria* spp. among 10 samples of sugarcane juice mug or jug samples. *Listeria* spp was observed in SC-4 (2.0086 log Cfu/unit) among 10 sugarcane juice mugs. Among ten Sandwich samples *Listeria* spp has no growth in all samples. Among three mixed salad samples, *Listeria* spp was only detected in the sample -03(mitali hotel) 2.69 log cfu/unit. Among 10 samples of Fuchka samples (Tok/Chutney) *Listeria* count ranged from 2.24 to 2.99 log cfu/unit besides Fuchka Spoon samples *Listeria*. count ranged from 2.13 to 2.60 log cfu/unit furthermore, Fuchka Plate/Cup samples *Listeria* spp. count ranged from 2.15 to 2.30 log cfu/unit.Lastly, Fuchka water samples. *Listeria*. count 2.18 log cfu/unit. Only detected in Beside of balur field, Lalbagh (FC-10).

According to Bangladesh food safety authority. for ready to drink juice items, for25ml of 5 samples *salmonella* or *vibrio cholerae*, *listeria* should be zero or absent. According to Bangladesh food safety authority, for fast food items, it indicates maximum allowance of units of e coli should be zero. If it crosses the limit then less than 10 in each 5 fast food

samples considered as the unsatisfactory from a satisfactory level. and marginally unacceptable. If all 5 sample has 10 *E.coli* or more than 10 than it is considered as the rejected for all parameters. Salmonella should be zero for maximum allowance of units. If it crosses the limit then less than 10 in each 5 fast food samples considered as the unsatisfactory from a satisfactory level. and marginally unacceptable. If all 5 sample has 10 *Salmonella* or more than 10 than it is considered as the rejected for all parameters.

Street food sellers are essential to people's ability to get and affordably prepare meals. They do, however, have particular difficulties with dealing with food safety. Street sellers frequently lack enough details about food safety, resulting in improper food handling procedures. Many operate their businesses in filthy circumstances, which puts customers in danger. Considering these difficulties, street food sellers offer a substantial economic contribution, particularly in developing nations where they sustain millions of people on low incomes. Around the world, street food may be found in exciting areas, busy corners, and bustling marketplaces. It is a unique and colorful component of urban life. However, eating food on the street may affect your health in both positive and negative ways. Cultural viewpoint Customers may sample a variety of local foods and flavors thanks to it. hazards to food safety Not all street food satisfies hygienic requirements. Foodborne infections can result from contaminated food. Absence of Regulations Street food sellers might not be subject to severe health inspections, in contrast to professional restaurants. Issues with Nutrition Certain snacks from the street are heavy in sugar, salt, and bad fats. pollutants While selecting products from the street food menu, customers with food allergies should use precautions. As a result, various foodborne diseases and large amounts of fecal indicator bacteria have been found in street snacks. Further research into the mechanisms underlying awareness & training of workers to maintain good personal hygiene is a simple and mitigate microbial level and improve the hygiene condition of street food vendors. To improve the hygienic state of street food personal hygiene is a straight forward answer.

References

Abdullah, D. (2022, March 30). Diarrhoea outbreak reported in several districts. *Dhaka Tribune*. https://www.dhakatribune.com/bangladesh/nation/266791/diarrhoea-outbreak-reported-in-several-districts

Abebe, E., Gugsa, G., & Ahmed, M. (2020). Review on major food-borne zoonotic bacterial pathogens. *Journal of tropical medicine*, *2020*.

Ackah, Michael. "Socioeconomic profile, knowledge of hygiene and food safety practices among street-food vendors in some parts of Accra-Ghana." *Internet journal of food safety* 13 (2011): 191.

Al Mamun, M., Rahman, S. M. M., & Turin, T. C. (2013). Microbiological quality of selected street food items vended by school-based street food vendors in Dhaka, Bangladesh. *International Journal of Food Microbiology*, *166*(3), 413-418.

Albuquerque, G., De Morais, I. L., Gelormini, M., Casal, S., Damasceno, A., Pinho, O., Moreira, P., Jewell, J., Breda, J., Lunet, N., & Padrão, P. (2019). Street food in Dushanbe, Tajikistan: availability and nutritional value. *British Journal of Nutrition*, *122*(9), 1052–1061. https://doi.org/10.1017/s0007114519001892

Ali, M. Y., Khan, M. R., & Saha, M. L. (2012). Antibiotic resistant patterns of bacterial isolates from ready-to-eat (RTE) street vended fresh vegetables and fruits in Dhaka City. *The Bangladesh Journal of Scientific Research*, *24*(2), 127–134. https://doi.org/10.3329/bjsr.v24i2.10769

Alter Chen, M. (2005). *Rethinking the informal economy: Linkages with the formal economy and the formal regulatory environment* (No. 2005/10). WIDER Research Paper.

Amare, A., Worku, T., Ashagirie, B., Adugna, M., & Dagnew, M. (2019). Bacteriological Q1 profile, antimicrobial 3 susceptibility patterns of the isolates 4 among street vended foods and hygienic 5 practice of vendors in Gondar town, 6 Northwest Ethiopia: a cross sectional study.

Aziz, M. A. (2020). *Hygiene Assessment Along the Bulk Food Packaging Bag Manufacturing Company of Bangladesh* (Doctoral dissertation, North South University).

Azman, A. S., Rudolph, K. E., Cummings, D. a. T., & Lessler, J. (2013). The incubation period of cholera: A systematic review. *Journal of Infection*, *66*(5), 432–438. https://doi.org/10.1016/j.jinf.2012.11.013

Baker, J. L. (2007). Bangladesh-Dhaka: improving living conditions for the urban poor.

Barro, N. (2006). *Hygienic status assessment of dish washing waters, utensils, hands and pieces of money from street food processing sites in Ouagadougou (Burkina Faso)*. https://www.ajol.info/index.php/ajb/article/view/42982

Barro, N., Razack, B. A., Yollande, I., Savadogo, A., Tidiane, O. C. A., De Souza Comlan, A., Sababénédjo, T. A., & Philippe, N. A. (2006). Street-Vended Foods Improvement: Contamination Mechanisms and Application of Food Safety Objective Strategy : Critical review. *Pakistan Journal of Nutrition*, *6*(1), 1–10. https://doi.org/10.3923/pjn.2007.1.10

Benjamin, E. (2011, July). Street Food Governance in Dhaka (Bangladesh): the appropriation of street vending spaces and the informal politics of exploitation. In *Proceedings of the International RC21 Conference*.

Begum, M. K. (2015). Preservation of sugarcane juice using herbal clarificant. *International Journal of Nutrition and Food Sciences*, *4*(5), 530. https://doi.org/10.11648/j.ijnfs.20150405.13

Bhowmik, S. (2012). *Street vendors in the global urban economy*. Taylor & Francis.

Birgen, B. J., Njue, L. G., Kaindi, D. M., Ogutu, F. O., & Owade, J. O. (2020). Determinants of microbial contamination of street-vended chicken products sold in Nairobi County, Kenya. *International journal of food science*, *2020*.

Biswas, S., Parvez, M. A. K., Shafiquzzaman, M., Nahar, S., & Rahman, M. N. (2010). Isolation and characterization of Escherichia coli in ready-to-eat foods vended in Islamic University, Kushtia. *Journal of Bio-Science*, *18*(1), 99-103.

Brown, A., Lyons, M., & Dankoco, I. (2010). Street traders and the emerging spaces for urban voice and citizenship in African cities. *Urban Studies*, *47*(3), 666-683.

Budiarso, T. Y., Amarantini, C., Prihatmo, G., Restiani, R., Putri, Y., Kindagen, V., & Linggardjati, S. (2021, March). Detection of Coliforms and Enteric Pathogens in Favorite Snack Food Sold in Yogyakarta City. In *7th International Conference on Research, Implementation, and Education of Mathematics and Sciences (ICRIEMS 2020)* (pp. 40-48). Atlantis Press.

CARIBBEAN REGIONAL WORKING GROUP ON STREET FOOD VENDORS. (n.d.). https://www.fao.org/3/ae973s/ae973s00.htm

Cross, J., & Morales, A. (Eds.). (2007). *Street entrepreneurs: People, place, & politics in local and global perspective*. Routledge.

Canet, C. (1997). L'Alimentation de Rue en Afrique. Revue "Aliments dans les villes", 2, 1-17.

Dahal, P. (2023, April 20). *Coliform bacteria- Characteristics, examples, identification*. Microbe Notes. https://microbenotes.com/coliform-bacteria/

Dhali, B. (2021, April 21). *Diarrhoea outbreak: 8 dead, thousands hospitalized across Barisal division*. https://www.dhakatribune.com/bangladesh/nation/244669/diarrhoea-outbreak-8-dead-thousands-hospitalized

Donkor, E. S., Kayang, B. B., Quaye, J., & Akyeh, M. L. (2009). Application of the WHO Keys of Safer Food to improve food handling practices of food vendors in a poor resource community in Ghana. *International Journal of Environmental Research and Public Health*, *6*(11), 2833–2842. https://doi.org/10.3390/ijerph6112833

E coli enteritis: MedlinePlus Medical Encyclopedia. (n.d.). https://medlineplus.gov/ency/article/000296.htm#:~:text=E%20coli%20is%20a%20type,severe%20%20case%20of%20food%20poisoning.

Faruque, Q., Haque, Q. F., Shekhar, H. U., & Begum, S. (2010). Institutionalization of healthy street food system in Bangladesh: A pilot study with three wards of Dhaka City Corporation as a model. *National Food Policy Capacity Strengthening Programme (NFPCSP)*, *7*, 1-84.

FAO. (n.d.). *The State of Food Insecurity in the World 2006 | Agrifood Economics | Food and Agriculture Organization of the United Nations*. https://www.fao.org/agrifood-economics/publications/detail/en/c/122052/

FAO (Food and Agriculture Organization of the United Nations) (1990). L'Alimentationde Rue en Afrique. Food and Agriculture Organization of the United Nations.

Ferrari, A. M., OLIVEIRA, J. D. S. C., & SÃO JOSÉ, J. F. B. D. (2021). Street food in Espírito Santo, Brazil: a study about good handling practices and food microbial quality. *Food Science and Technology*, *41*, 549-556.

Feglo, P. K., & Sakyi, K. (2012). Bacterial contamination of street vending food in Kumasi, Ghana. *ResearchGate*. https://doi.org/10.4314/jmbs.v1i1

Food and Agricultural Organization of the United Nations, "*Improving food safety, quality and food control in Bangladesh,"* Report—Seminar on Food Safety Challenges in Bangladesh, pp. 1–16, May 2010.

Food and Agriculture Organization of the United Nations. (2009). Good hygienic practices in the preparation and sale of street food in Africa. Retrieved from http://www.fao.org/3/a0740e/a0740e00.htm

FSHN033/FS099: General Overview of the Causative Agents of Foodborne Illness. (n.d.). Ask IFAS - Powered by EDIS. https://edis.ifas.ufl.edu/publication/FS099

Gabbey, A. E. (2019, March 8). *Salmonella food poisoning*. Healthline. https://www.healthline.com/health/salmonella-enterocolitis

Goswami, C., & Begum, R. (2016). A study of home-based enterprises and street vendors of Assam, India. *International Journal of Economic Policy in Emerging Economies*, *9*(3), 290-323.

Haq, J.A., Rahman, K.M., 1991. Campylobacter jejuni as a cause of acute diarrhoea in children: a study at an urban hospital in Bangladesh. J. Trop. Med. Hyg. 94, 50–54.

Hasan, S. M. T., Das, S., Faruque, A. S. G., Khan, A. I., Clemens, J. D., & Ahmed, T. (2021). Taking care of a diarrhea epidemic in an urban hospital in Bangladesh: Appraisal of putative causes, presentation, management, and deaths averted. *PLOS Neglected Tropical Diseases*, *15*(11), e0009953. https://doi.org/10.1371/journal.pntd.0009953

Henry, F.J., Huttly, S.R.A., Patwary, Y., Aziz, K.M.A., 1990. Environmental sanitation, food and water contamination and diarrhoea in rural Bangladesh. Epidemiol. Infect. 104, 253–259.

Hossain, K. (2023, September 20). Best Bangladeshi Fuchka Recipe - Cuisine Tutor. *Cuisine Tutor*. https://cuisinetutor.com/bangladeshi-fuchka-recipe/

Instructables. (2017, October 19). *How to make a perfect sandwich*. Instructables. https://www.instructables.com/How-to-make-an-amazing-sandwich/

International Food Safety Authorities Network (INFOSAN), 2010. Basic Steps to Improve Safety of Street-Vended Food. WHO, Geneva.

Islam, N. (2005). *Dhaka now: contemporary urban development*. Bangladesh Geographical Society.

Mensah, P., Yeboah-Manu, D., Owusu-Darko, K., Ablordey, A., 2002. Street foods in Accra, Ghana: how safe are they? Bull. World Health Organ. 80, 546–554.

Khairuzzaman, M., Chowdhury, F. M., Zaman, S., Mamun, A. A., & Bari, M. L. (2014). Food Safety Challenges towards Safe, Healthy, and Nutritious Street Foods in Bangladesh. *International Journal of Food Science*, *2014*, 1–9. https://doi.org/10.1155/2014/483519

Luby, S.P., Rahman, M., Hossain, M.J., Blum, L.S., Husain, M.M., Gurley, E., Khan, R.,Ahmed, B., Rahman, S., Nahar, N., Kenah, E., Comer, J.A., Ksiazek, T.G., 2006.Foodborne transmission of Nipah virus, Bangladesh. Emerg. Infect. Dis. 12,1888–1894.

Mixed Salad with Bangla Touch Recipe from Bangladesh. (n.d.). https://excitedfood.com/recipes/mixed-salad-with-bangla-touch

Mosupye, F.M., von Holy, A., 2000. Microbiological hazard identification and exposure assessment of street food vending in Johannesburg. Int. J. Food Microbiol.61, 137–145.

Muleta, D., Ashenafi, M., 2001. Salmonella, Shigella and growth potential of other food-borne pathogens in Ethiopian street vended foods. East Afr. Med. J. 78, 576–580.

Muinde, O.K. and Kuria, E. (2005) Hygienic and Sanitary Practices of vendors of street foods in Nairobi, Kenya. African Journal of Food Agriculture and Nutritional Development, 5, 1-15. - References - Scientific Research Publishing. (n.d.). https://www.scirp.org/reference/ReferencesPapers?ReferenceID=1965016

Murindamombe, G.Y., Collison, E.K., Mpuchane, S.F., Gashe, B.A., 2005. Presence of Bacillus cereus in street foods in Gaborone, Botswana. J. Food Prot. 68, 342–346

Nutrition, C. F. F. S. a. A. (2022, November 7). *BAM Chapter 18: Yeasts, Molds and Mycotoxins*. U.S. Food And Drug Administration. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-18-yeasts-molds-and-mycotoxins

Nyenje, M. E., Odjadjare, C. E., Tanih, N. F., Green, E., & Ndip, R. N. (2012). Foodborne Pathogens Recovered from Ready-to-Eat Foods from Roadside Cafeterias and Retail Outlets in Alice, Eastern Cape Province, South Africa: Public Health Implications.

International Journal of Environmental Research and Public Health, *9*(8), 2608–2619. https://doi.org/10.3390/ijerph9082608

Ohiokpehai, O. (2003). Nutritional aspects of street foods in Botswana. *Pakistan Journal of Nutrition*, *2*(2), 76–81. https://doi.org/10.3923/pjn.2003.76.81

Practices, knowledge and risk factors of street food vendors in Uganda. (2011, February 3). Practices, Knowledge and Risk Factors of Street Food Vendors in Uganda - ScienceDirect. https://doi.org/10.1016/j.foodcont.2011.01.016

Ram, P.K., Naheed, A., Brooks, W.A., Hossain, M.A., Mintz, E.D., Breiman, R.F., Luby, S.P., 2007. Risk factors for typhoid fever in a slum in Dhaka, Bangladesh. Epidemiol. Infect.135, 458–465.

Rane, S. (2011). Street vended food in developing world: hazard analyses. *Indian journal of microbiology*, *51*(1), 100-106.

Raza, J., Asmat, T. M., Mustafa, M. Z., Ishtiaq, H., Mumtaz, K., Jalees, M. M., ... & ur Rehman, H. (2021). Contamination of ready-to-eat street food in Pakistan with Salmonella spp.: implications for consumers and food safety. *International Journal of Infectious Diseases*, *106*, 123-127.

Roberts, K. (2008). *Food safety training and foodservice employees' knowledge and behavior*. https://www.semanticscholar.org/paper/Food-safety-training-and-foodservice-employees'-and-Roberts-Barrett/d9998354f40c22ba0f573ce13f8ca1a3bd146d4f

Saha, S.K., Saha, S., Shakur, S., Hanif, M., Habib, M.A., Datta, S.K., Bock, H.L., 2009. Community-based cross-sectional seroprevalence study of hepatitis A in

Bangladesh. World J. Gastroenterol. 15, 4932–4937

Saha, S. (n.d.). *This Aloe-Vera sharbat is a popular Bangladeshi drink, why you should try it too!* NDTV Food. https://food.ndtv.com/food-drinks/aloe-vera-benefits-this-aloe-vera-juice-is-a-popular-bangladeshi-drink-why-you-should-try-it-too-2179691

Salamandane, A., Silva, A. C., Brito, L., & Malfeito-Ferreira, M. (2021). Microbiological assessment of street foods at the point of sale in Maputo (Mozambique). *Food Quality and Safety*, *5*, fyaa030.

Sanders, E. R. (2012). Aseptic laboratory techniques: plating methods. JoVE (Journal of Visualized Experiments), (63), e3064.

Schelin, J., Wallin-Carlquist, N., Cohn, M. T., Lindqvist, R., Barker, G. C., & Rådström, P. (2011). The formation ofStaphylococcus aureusenterotoxin in food environments and advances in risk assessment. *Virulence*, *2*(6), 580–592. https://doi.org/10.4161/viru.2.6.18122

Sheikh, A., Sugitani, M., Kinukawa, N., Moriyama, M., Arakawa, Y., Komiyama, K., ... & Suzuki, K. (2002). Hepatitis e virus infection in fulminant hepatitis patients and an apparently healthy population in Bangladesh. *The American journal of tropical medicine and hygiene*, *66*(6), 721-724.

Sheth, M., R, G., & R, M. (2005). Identification of Hazards in the Street foods of Vadodara, India.*ResearchGate*.https://www.researchgate.net/publication/268983398_Identification_of_Hazards_in_the_Street_foods_of_Vadodara_India

Sonkar, P. K., & Kumar, N. (2022). PH determination of water sample using a pH meter. *ResearchGate*.https://www.researchgate.net/publication/362644467_pH_Determination_of_Water_Sample_Using_A_pH_Meter

Soula, A., Yount-André, C., Lepiller, O., & Bricas, N. (2020). Manger en ville. Regards socio-anthropologiques d'Afrique, d'Amérique latine et d'Asie. In *éditions Quae eBooks*. https://doi.org/10.35690/978-2-7592-3091-4

Sousa, S., Gelormini, M., Damasceno, A., Lopes, S. A., Chongole, C., Muholove, P. et al.(2019). Street Food in Maputo, Mozambique: Availability and Nutritional Value of Ho-memade Foods. Nutrition and Health, 25, 37-46. https://doi.org/10.1177/0260106018816427

Street foods in Accra, Ghana: how safe are they? - PubMed. (2002, January 1). PubMed. https://pubmed.ncbi.nlm.nih.gov/12163918/

Springer, K. (2022). 50 of the best street foods in Asia. *Cnn Travel*. https://www.cnn.com/travel/article/asia-best-street-foods-cmd

Tabashsum, Z., Khalil, I., Nazimuddin, M., & Bari, L. (2013). Prevalence of foodborne pathogens and spoilage microorganisms and their drug resistant status in different. . . *ResearchGate*. https://www.researchgate.net/publication/259708756_Prevalence_of_Foodborne_Pathogens_and_Spoilage_Microorganisms_and_Their_Drug_Resistant_Status_in_Different_Street_Foods_of_Dhaka_city

Tambekar, D., Murhekar, S., Dhanorkar, D. V., & Dudhane, M. N. (2009). Microbial Quality and safety of street vended fruit juices: A case study of Amravati city. *ResearchGate*.https://www.researchgate.net/publication/237236057_Microbial_Quality_and_safety_of_street_vended_fruit_juices_A_case_study_of_Amravati_city

T, C. A. (2018). *Agriculture, alimentation et emploi en Afrique de l'Ouest*. https://agris.fao.org/search/en/providers/122621/records/6473b99513d110e4e7ac31db

Teplitski, M., Barak, J. D., & Schneider, K. R. (2009). Human enteric pathogens in produce: un-answered ecological questions with direct implications for food safety. *Current Opinion in Biotechnology*, *20*(2), 166–171. https://doi.org/10.1016/j.copbio.2009.03.002

The State of Food and Agriculture 2007. (2007). In *The state of food and agriculture*. https://doi.org/10.18356/ce58efdc-en

The State of Food Insecurity in the World 2010. (n.d.). https://www.fao.org/3/i1683e/i1683e00.htm

The State of Food Insecurity in the World 2012. (n.d.). http://www.fao.org/docrep/016/i3027e/i3027e00.htm

Vaillant, V., De Valk, H., Baron, E. J., Ancelle, T., Colin, P., Delmas, M., Dufour, B., Pouillot, R., Strat, Y. L., Weinbreck, P., Jougla, É., & Desenclos, J. (2005). Foodborne infections in France. *Foodborne Pathogens and Disease*, *2*(3), 221–232. https://doi.org/10.1089/fpd.2005.2.221

Warde, A., & Martens, L. (2000). *Eating out*. https://doi.org/10.1017/cbo9780511488894

World Health Organization (WHO), 1996. Essential Safety Requirements for Street Vended Foods (Revised Edition). Food Safety Unit, Division of Food and Nutrition, WHO, Geneva

World Health Organization (WHO), 1999. Food Safety: An Essential Public Health Issue for the New Millennium. Food Safety Programme, Department of Protection of the Human Environment, Cluster of Sustainable Development and Healthy Environments, WHO, Geneva

World Health Organization: WHO & World Health Organization: WHO. (2023, December 11). *Cholera*. https://www.who.int/news-room/fact-sheets/detail/cholera

World Health Organization: WHO. (2018, February 7).*E. coli*.https://www.who.int/news-room/fact-sheets/detail/e-coli#:~:text=coli%20are%20harmless.,contaminated%20raw%20vegetables%20and%20sprouts

Zain, M. M., & Naing, N. N. (2002). Sociodemographic characteristics of food handlers and their knowledge, attitude and practice towards food sanitation: a preliminary report. *Southeast Asian journal of tropical medicine and public health*, *33*(2), 410-417.

.

Zaman, S., Ahmed, S., Kabir, M. R., & Bari, L. (2018). Microbiological risk assessment and simple cost-effective ways to reduce the risk in bulk food bags manufacturing company. *Journal of Food Safety*, *38*(5). https://doi.org/10.1111/jfs.12497

Appendices

Appendix I

Media Composition

The composition of the media used in the present study has been given below. Unless otherwise mentioned all media were autoclaved at 121 °C for 15 min.

1.0 The names and manufacturers of all Media that used.

Media/Broth	Manufacturer	Country
Tryptic Soy Agar	Oxoid	United Kingdom
Sorbitol MacConkey Agar (SMAC)	Condalab	Spain
Xylose lysine deoxycholate Agar	Himedia	India
Thiosulfate citrate bile salts sucrose (TCBS) Agar	Merck, KGaA	Germany
Tryptone Bile X-glucuronide(TBX) Agar	Oxoid	United Kingdom
Listeria Selective Agar	Oxoid	United Kingdom
Dichloran Rose Bengal Chloramphenicol Agar	Himedia	India
Tryptic Soya Broth	Difco	USA

2.0 Tryptic Soy Agar (TSA) 1000 ml

Ingredients	**(g/l)**
Casein Peptone	15.0 g
Soy Peptone	5.0 g
Sodium Chloride	5.0 g
Agar	15.0 g
Distilled water	1000 ml
Final pH 7.3 ± 0.2 at 25 °C	

3.0 Xylose Lysine Deoxycholate Agar (XLD) 1000 ml

Ingredients	**(g/l)**
Yeast extract	3.0 g
L-Lysine HCl	5.0 g
Xylose	3.75 g
Lactose	7.5 g
Sucrose	7.5 g
Sodium desoxycholate	1.0 g
Sodium chloride	5.0 g
Sodium thiosulphate	6.8 g
Ferric ammonium citrate	0.8 g
Phenol red	0.08 g
Agar	12.5 g
Distilled water	1000 ml
pH 7.4 ± 0.2 at 25°C	

4.0 Thiosulfate citrate bile salts sucrose (TCBS) Agar

Ingredients	**(g/l)**
Proteose Peptone	10.0 g
Sodium chloride	10.0 g
Yeast Extract	5.0 g
Sodium Thiosulfate	10.0 g
Sodium Citrate	10.0 g
Oxgall	8.0 g
Sucrose	20.0 g
Ferric Citrate	1.0 g
Bromo Thymol Blue	0.04 g
Thymol Blue	0.04 g
Agar	12.0 g
Distilled water	1000 ml
pH 7.5 ± 0.2 at 25°C	

5.0 Tryptone Bile Glucuronic Agar (TBX Agar)

Ingredients	**(g/l)**
Tryptone	20.0 g
Bile Salts No. 3	1.5 g
X-glucuronide	0.075.0 g
Agar	15 g
Distilled water	1000 ml
pH 7.5 ± 0.2 at 25°C	

6.0 Listeria Selective Agar 1000 ml

Ingredients	**(g/l)**
Columbia Blood Agar Base	39.0 g
Lithium chloride	15.0 g
Ferric ammonium citrate	0.5 g
Aesculin	1.0 g
Agar	15.0 g
Distilled water	1000 ml
pH 7.1 ± 0.2 at 25°C	

7.0 Dichloran Rose Bengal Chloramphenicol (DRBC) Agar Medium - 1000ml

Ingredient	Amount (g/l)
Glucose	10.0 g
Rose Bengal (5% soln., w/v)	0.5 ml
Dichloran (2,6-dichloro-4-nitroaniline) solution (0.2%(w/v) in ethanol)	1.0 ml
Chloramphenicol	0.1 g
Potassium phosphate, monobasic	1.0 g
Magnesium sulfate heptahydrate	0.5 g
Agar	15.0 g
Final pH 7.2±0.2 at 25º C	

8.0 MacConkey Sorbitol Agar (SMAC) 1000 ml

Ingredients	**(g/l)**
Peptone	17.0 g
Proteose peptone	3.0 g

D-Sorbitol	10.0 g
Bile salts mixture	1.5g
Sodium chloride	5.0g
Neutral red	0.03g
Crystal violet	0.001g
Agar	15.0 g
Distilled water	1000 ml
pH 5.6 ± 0.2 at 25°C	

9.0 Tryptic Soya Broth (TSB) 1000 ml

Ingredients	**(g/l)**
Tryptone	17.0 g
Soytone	3.0 g
Glucose (=Dextrose)	2.5 g
Sodium Chloride	5.0 g
Dipotassium Hydrogen Phosphate	2.5 g
Distilled water	1000 ml
pH 7.3 ± 0.2 at 25°C	

Appendix II

API kits, reagents and medium

1.0 API 20E Strep

Content of the kit (Kit for 25 tests)

- 25 API 20 E strips
- 25 incubation boxes
- 25 result sheets
- 1 clip seal
-1 Package insert

Composition of the medium

	L-cystine	0.5 g

API 20 Medium 2 ml	Tryptone (bovine/porcine origin)	20.0 g
	Sodium chloride	5.0 g
	Sodium sulfite	0.5 g
	Phenol red	0.17 g
	Demineralized water to make	1000 ml
	pH: 7.4 - 7.6	

Reagents / Instrumentation

- API® Suspension Medium, 5 ml
- Reagents: TDA

  JAMES

  VP 1 + VP 2

  NIT 1+NIT 2

-Zn reagent

-Oxidase

- Mineral oil
- McFarland Standard)
- API 20E Strep Analytical Profile Index or apiweb TM identification software

Appendix III

Apparatus used

Autoclave	ALP (Tokyo, Japan)
Class-2 A1/A2 biological safety cabinet	Thermo Pharma, USA
Drier	Memmert (UK)
Duran bottle	Duran (Germany)
Electric balance Model- AND HR-300	A&D Weighing (USA)
Eppendorf tubes	Eppendorf (Germany)
Freezer (-20 °C)	Thermo Ferma, USA
Fridge (4 °C) Model- W2D-2B0	WALTON (Bangladesh)
Fridge (4 °C) Model- LSC239CF	Kelvinator Plus (China)
Incubator (37 °C)	MMM Group (Germany)
Incubator (30 °C)	Memmart, Germany

Micropipettes (1000 µl)	BrandTech Scientific Inc. (Germany)
Micropipettes (100 µl)	Oxford Lab Products (USA)
Micropipette tips	Labsystems (Finland)
Microwave oven Model- R-380V(S)	SHARP (Thailand)
Stomacher 400 Circulator	Seward (UK)
Vortex Mixer Model- VM-2000	Digisystem Laboratory Intstrument Inc. (Taiwan)