Microbial quality of selected sandwiches sold at fast food shops in Dhaka city

A DISSERTATION SUBMITTED TO BRAC UNIVERSITY IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN MICROBIOLOGY

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DECLARATION

I hereby declare that the thesis project titled "**Microbial quality of selected sandwiches sold at fast food shops in Dhaka city"** submitted by me has been carried out under the supervision of Dr. M. Mahboob Hossain, Associate professor, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka. It is further declared that the research work presented here is based on actual and original work carried out by me. Any reference to work done by any other person or institution or any material obtained from other sources have been duly cited and referenced.

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Dedicated to……..

My Parents

Acknowledgement

The piece of work I accomplished in pursuance of my B.Sc. project happens to be the first undertaking of this nature I have ever been exposed to. It may be a small step as such but for me it was a great leap. I needed help and encouragement not to be frustrated in the event of repeated failures in my experiments. Fortunately there were people around me who provided the needed supports.

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Abstract

The project was designed to undertake a study on the microbiological status of the different types of sandwich e.g. egg salad sandwiches, chicken salad sandwich, beef sandwich and vegetable sandwich taken from seven different fast food shops located in different areas of Dhaka city. Both qualitative and quantitative microbiological analyses of seven sandwiches were done and microbes were identified by standard biochemical tests. The quantitative analysis and biochemical tests showed that the sandwich sample contained a number of microorganism of which 37 isolates identified. From the results of standard biochemical test for identification of all the 37 isolates from sandwich sample it has been observed that the sandwich sample contains *Alcalegenes sp. (2.7%) Bacillus sp (2.7%), Shigella sp*(2.7%), *Klebshiella sp (5.4%), Enterobacter sp* (10.5%), *Escherichia coli (10.8%)* and *Salmonella sp(10.8%), Vibrio sp (13.5%)* and *Saccharomyces sp(13.5%) and Staphylococcus sp(19%)* depending on the types of the sandwich. The highest found in beef sandwich (6.0×10^8 CFU/g) moderate in chicken salad sandwich (3.7×10^6 CFU/g) and egg salad sandwich (2.1×10^6 CFU/g) and small count in vegetable sandwich (2.3×10^4 CFU/mg). The degree of initial contamination in sandwich samples which may pose hazard to public health has been discussed. It was concluded that the hygienically maintained food retained the best quality attributes required for consumer's acceptability and safety.

Contents

LIST OF ABBREVIATIONS

^{0}C	Degree Celsius
min	Minute
sec	Second
h	Hour
d	Day
mg	Milligram
g	Gram
kg	Kilogram
pg	Picogram
fg	Femtogram
L	Liter
ml	Milliliter
µL	Microliter
mM	Millimolar
M	Molar
mm	Millimeter
µm	Micrometer
nm	Nanometer
OD	Optical density
e.g.	For example
et al.	And others
pH	Negative logarithm of hydrogen ion concentration

%	Percentage
bp	Base pair
rpm	Rotation per minute
UV	Ultra violet
spp.	Species
CFU	Colony forming unit

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1. Introduction

1.1 Introduction

Fast food or ready to eat food are the food which are easy to make and can be eaten in an easy manner or can be taken away. The consumption and uses of fast foods have currently become a vital part of convenient food preparation patterns all over the world including Bangladesh. The most popular fast foods in Bangladesh are Sandwich, burger, pizza, French fries and so on. The consumption of these ready-to-eat foods has been reported to be associated with serious health problems (Adams and Moss, 2000; FDA, 2000). Changes in life-style and food habits have been bringing about this evolving shift from traditional foods. People consciously or subconsciously make the decision to invest their time more in actions other than food preparation. Besides, consumers prefer to have certain readily available foods for which they do not possess the skill and or equipment to prepare. Among these fast foods Sandwich is one of the very popular choice of consumption because of its contents and easy to eat shape.

The disease causing agents spread by sandwich not only incapacitate large groups of people, but also sometimes result in serious disability and even death. The transmission of human diseases through food is a global problem, particularly in developing countries where gastrointestinal diseases are one of the most important causes of mobility and mortality. However, food habits adopted by populations may mitigate or increase the hazards (WHO, 1968; WHO, 1976). The above mentioned hazards can be minimized to a great extent simply by monitoring the microbiological quality of food e.g. sandwich and creating awareness among the people about the fundamental principles of sanitation and hygienic quality of foods.

Food-borne diseases and problems relating to the sanitary and microbiological quality of foods continue to be of major interest and concern in Bangladesh and other countries of the world. As sandwich is a very popular form of fast food in our country unhygienic sandwich poses a great threat to the health safety among the general people in Bangladesh mostly to the children. New problems have been created due to recent development the processing and handling of foods change of food habits and availability of convenience foods. Current consumer's oriented publicity on freshness of fast foods available for sale in stores has created general impression that age per se of a food is closely related to acceptability. The

question of dating and labeling or retail packages has been considered essential features of acceptable quality assurance and quality control programs. However, in Bangladesh such programs are still undeveloped, as little information is available on the microbiological aspects and keeping quality of commercially processed sandwich is under the prevailing conditions. The elements that are used to preparing these sandwich e.g. meat, raw vegetables, egg etc are mostly not hygienic and well maintained. Therefore, the present study was designed to determine the sanitary quality of commercial sandwich sold at retail outlets of fast food shops in Dhaka city of Bangladesh.

1.2 Problem statement and justification

Although governments throughout the world are attempting to improve the safety of the food supply, the occurrence of food borne disease remains a significant health issue in both developed and developing countries (WHO, 2011). The global incidence of food borne disease is difficult to estimate, but it has been reported that in 2005 alone 1.8 million people died from diarrheal diseases. A great proportion of these cases can be attributed to contamination of food and drinking water (WHO, 2011). In countries where fast food vending is prevalent, there is commonly a lack of information on the incidence of food borne diseases related to fast.

Vended foods (WHO, 2011). Due to lack of proper knowledge and guidance on fast food vending, vendors prepare their foods in explicitly unhygienic and sanitary conditions. Consumers who depend on such food are more interested in its convenience and usually pay little attention to its safety, quality, and hygiene.

It has been observed that fast food vendors in Dhaka practice minimal hygienic and sanitary practices. There lacks knowledge on the epidemiological importance and public awareness of fast food which hampers precise scientific approach of the food safety problem. It has been reported that varying levels of *coliforms*, *Staphylococcus aureus, Bacillus cereus and Clostridium perfringens*in food. However there were limited studies on specific hazards posed by microorganisms of public health concern in fast food. There is need to study the strain distribution and pathogen city of presumptive food pathogens and the relationship between their occurrence and the hygiene practices in Dhaka. This could reveal potential of food poisoning outbreaks relating to fast food consumption and relate this to handling practices through evaluation of virulence/pathogenicity of the micro organisms isolated from fast food matrices.

1.4 Illness caused by fast food consumption

Fast food poses a great threat to the human body. It may cause food borne illness as well as many life threatening diseases. Food borne illness (also food borne disease and colloquially referred to as food poisoning)is any illness resulting from the consumption of contaminated food, pathogenic bacteria, viruses, or parasites that contaminate food, as well as chemical or natural toxins such as poisonous mushrooms.

Symptoms vary depending on the cause, and are described below in this article. A few broad generalizations can be made, e.g.: The incubation ranges from hours to days, depending on the cause and on how much was consumed. The incubation period tends to cause sufferers to not associate the symptoms with the item consumed, and so to cause sufferers to attribute the symptoms to stomach for example. Symptoms often include vomiting, fever, and aches, and may include diarrhea. Bouts of vomiting can be repeated, with an extended delay in between, because even if infected food was eliminated from the stomach in the first bout, microbes (if applicable) can have passed through the stomach into the intestine, attached to the cells lining the intestinal walls, and begun to multiply there. Some types of microbes stay in the intestine, some produce a toxin that is absorbed into the bloodstream, and some can directly invade deeper body tissues.

1.4Overall objective

The overall objective of this study was to characterize the microbial load and diversity of selected food borne microbes in fast food, and relate them with hygienic practices in Dhaka, Bangladesh.

2. Literature review

2.1 Fast food

Food and green groceries are available on the street for a fraction of the cost in a restaurant or a supermarket (FAO, 2007).This food is termed as 'fast food' and the consumption is common among those in a search of eating tasty ready to eat food(Mensah et al. 2002). Fast food is the term given to food that is prepared and served very quickly, first popularized in the 1950s in the United States. While any meal with low preparation time can be considered fast food, typically the term refers to food sold in a restaurant or store with preheated or precooked ingredients, and served to the customer in a packaged form for take-out/take-away. Fast food restaurants are traditionally separated by their ability to serve food via a drive-through. The term "fast food" was recognized in a dictionary by Merriam in 1951.

Outlets may be stands or kiosks, which may provide no shelter or seating, or fast food restaurants (also known as *quick service restaurants*). Franchise operations that are part of restaurant chains have standardized foodstuffs shipped to each restaurant from central locations. Fast food is obtainable from a street side vendor, often from a makeshift or portable stall (FAO, 2007). Some fast foods are regional, while others have spread beyond their region of origin (FAO, 2007). The food and green groceries sold in farmers' markets may also fall into this category, including the food exhibited and sold in fairs such as agricultural show and state fair (FAO, 2007). Most fast foods are both finger and fast food. Finger food is food eaten directly using the hands, in contrast to food eaten with a knife and fork, chopsticks, or other utensils (Kay, 1999). Fast food is food that can be quickly prepared and served (Jakle, 1999).

2.2 Importance of fast food in urban areas

In developing countries, a large proportion of ready to eat foods are sold on the street (Mensah et al. 2002). According to the Food and Agriculture Organization, 2.5 billion people worldwide eat fast food every day (FAO, 2007).Increased reliance of fast food has been identified as one of the characteristics of urban food distribution systems driven by changes in the urban way of life and poverty in developing countries (FAO, 1998).Fast foods have already become a common feature of urban life (Hilda, 2002). The increasing poverty and time constraints to survive in developing countries indicate that the fast food phenomenon

will only increase (Hilda, 2002). With the increasing pace of globalization and tourism, the safety of fast food has become one of the major concerns of public health, and a focus for governments and scientist to raise public awareness of food (FAO, 2007)

2.2.1 Nutritional benefits

The fast food industry plays an important role in developing countries in meeting the food demands of the urban dwellers (Latham, 1997). Fast foods play significant nutritional role for consumers, particularly for middle and low-income sectors of the population, who depend on fast foods for their main food intake (Mensah et al. 2002; Dardano, 2003). FAO reports that fast foods provide nutritionally balanced diets, sufficient in quantity and presenting options for variety and choice for consumers, particularly from middle and low-income sectors of the population, who depend heavily on them (FAO, 1997).

The contribution to the daily food intake of urban dwellers is scarcely quantified in energy and nutrients (Hilda, 2002).The foods have been shown to contribute a substantial proportion of the daily requirement of energy and protein (25%-50%) for adolescents attending schools (Oguntona and Kanye, 1995) and in Bangladesh, urban construction workers in Dhaka (Korir et al. 1998) and Calcutta street traders (Chakravarty and Canet, 1996). Their nutritional value however depends on the ingredients used and how they are prepared, stored and sold (Owusu-Darkoand Ablordey, 2002).

2.2.2 Economic benefits of fast food

The fast food industry offers a significant amount of employment, often to persons with little education and training (Latham, 1997).Fast food in Dhaka provides a substantial amount of income for most vendors, with most of them earning an income above the official minimum wage while some of them earn twice or more of this amount (Mwangi, 2002). Fast food operations sometimes involve the entire family in the procurement of raw materials, preparation and cooking of the meals (Mensah et al.2002). The role of women in the sector is significant, as they control a large share of market activity and commodity trading (Mensah et al.2002). Fast food vendors benefit from a positive cash flow, often evade taxation, and can determine their own working hours (Mensah et al.2002). In selling snacks, complete meals, and refreshments at relatively low prices, they provide an essential service to workers,

shoppers, travelers, and people on low incomes. However, the people who depend on such food are often more interested in its convenience than in questions of its safety, quality and hygiene (Mensah et al.2002; Muinde and Kuria, 2005).

2.4 Microbial safety of foods

2.4.1 Food borne diseases

The global incidence of food borne disease is difficult to estimate, but it has been reported that in the year 2000 alone 2.1 million people died from diarrhea diseases (WHO, 2011). Unsafe food causes many acute and life-long diseases, ranging from diarrheal diseases to various forms of cancer (WHO, 2011). WHO estimates that food borne and waterborne diarrheal diseases taken together kill about 2.2 million people annually, 1.9 million of them children (WHO, 2011). The risk of serious food poisoning outbreaks linked to fast foods remains a threat in many parts of the world, with microbiological contamination being one of the most significant problems (FAO, 1998). Food-borne pathogens are recognized as a major health hazard associated with fast foods, the risk being dependent primarily on the type of food and the method of preparation and conservation (FAO, 1998; FAO/WHO 2005). In Bangladesh, incidences of food borne disease outbreaks have been reported each year (MOH, 2003).

Pathogenicity and virulence of an organism are regulated by virulence coding genes present in the genomic regions known as pathogenicity islands (Hacker and Kaper, 2000). *Staphylococcus aureus* is one of the most prevalent pathogens causing several outbreaks (Veras et al. 2008). *Staphylococcus aureus* is a gram positive, catalase and coagulase positive microorganism(Veras et al. 2008). Contamination of food with enterotoxigenic *Staphylococcus aureus* causesstaphylococcal enterotoxins (SEs) intoxication hence the associated symptoms like vomiting and diarrhea. Major serological enterotoxins that have been characterized are: SEA, SEB, SEC, SED, and SEE (Robbins et al. 1977)and recently SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, and SEU (Letertre et al. 2003*;* Yarwood et al. 2002).SEA is the most common SE associated with food borneoutbreaks followed by SED. However, the type of SE is not relevant because SEs is very similar in structure and function (Balaban and Rasooly, 2000). Shiga toxin-producing *Escherichia coli* are a group of bacteria strains capable of causing significant human disease (Richard, 1999). The pathogen is transmitted primarily by food (Richard, 1999). The subgroup enterohaemorrhagic *E. coli* includes the relatively important serotype O157:H7, and more than 100 other non-O157 strains (Richard, 1999). Infection is transmitted primarily by food and less commonly by direct contact or water (Richard, 1999). Shiga toxin is a family of toxins produced by a variety of organisms, including *Shigella dysenteriae* type I and Shiga toxin-producing *Escherichia coli.* These toxins have a cytotoxic effect on intestinal epithelial cells that probably causes the characteristic bloody diarrhea (Richard, 1999). Laboratory identification of *E. coli* O157:H7 is easily performed using specialized media but identification of non-O157 Shiga toxin-producing *Escherichia coli* strains requires detection of the Shiga toxin gene by polymerase chain reaction or DNA probe-for virulence genes *stx1, stx2 and eae*(Richard, 1999).

In 2004, *Enterococcus*genustook the place of faecal coliforms as the new federal standard for water quality and public beaches in Hawaii USA. It provides a higher correlation than faecal coliforms with many of the human pathogen often found in city Dhaka (Jin et al. 2004). Enterococci however do not multiply in water especially in low organic matter. They are less numerous than *Escherichia coli* (James et al. 2005).

2.4.2 Microbial food safety of fast foods

A lack of knowledge among fast food vendors about the causes of food-borne disease is a major risk factor (FAO, 1998). Poor hygiene, inadequate access to potable water supplyand garbage disposal, and unsanitary environmental conditions such as proximity to sewers and garbage dumps further exacerbate the public health risks associated with street foods (FAO, 1998). Traditional processing methods that are used in preparation, inappropriateholding temperatures and poor personal hygiene of food handlers are some of the main causes of contamination of street-vended food (Mensah et al. 2002; Barro *et al.* 2006). Recent studies have indicated that ready to eat foods and food preparation surfaces may be reservoirs for microbial contamination (Mankee *et al.*2005; Ghosh *et al.*2007; Christison *et al.*2008). Fast foods in some Asian countries have been tested for various microorganisms of public health concern, including fecal coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* species and *Bacillus cereus*(FAO/WHO 2005). *Escherichia coli* and *Staphylococcus aureus* were recovered in a significant proportion of the food, water, hand and surface swabs tested in Bangladesh(FAO/WHO, 2005). Fast foods can also be sources of several groups of enteropathogens (Mensah et al.2002).

2.5 Epidemiological importance of microbial food borne disease in fast foods

Despite the availability of food safety strategies for public health and economic development in many countries, food safety policies, plan of action and legislation have not been implemented especially in developing countries (Anonymous, 2001). In recent times, food safety issues have assumed a wider dimension because of the reliance on fast food whose preparation the consumer has no control over. In the busy way of life today, people eat more meals outside their homes. In developing countries a large portion of ready to eat is sold on the streets. If this food is not handled hygienically or not stored at the right temperature, food borne illnesses are bound to occur (Anonymous,2001).All age groups consume fast foods in Asia (FAO/WHO,2005). However, there may be differences in the type of client depending on locality (Mensah *et al*.2002). While it is often thought that children under five years of age are fed at home, Mensah *et al*.(2002) observed that many mothers working at the markets also bought some food items from vendors to feed their babies. This has serious implications on the health of the children (FAO/WHO, 2005).Mahale et al.(2008) cited documented outbreaks of illnesses in humans associated with the consumption of street vended foods.

2.6 Gaps in knowledge on fast food safety

There is a lack of knowledge in microbial safety, pathogenicity, and strain distribution of fast food microorganisms. The role of hygiene practices or their lack in occurrence of possible food borne pathogens in Dhaka fast foodies not clear. The possible impact of handling practices in fast foods in Dhaka on human health remains unclear.

3. Materials and Methods

3.1 Working laboratory

Overall research was performed in the Microbiology UB10th floor laboratory and Microbiology research UB 18th floor Laboratory, Department of Mathematics and Natural Sciences , BRAC University from February 2014 to June 2014.7

3.2 Sources, collection and transportation of samples

Seven different brands of sandwiches available at retail stores were selected for this study. These brands belonged to

(i) Solna

(ii) BRACU Cafeteria

(iii) Hossain bakery

(iv) Star Kabab and restaurant.

(v) Milton

(vi) Cinnamon

(vii) Best Bites

Each brand of samples for this study was either wrapped or unwrapped. All these types are of two categories, pre-microwave oven (fresh sample from retail outlets) and post-microwave oven treated prior to sale or offered to customers. These sandwiches were prepared almost entirely using open hand and the ingredients used for their preparation did not receive any heat treatment or otherwise processed. The ingredients were bread, meat e.g. chicken and beef, egg, raw vegetables, fish, tomato sauce and mayonnaise. The food items collected for this study were carefully handled and transported to the laboratory in aseptic condition. Due aseptic care was taken during transportation and the samples were kept cool until they were subjected to bacteriological analysis.

3.3 Preparation of agar media plates and enrichment broth

Large and small agar plates were sterilized in hot air oven. Respective media recipe ingredients for agar medium were chosen and mixed in respective portion. For the agar medium the solvent that was used is distilled water. Then the agar media were mixed properly by applying heat and then were being autoclaved at 121^{o}C for 15 munities for sterilization. The hot agar media was then poured into agar plates inside laminar airflow and cooled down to 20-25^{o} C. All the agar plates were kept at 4^{o}C for further use.

As enrichment medium Alkaline peptone for enrichment of the *Vibrio* species and Selenite Broth for enrichment of *Salmonella* and *Shigella* species were made.

3.4 Preparation of samples

Portions of all food samples were uniformly homogenized in food grinder. The grinder was pre washed with boiled water and then properly cleaned with 70% ethanol solution. Three of 50ml beaker was taken and 9 ml of normal saline, 9 ml of Selenite broth and 9 ml of alkaline peptone water was poured into each beaker. Quantity of 1gm grinded sample of each sandwich was taken aseptically with a sterile forceps and transferred carefully into each of the beaker. Thus 1:1 dilution of the samples was obtained. Then on using the vortex machine the mixture was mixed. Later different serial dilutions ranging from 10^{-2} to 10^{-6} were prepared according to the standard method (ISO, 1995). The enrichment media mixture (Alkaline peptone water and Selenite Broth) were put into 36^{o} C incubator for four hours.

All the samples were studied in Quantitative and Qualitative method

3.5 Detection of the presence of total coliform count

For the determination of total coliform count 100 µl of each tenfold diluted sample was transferred to MacConkey agar plate. For each dilution six test plates containing MacConkey agar were used. The spread plate technique was done. All the agar plates were incubated at 37^{o}C temperature for 24 hours and 48 hours respectively. Pink or red and colorless colonies were observed on MacConkey agar media The total coliform count was calculated according to ISO (1995). The results of the total coliform count were expressed as the number of organism or colony forming units per gram (CFU/gm) of sandwich sample.

3.6 Detection of the presence of total Gram-Negative bacteria count

For the detection of total coliform count 100 µl of each tenfold diluted sample was transferred to Eosin Methylene Blue (EMB, also known as "Levine's formulation") agar plate. For each dilution six test plates containing EMB agar were used. Spread plate method was done. Eosin-methylene blue (EMB) agar plate inoculated with *Escherichia coli* (a gram-negative coliform bacterium) showing good growth of dark blue-black colonies with metallic green sheen indicating vigorous fermentation of lactose and acid production which precipitates the green metallic pigment. The agar plate inoculated with *Enterobacter aerogenes* (a gram-negative coliform bacterium) showing good growth of brown, dark-centered, mucoid colonies indicating lactose fermentation and acid production. Again, this agar plate inoculated with *Klebsiella pneumoniae* (a gram-negative coliform bacterium) showing good growth of brown, dark-centered, mucoid colonies (smaller than *Enterobacter*) indicating lactose fermentation and acid production. However, this agar plate inoculated with *Pseudomonas aeruginosa* (a gram-negative noncoliform bacterium) showing good growth via yellowish colonies but no fermentation of sugars or acid production. Inoculated with *Proteus vulgaris* (a gram-negative coliform bacterium) showing growth of pink colonies indicating non-lactose fermentation and some acid production and inoculated with a mixed culture of *Escherichia coli* and *Pseudomonas aeruginosa*. Note the metallic green sheen of the strong lactose-fermenting *Escherichia coli* and the pinkish colonies of nonfermenter *Pseudomonas aeruginosa.*On the other hand this agar plate inoculated with *Acinetobacter baumannii* (a gram-negative non-glucose-fermenting bacillus) showing a colony with a classic blue-grey center. This should not be mistaken for evidence of lactose fermentation on EMB agar. Lastly, EMB agar plate inoculated with *Salmonella enteritidis* (a gram-negative coliform bacterium) showing good growth of grey mucoid colonies with no fermentation of lactose or acid production.

All the agar plates were incubate at 37^{o}C bacterial temperature for 24 hours and 48 hours according to need. The total Gram-negative bacterial count was calculated according to ISO (1995). The results of the total Gram-negative bacterial count were expressed as the number of organism or colony forming units per gram (CFU/gm) of sandwich sample.

3.7 Detection of the presence of total fungal count

For the detection of total fungal count 100µl of each tenfold diluted sample was transferred to Sabouraud Dextrose Agar (SDA) agar plate. For each dilution six test plates containing SDA were used. The spread plate technique was done. All the agar plates were incubated at 25-27^{o}C temperature for 24 hours and 48 hours respectively. White, black, yellowish, branched colonies were observed on SDA media The total fungal count was calculated according to ISO (1995). The results of the total fungal count were expressed as the number of organism or colony forming units per gram (CFU/gm) of sandwich sample.

3.8 Detection of the presence of total Gram-Positive bacteria count

For the detection of total fungal count 100 µl of each tenfold diluted sample was transferred to Mannitol salt agar (MSA) agar plate. For each dilution six test plates containing MSA were used. The spread plate technique was done. All the agar plates were incubated at 37^{o}C temperature for 24 hours and 48 hours respectively. The color of this media is red. The following changes is shown if any of the following bacterial growth is found.

- Gram + *staphylococcus* : fermenting mannitol: Media turns yellow (ex. S. aureus)
- Gram + *staphylococci* : not fermenting mannitol. Media does not change color (ex. S. epidermidis)
- Gram + *streptococci* : inhibited growth
- Gram - : inhibited growth

Mainly white and yellowish colonies were observed on MSA media. The total Gram-Positive bacteria count was calculated according to ISO (1995). The results of the total Gram-Positive bacteria count were expressed as the number of organism or colony forming units per gram (CFU/gm) of sandwich sample.

3.9D Detection of the presence of presence of *Vibrio* species

For the detection of the presence of Vibrio Species 100µl of enriched media and sample mixture from Alkaline peptone water was transferred to one plate of Thiosulfate-citrate-bile salts-sucrose (TCBS) agar. The Color of this media is deep green. The spread plate technique

was done. The agar plate was incubated at 37°C temperature for 24 hours and 48 hours as needed. If the *vibrio* species is present such as *V. cholerae* then it ferment sucrose, this result in a pH shift and production of yellow-brown colonies. By the presence of the Vibrio species the media color is usually turned into yellow. The result of the total *Vibrio* Specis count were expressed in colony formation and color changing capability of the media of the sandwich sample.

3.10 Detection of the presence of *Salmonella* and *Shigella* Species

For the detection of the presence of *Salmonella* and *Shigella spp* 100 µl of enriched media and sample mixture from Selenite broth was transferred to one plate of xylose lysine deoxycholate (XLD) agar. It has a pH of approximately 7.4, leaving it with a bright pink or red appearance due to the indicator phenol red. The spread plate technique was done. The agar plate was incubated at 37°C temperature for 24 hours and 48 hours respectively. Sugar fermentation lowers the pH and the phenol red indicator registers this by changing to yellow. Most gut bacteria, including *Salmonella*, can ferment the sugarx ylose to produce acid; *Shigella* colonies cannot do this and therefore remain red. After exhausting the xylose supply*Salmonella* colonies will decarboxylate lysine, increasing the pH once again to alkaline and mimicking the red *Shigella* colonies. Salmonellae metabolise thiosulfate to produce hydrogen sulfide, which leads to the formation of red colonies with black centers and allows them to be differentiated from the similarly coloured *Shigella* colonies.Other Enterobacteria such as *E. coli* ferment the lactose and sucrose present in the medium to an extent that prevent pH reversion by decarboxylation and acidify the medium turning it yellow.

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

The result of the total *Salmonella* and *Shigella* Specis count were expressed in colony formation, colony color and color changing capability of the media.

3.11Detection of the presence of other bacterial Species

For the detection of the presence of other bacterial Species HiCrome media which is a chromogenic agar. HiCrome ECC selective agar is a selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in food samples. The chromogenic mixture contains two chromogenic substrates The enzyme _-galactosidase produced by coliforms cleaves the chromogen resulting in the salmon to red colouration. The enzyme _-glucuronidase produced by *Escherichia coli,* cleaves X-glucuronide. Colonies of *Escherichia coli* are dark blue to violet coloured due to cleavage of both the chromogen. The addition of L-tryptophan improves the indole reaction, thereby increasing detection reliability. Cefsulodin, (FD190) when added inhibits *Pseudomonas* and *Aeromonas species.* This agar media is easy to use several bacteria species simultiniously.

For the detection of the presence of many species 100 µl of sandwich sample mixture from10^{-1} was transferred to one plate of HiCrome media. The spread plate technique was done. The agar plate was incubated at 37^{o}C temperature for 24 hours and 48 hours respectively. The organisms shows different colors in the Hicrome chromogenic agar media. The colors specification foe different organisms are below

Name of the Organism	**Color in HiChrome chromogenic agar**
Enterococcus faecalis……	blue/blue-green
Escherichia coli……	purple
Klebsiella pneumoniae……	rose pink
Pseudomonas aeruginosa……	greenish pigment
Staphylococcus aureus……	golden yellow
Salmonella typhi……	colourless
Salmonella serotype Enteritidis……....	light greenish blue

The result of the total isolates were expressed in colony formation and color changing of the colonies of the bacteria of the sandwich sample.

3.12Detectionof total bacteria count

For the determination of total bacterial count 100 µl of each tenfold dilution was transferred to nutrient agar (NA) plate. For each dilution six test plates containing NA agar were used. The spread plate technique was done. All the agar plates were incubated at 37^{o}C temperature for 24 hours and 48 hours respectively. White, yellowish white, off white colonies were observed on nutrient agar media the total bacterial count was calculated according to ISO (1995). The results of the total bacterial count were expressed as the number of organism or colony forming units per gram (CFU/gm) of sandwich sample.

3.13 Long-term preservation

For long-term preservation, 500 µl of bacterial culture grown in Trypticase Soy Broth (Oxoid, England) at 37^{o}C for 6 hours was taken in a sterile cryovial. Then 500 µl of sterile glycerol was added to the broth culture and the cryovial was stored at -20^{o}C

3.14 isolates from spread plates

For isolating isolation from each spread plates one colony were taken from the one of the dilution of one of the spread plate and were streak in the Nutrient agar plate using four way streaking. All the sample plates were incubated in 37^{o}C for 24 hours and then preserved in 4^{o}C.

3.15 Microscopic observation of isolates

For evaluation of microscopic character, pure colony of each isolates was picked and Gram staining was performed according to Hacker's modified method (Doetsch, 1981). The size, shape, arrangement and Gram reaction properties of isolates were carefully observed in a microscopic field.

2.16 Biochemical Identification

Biochemical tests were performed according to the methods described in Microbiology Laboratory Manual (Cappuccino *et al*., 2005). The biochemical tests carried out were

1) Nitrate reduction test
2) Triple sugar iron test
3) Catalase test
4) Oxidase test
5) Carbohydrate fermentation (Dextrose, Sucrose, Lactose) test
6) Citrate utilization test
7) Motility test
8) Urease activity test
9) Methyl-red test
10) Voges- Proskauer test

Nitrate reduction test

1. Nitrate broth was inoculated with an isolate from each sample plates and incubate for 48 hours.
2. Then reagent A and reagent B were mixed carefully. If the bacterium produces nitrate reductase, the broth will turn a deep red within 5 minutes at this step.
3. If no color change is observed, then the result is inconclusive. Add a small amount of zinc was added to the broth. If the solution remains colorless, then both nitrate reductase and nitrite reductase are present. If the solution turns red, nitrate reductase is not present.

Triple Sugar Iron (TSI) Test

1) To inoculate, isolated colony from the respective agar plate was picked with a cool, sterile needle, stabbed into the TSI, (Himedia, India) containing dextrose, lactose and sucrose butt.
2) Incubated with caps loosened at 37°C for overnight and examined after 24 hours for carbohydrate fermentation, CO_2 and H_2S production.

3) A yellow (acidic) color in the butt indicated that the organism being tested capable of fermenting all the three sugars, whereas red (alkaline) color in the slant and butt indicated that the organism being tested is a non fermenter.
4) Detection of H_2S production identified by black precipitation in the butt of the tube.
5) CO_2 Gas production was indicated by splitting and cracking of the medium.

Catalase Test

1) A small amount of bacterial colony was transferred from therespective agar plate to a surface of clean, dry glass slide using a clean toothpick.
2) A drop of the catalase reagent (Hydrogen Peroxide) was placed on to the slide and mixed.
3) A positive result gave a rapid evolution of oxygen within 5-10 seconds and was evidenced by bubbling reaction.
4) A negative result showed no bubbles or only a few scattered bubbles.

Oxidase Test

1) A loopful of bacteria from the Nutrient agar plate was streaked onto a piece of filter paper (Whatman, 1MM).
2) A few drops of oxidase reagent (*N,N,N′,N′*-tetramethyl-*p*-phenylenediamine) were added onto the streaked bacteria on the filter paper.
3) Positive reactions turned the bacteria from violet to purple within 1 to 30 seconds. Delayed reactions should be and was ignored.

Carbohydrate fermentation (Dextrose, Sucrose, Lactose)Test

1) The Durham tubes were inserted in an inverted position into all the tubes, fully filled with broth (lactose, dextrose and sucrose)
2) Each labeled carbohydrate broth (lactose, dextrose and sucrose) was inoculated aseptically with each of the seven bacterial cultures.
3) After inoculation into a particular sugar, the loop was sterilized in order to avoid cross contamination of the tube with other sugars.
4) The tubes were incubated for 24 hours at $37^{o}C$.
5) Following incubation, the tubes showed either of the results: Acid production, Acid and gas production or no fermentation at all.

6) The presence of acid and gas changes the medium into a yellow color indicating a positive result.
7) Gas production can be detected by the presence of small bubbles in the inverted durham tubes.
8) The broth retaining the red color is an indication of the absence of fermentation.

Citrate utilization Test

1) Colorless bacterial colonies were picked from the respective agar plate by a straight wire and inoculated into the slope of Simmon's citrate agar (Oxoid ltd, England) and incubated overnight at 37^{o}C.
2) If the organism had the ability to utilize citrate, the medium changed its color from green to prussion blue; a negative slant would have no growth of bacteria and would remain green.

Motility test (Indole activity test)

1) The test was carried out in motility indole urea semisolid medium.
2) One suspected isolated colony was touched with a straight wire stabbed carefully into down the tubes without touching the bottom.
3) Following incubation, the tubes are observed for the presence of motile organisms which will disperse through the medium leaving the stab line spread and made the tube turbid.
4) Production of cherry red reagent layer after addition of Kovac's reagent in MIU medium demonstrates that the substrate tryptophan has been was hydrolyzed which indicates indole positive reactio

Ureas activity test

1) Inoculate the urea broth with the inoculation loop containing the organism from the tryptic soy broth culture.
2) Incubate for 24-48 hours at 37°C.
3) Take out a loopful organism from the tryptic broth culture tube with the cooled loop aseptically.
4) Take a sterile urea slant tube remove the cap and flame the neck of the tube.

5) Inoculate the entire surface of the urea slant (slope) with the provided growth from the tryptic broth culture using the inoculating loop (do not stab the butt). The slant of the medium is inoculated by streaking the surface of the agar in a zigzag manner.
6) Tighten the cap and incubate at 37°C for 24-48 hours.
7) Obtain the tubes from the incubator and observe the colour change.
8) If the color turns pink that means it's urease positive

Methyl red (MR) Test

1) The bacterium to be tested was inoculated into potassium phosphate broth (MR-VP broth), which contained dextrose, peptone and potassium phosphate and incubated at 37^{o}C for 24 hours.
2) Over the 24 hours the mixed-acid producing organism might produce sufficient acid to overcome the phosphate buffer and remained acidic.
3) The pH of the medium was tested by the addition of five drops of MR reagent. Development of red color was taken as positive. MR negative organism produced yellow color.

Voges-Proskauer Test

1) Bacterium to be tested was inoculated into potassium phosphate broth (MR-VP broth) and incubated for 24 hours.
2) Barritt's reagent A was added to the test broth and shaken.
3) Barrit's reagent B was added and the tube was allowed to stand for 15 min.
4) Appearance of red color was taken as a positive test, negative tube might be held for an hour after addition of reagents.

4. Results

The chief purpose of microbiological examination sandwich are to give assurance that the sandwich will be acceptable from the public health standpoint and that the sandwich will be of satisfactory quality, i.e., will consist of good original materials that have not deteriorated or become unduly contaminated during processing, packaging, storage, handling or marketing. The practice that has been in effect for many years and continues to be followed is to determine the sanitary quality of foods by their content of certain indicator organisms and pathogens. The present study is therefore undertaken to determine the total viable bacterial load, the presence of coliforms, Gram-Negative and gram positive bacteria, Fungi load, *Vibrio* *Salmonella* and *Shigella* and detection of staphylococci in fast foods sample.

Both Qualitative and quantitative results were observed and all the results were input accordingly.

For the Quantitative analysis and observation Nutrient Agar (NA), McConkey Agar (MAC), Eosin Methylene Blue (EMB), Sabouraud Dextrose Agar (SDA) and Mannitol salt agar (MSA) agar plates were used.

For the Qualitative analysis and observation Thiosulfate-citrate-bile salts-sucrose (TCBS) agar, Xylose lysine deoxycholate (XLD) and chromogenic HiCrome agar plates were used.

For the Qualitative analysis spread plate technique were done from each of the 10^{-1} to10^{-6} dilution to each agar media plates. It has been seen that because of the too much microbial count in the higher dilution the microbial colony count were too numerous to count or TNTC. Thus the determination of the quantitative analysis was impossible to interpret. To avoid this problem the best colony count and Colony formation unit (CFU) from the suitable dilution were taken and then the result was interpreted accordingly.

All the agar media were kept at 37^{o} C for 24-48 hours and then the colony count were taken.

Table-4.1 Quantitative microbial analysis of all the unwrapped sandwich samples

Sample Number	Types of Sandwich	Colony forming unit/ml in qualitative specific agar media				
		NA CFU/ml	MAC CFU/ml	EMB CFU/ml	MSA CFU/ml	SDA CFU/ml
S1	Chicken salad	3.7×10^{6}	NG	NG	1.2×10^{6}	1.7×10^{6}
S2	Egg salad	2.1×10^{6}	NG	NG	2.0×10^{4}	11.5×10^{6}
S3	Chicken salad	6.3×10^{6}	1.0×10^{4}	5.0×10^{4}	1.7×10^{6}	1.7×10^{6}
S4	Beef	8.5×10^{8}	3.6×10^{8}	6.0×10^{8}	4.7×10^{8}	5.4×10^{8}
S5	Egg salad	2.7×10^{7}	NG	NG	9.8×10^{7}	8.6×10^{7}
S6	Chicken salad	6.7×10^{5}	4.0×10^{5}	1.4×10^{5}	3.7×10^{5}	8.6×10^{5}
S7	Vegetable	2.3×10^{4}	NG	NG	1.3×10^{4}	2.1×10^{4}

NG= No Growth

Quantitative microbial analysis of all the unwrapped sandwich samples showed that the beef sandwich from the star kabab shows the highest microbial count in all the agar media. Egg salad sandwich from Milton shows the second highest growth though it didn't show any growth in McConkey and EMB agar plate. From the table-4.1 has been also seen that sandwich from BRACU

cafeteria and Solna shows the same amount of microbial growth. Vegetable sandwich from Best bites shows the least microbial growth in all the agar media.

Table-4.2 Colony characteristics of microbes isolated from unwrapped sandwich samples in different media

Sample Number	Kind of Sandwich	Chromogenic HiCrome Agar	Enrichment of Sample	
			XLD	TCBS
S1	Chicken salad	Yellow, blue-green, purple	Yellow colonies with white edge, media color changed to yellow	Yellow, white media color changed to yellow
S2	Egg Salad	Blue-white, yellow	Yellow colonies. Media color unchanged	Yellow, green, black colonies. Media color unchanged.
S3	Chicken Salad	Green, purple, blue, yellow	Media color changed to yellow	Media color changed to yellow
S4	Beef	Blue, purple, yellow	Media color changed to yellow	Media color changed to yellow
S5	Egg salad	Ash, white, yellow, blue.	Media color changed to yellow with white colonies.	Media color change to yellow with white colonies
S6	Chicken salad	Blue, yellow, blue-green	Media color changed to yellow	Media color changed to yellow
S7	Vegetable	Purple, yellow	Media color unchanged	Media color unchanged

Table-4.3 Colony characteristic of the microbes isolated from food samples in different media

Sample Number	Colony Characteristic	Agar Media				
		NA	MAC	EMB	MSA	SDA
S1	Size	small	Absent	Absent	small	large
	Shape	circular			circular	irregular
	Elevation	raised			raised	flat
	Margin	entire			entire	undulate
	color	white			white	white
	Texture	smooth			smooth	rough
S2	Size	large	Absent	Absent	small	small
	Shape	circular			circular	circular
	Elevation	raised			raised	raised
	Margin	entire			entire	entire
	Color	yellow			yellow	white
	Texture	rough			smooth	smooth
S3	Size	large	small	small	small	large
	Shape	circular	circular	circular	circular	irregular
	Elevation	raised	raised	raised	raised	raised
	Margin	entire	entire	entire	curled	curled
	Color	yellow	pink	brown	white	white
	Texture	smooth	smooth	smooth	smooth	rough
S4	Size	small	small	small	small	small
	Shape	circular	circular	circular	circular	circular
	Elevation	raised	raised	raised	raised	raised

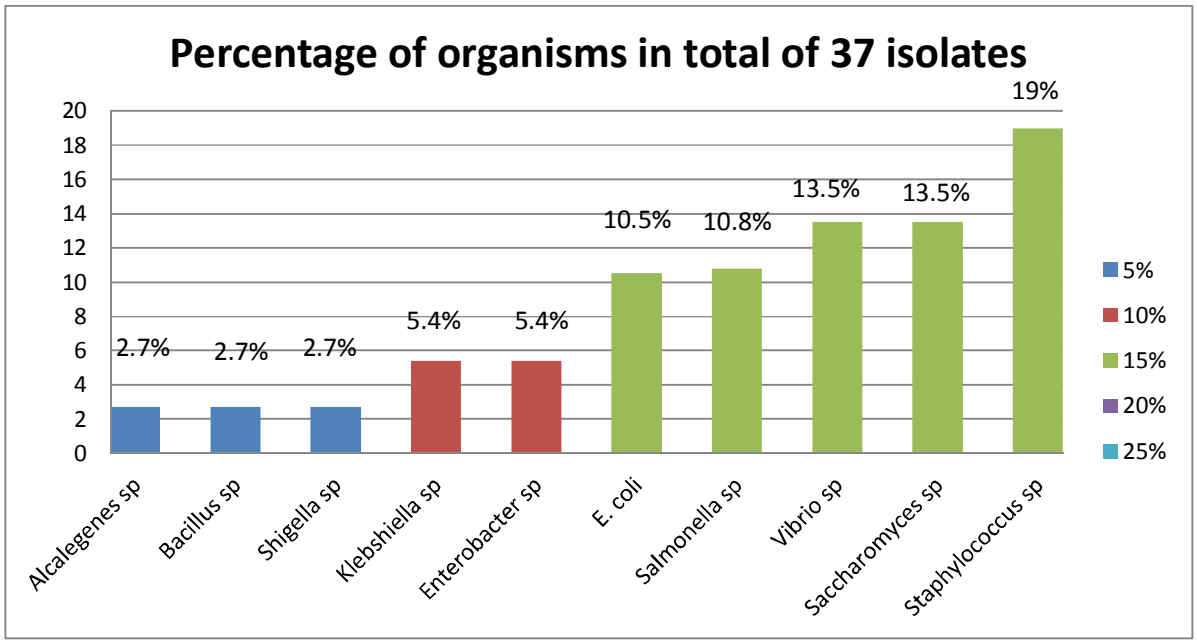
	Margin	entire	entire	entire	entire	entire
	Color	white	pink	colorless	yellow	white
	Texture	smooth	smooth	smooth	smooth	smooth
S5	Size	small	Absent	Absent	small	large
	Shape	circular			circular	Filamentous
	Elevation	raised			raised	flat
	Margin	curled			entire	filliform
	Color	yellow			yellow	white
	Texture	rough			smooth	rough
S6	Size	large	large	small	large	large
	Shape	circular	circular	circular	circular	circular
	Elevation	raised	raised	raised	raised	raised
	Margin	entire	entire	entire	entire	curled
	Color	white	pink	white	white	white
	Texture	smooth	smooth	smooth	smooth	rough
S7	Size	small	Absent	Absent	small	small
	Shape	circular	Absent	Absent	circular	circular
	Elevation	raised			raised	raised
	Margin	entire			entire	entire
	Color	white			white	white
	Texture	smooth			smooth	smooth

Table 4.4: Results of standard biochemical test for identification of all the isolates from various food samples

Isolates	Agar plate	Gram stain	Nitrate reduction	Gas	Acid	H_2S production	Catalase	Oxidase activity	Dextrose	Sucrose	Lactose	Citrate utilization	Motility	Indole activity	Urease activity	Methyl red reaction	Voges-Proskauer	Suspected organism
Sample-1, Chicken salad sandwich from Solna																		
I1	NA	_ve	+	_	+	_	+	_	_	_	_	+	_	_	_	_	_	*Alcalegenes sp*
I2	MSA	+ve	+	_	+	_	+	_	A	A	A	_	_	_	_	+	_	*Staphylococcus sp*
I3	SDA	-ve	+	_	+	_	+	_	A	A	_	+	+	_	+	+	_	*S. cerevisiae*
I4	TCBS	-ve	+	_	+	_	+	_	AG	AG	A	_	+	_	_	+	_	*V. cholerae*
I5	XLD	-ve	+	_	+	_	+	_	A	A	AG	+	+	_	+	+	+	*Salmonella sp*
Sample-2, Egg salad sandwich from BRACU cafeteria																		
I1	NA	_ve	_	_	_	_	+	_	A	A	_	_	+	_	_	_	+	*Bacillus sp*
I2	MSA	+ve	+	_	+	_	+	_	A	A	A	_	_	_	_	+	_	*Staphylococcus sp*
I3	SDA	-ve	+	_	+	_	+	_	A	A	_	+	+	_	+	+	_	*S. cerevisiae*
Sample-3, Chicken salad sandwich from Hossain Bakery																		
I1	NA	+ve	-	-	+	-	+	-	AG	AG	A	-	+	-	_	+	+	*Enterobacter Sp*
I2	MAC	-ve	+	-	+	-	+	-	AG	AG	-	-	-	-	_	+	_	*E. coli*

I3	EMB	+ve	+	-	+	-	+	-	+	+	-	-	+	-	_	+	+	*K. pneumonie*
I4	MSA	+ve	+	-	+	-	+	-	**A**	**A**	-	-	-	-	+	_	_	*S. aureus*
I5	SDA	+ve	+	-	+	-	+	-	**AG**	**A**	-	+	+	-	+	+	+	*S. cerevisiae*
I6	TCBS	-ve	+	-	+	-	+	-	A	AG	A	-	+	-	_	+	_	*Vibrio sp*
I7	XLD	-ve	+	-	+	-	+	-	A	A	A	+	+	-	+	+	+	*Salmonella spp*
Sample-4, Beef sandwich from Star Kabab and resturant																		
I1	NA	+ve	-	-	+	-	+	-	AG	AG	A	-	+	-	_	+	+	*Enterobacter Spp*
I2	MAC	-ve	+	-	+	-	+	-	AG	AG	-	-	-	-	_	+	_	*E. coli*
I3	EMB	+ve	+	-	+	-	+	-	**A**	**A**	-	-	+	-	_	+	+	*K. pneumonie*
I4	MSA	+ve	+	-	+	-	+	-	**A**	AG	-	-	-	-	+	_	_	*S. aureus*
I5	SDA	+ve	+	-	+	-	+	-	**A**	**A**	-	+	+	-	+	+	+	*S. cerevisiae*
I6	TCBS	-ve	+	-	+	-	+	-	A	A	A	-	+	-	_	+	_	*Vibrio sp.*
I7	XLD	-ve	+	-	+	-	+	-	A	A	A	+	+	-	+	+	+	*Salmonella sp*
Sample-5, Egg salad sandwich from Milton																		
I1	NA	+ve	-	-	+	-	+	-	A	A	-	-	+	-	_	-	-	*Staphylococcus sp*
I2	MSA	+ve	+	-	+	-	+	-	**A**	**A**	-	-	-	-	+	_	_	*S. aureus*
I3	SDA	+ve	+	-	+	-	+	-	**A**	**A**	-	+	+	-	+	+	+	*S. cerevisiae*

I4	TCBS	-ve	+	-	+	-	+	-	A	A	A	-	+	-	_	+	_	*V. cholerae*
I5	XLD	-ve	+	-	+	-	+	-	A	A	A	+	+	-	+	+	+	*Shigella sp*
Sample-6, Chicken salad sandwich from Cinnamon																		
I1	NA	+ve	-	-	+	-	+	-	A	A	A	-	+	-	_	+	-	*Staphylococcus sp*
I2	MAC	-ve	+	-	+	-	+	-	AG	AG	-	-	-	-	_	+	_	*E. coli*
I3	EMB	-ve	+	-	+	-	+	-	AG	AG	AG	-	-	-	_	+	_	*E. coli*
I4	MSA	+ve	+	-	+	-	+	-	**A**	**A**	**A**	-	-	-	+	_	_	*S. aureus*
I5	SDA	+ve	+	-	+	-	+	-	**A**	**A**	-	+	+	-	+	+	+	*S. cerevisiae*
I6	TCBS	-ve	+	-	+	-	+	-	A	A	A	-	+	-	_	+	_	*V. cholerae*
I7	XLD	-ve	+	-	+	-	+	-	A	A	A	+	+	-	+	+	+	*Salmonella spp*
Sample-7, Vegetable sandwich from Best bites																		
I1	NA	+ve	-	-	+	-	+	-	A	A	A	-	+	-	_	+	-	*Staphylococcus sp*
I2	MSA	+ve	+	-	+	-	+	-	**A**	-	**A**	-	-	-	+	_	_	*S. aureus*
I3	SDA	+ve	+	-	+	-	+	-	**A**	**A**	-	+	+	-	+	+	+	*Saccharomyces sp*



A= Acid

AG= Acid Gas

From the results of standard biochemical test for identification of all the 37 isolates from sandwich sample it has been seen that the sandwich sample contains *Alcalegenes sp, (2.7%) Bacillus sp(2.7%) and Shigella sp* (2.7%), *Klebshiella sp(5.4%)* ,*Enterobacter sp* (10.5%), *Escherichia coli(10.8%)* and *Salmonella sp (10.8%), Vibrio(13.5%)* and *Saccharomyces sp(13.5%) and Staphylococcus sp(19%)*

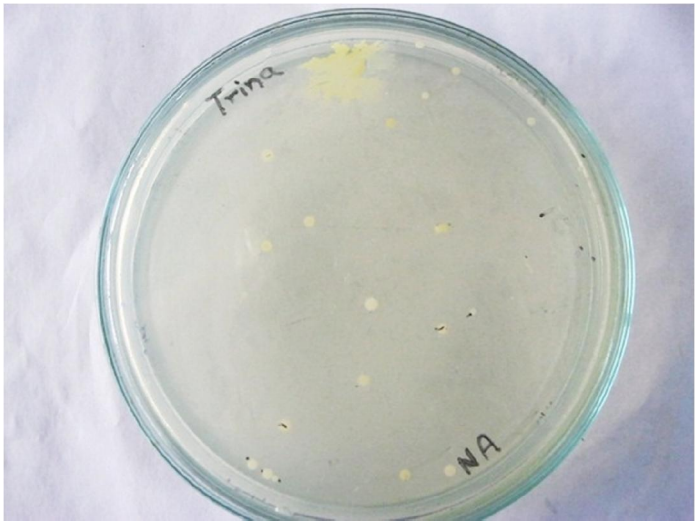


Figure 4.1: Colonies in Nutrient Agar plate

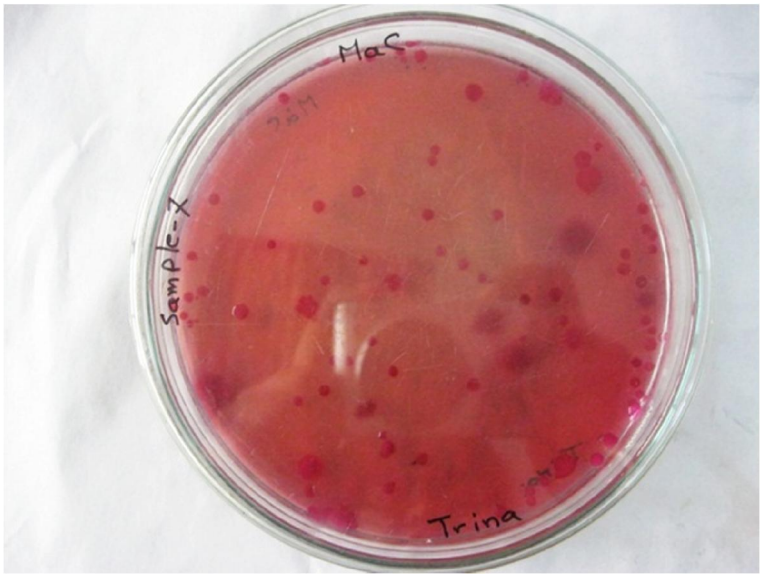


Figure 4.2: Colonies in McConkey agar plate

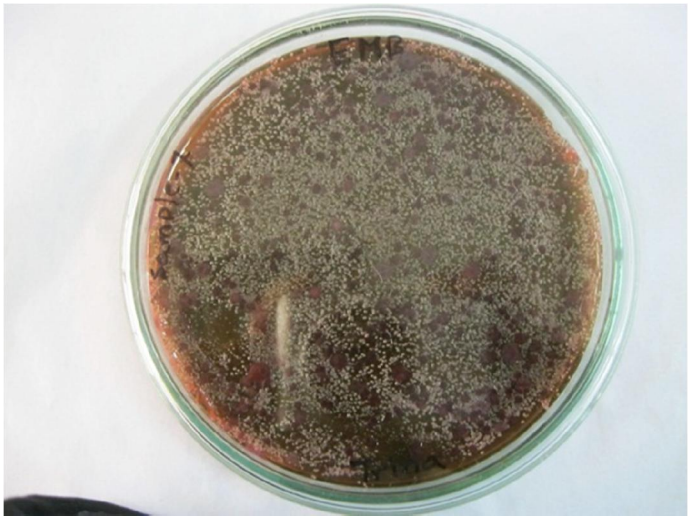


Figure 4.3: Colonies in Eosin Methylene Blue agar plate

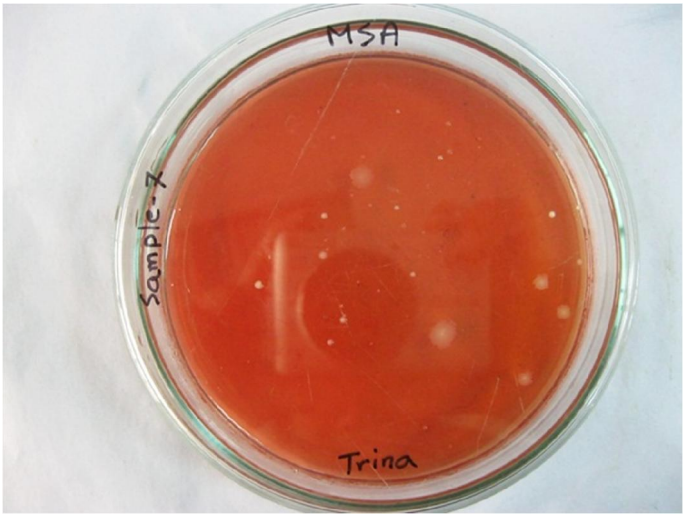


Figure 4.4 : Colonies in Mannitol salt agar agar plate

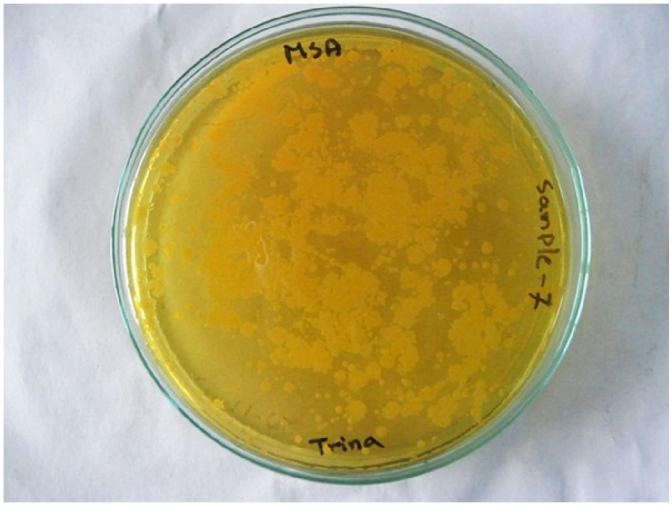


Figure 4.5: Colonies in Mannitol salt agar plate.

The agar plate color changed to yellow

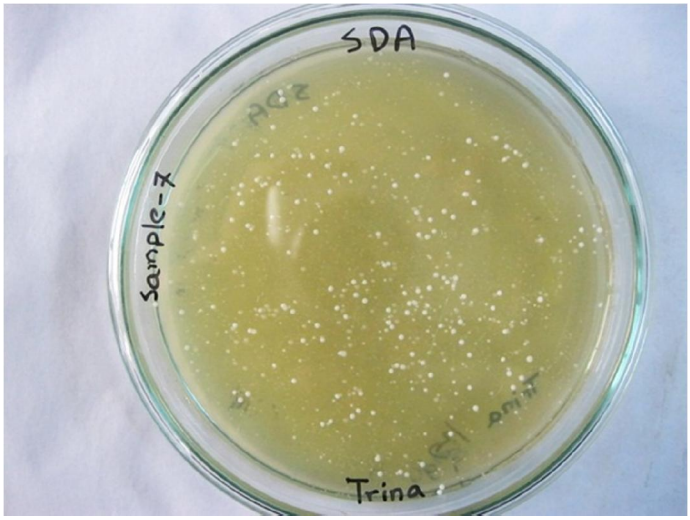


Figure 4.6: Colonies in Sabouraud Dextrose Agar plate.

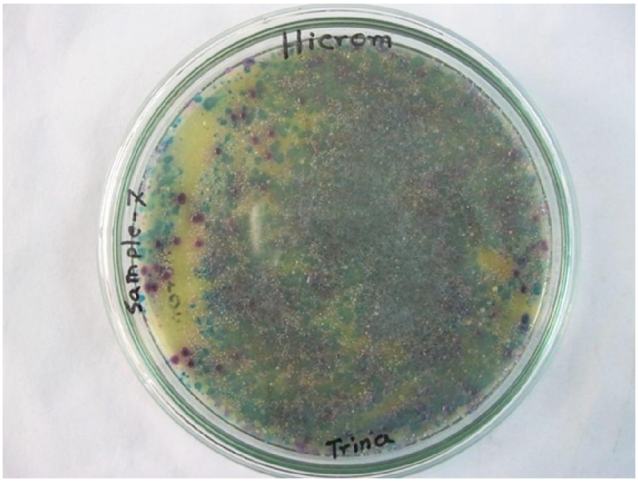


Figure 4.7: Different colored colonies in HiCrome chromogenic agar plate.

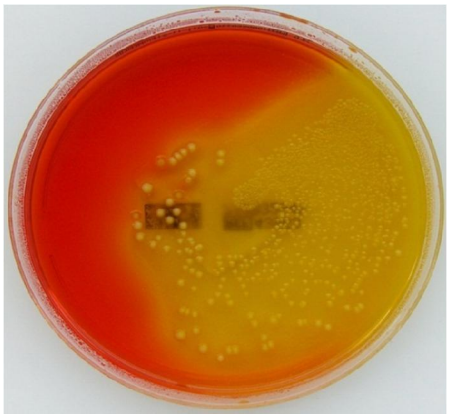


Figure 4 8: Colonies on Xylose lysine deoxycholate agar plate.

Figure 4.9: Colonies on Thiosulfate-citrate -bile salts-sucrose agar plate.

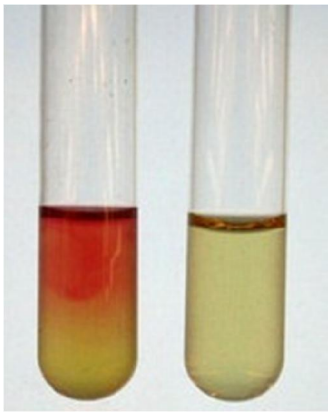


Figure 4.10: Nitrate reduction test

Figure 4.11: Triple sugar iron test

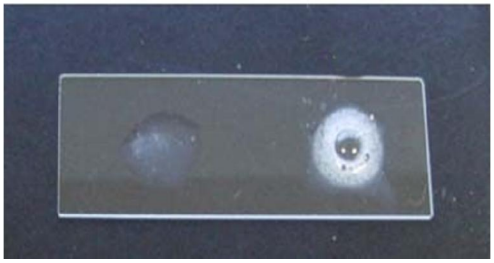


Figure 4.12: Catalase test

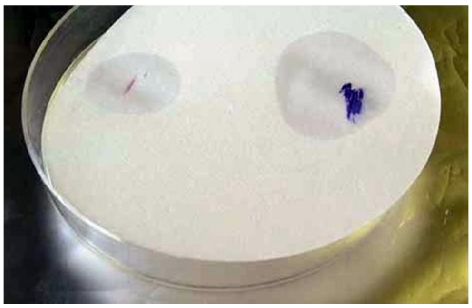


Figure 4.13: Oxidase test

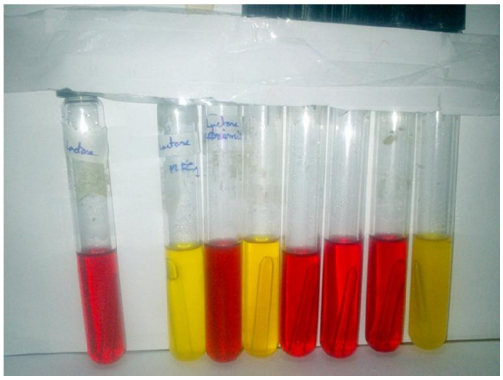


Figure 4.14: Carbohydrate fermentation Sucrose, Lactose) test

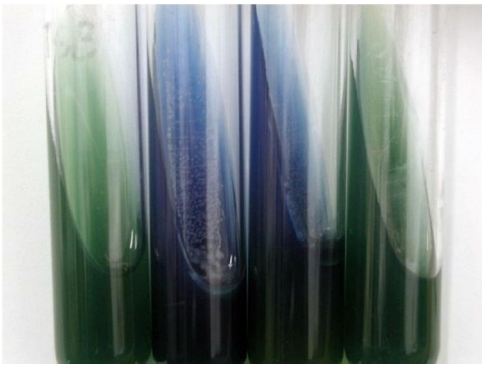


Figure 4.15: Citrate utilization test (Dextrose,

Figure 4.16: Motility test

Figure 4.17: Urease activity test

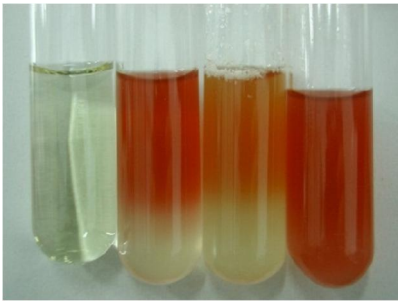


Figure 4.18: Methyl red test

Figure 4.19: Voges- Proskauer test

5. Discussion

Food is one of the basic needs of the human body. Here the fast food plays a very important role in urban human life. In this project the microbial quality of the selected sandwich sample were studied. Food specially fast food may cause many types of diseases in the human body as fast food is made in a very short period of time in which all the hygienic procedure may not be maintained. All these fast food may cause all the food borne diseases e.g. food poisoning, diarrhea, cholera, typhoid, infection in gastrointestinal tract etc.

In this study a total of 7 sandwich samples from different fast food shop were tested for quantitative and qualitative test. Quantitative microbial analysis of beef sandwich from star kabab shows the highest number of bacterial colony count in all of the agar media. The number is around 6.0×10^8 CFU/ml in table 4.1. This sandwich sample contains *Enterobacter sp , E.coli, Klebsiella sp, Staphylococcus sp and vibrio sp* which all are the pathogenic strains may cause different types of diseases in the human body e.g. diarrhea, cholera, food poisoning etc.

The egg salad sandwich from all the fast food shops shows moderate growth, around 3.7×10^6 CFU/ml on average in most of the agar media. It has been found that most of the egg salad sandwich shows no growth in McConkey agar and eosin methylene blue agar plate which shows there is no growth of *E. coli* and Gram negative pathogenic organism in the agar plate. On the other hand some egg sandwich shows the growth of *Staphylococcus* species which may cause food poisoning in the human body.

The vegetable sandwich from Best bites shows the lowest growth of microorganism in all the agar plates which is on average 2.3×10^4 CFU/ml. This sample is also free from most of the pathogenic bacteria which are *E.coli* or other *Vibrio sp* but on the other hand it contains *Staphylococcus* species which may cause food poisoning.

From the overall studies of all the sandwich sample of the seven fast food shops shown that there is the *Alcaligenes sp* is 2.5% which may come from the chicken and dough ingredient of the sandwich sample.

There is also 19% of *Staphylococcus sp* and 13.5% of *E. coli sp* in the sandwich sample. These strains are the resident and transient bacteria, respectively on hands and are associated with poor hygiene practice (Department of Health, 2000). Toxin production strain of *Staphylococcus* are the leading cause of gastroenteretis following handling of food by the person who carry the microorganism in their nose and skin (Le Loir et al, 2003). These bacteria present in about 60% of a given human population and can also survive on hand knives, chopping board and dish cloths. They suggest that the food contamination in the Dhaka fast food restaurants is mainly due to poor water quality and hygiene, peeling of raw vegetables long before consumption and because the shop is crowded and poorly maintained. In another study (Khan et al., 1992) observed high percentage of *Coliform sp* from the raw vegetables (9.2×10^6 /100g) samples. The isolates of *Enterobacter* from all salad in the sandwich shows that the 40% of the microorganisms are *Vibrio sp*, previous studies has been shown that the presence of *Vibrio sp* in salad vegetables (Ashenafi,1989) isolated from the lettuce. In the present study *Klebshiella* and *Shigella* species are found but the origin of these in the sandwich sample and the pathogenicity was not determind. The *Saccharomyces sp* may come from the dough of the bread.

From the results of standard biochemical test for identification of all the 37 isolates from sandwich sample it has been seen that the sandwich sample contains *Alcalegenes sp, (2.7%) Bacillus sp(2.7%) and Shigella sp* (2.7%), *Klebshiella sp(5.4%)* ,*Enterobacter sp* (10.5%), *Escherichia coli(10.8%)* and *Salmonella sp (10.8%), Vibrio(13.5%)* and *Saccharomyces sp(13.5%) and Staphylococcus sp(19%)* biochemical test confirms that except *Saccharomyces sp* almost all the organisms are pathogenic.

The serving utensils used at the vending site are often contaminated with *Micrococcus sp*. which may have originated from the vendors hands when they touch the food preperaton areas, dish, cloth or water during dish washing or hand washing indicates cross contamination between dishwasher, food preperation surface and food itself (Manesha et al., 2002)

In the present study staphylococci occupied the highest percentage of occurrence. presence of high number of pathogenic *Staphylococci* in fast food is alarming. Next to *Staphylococci*, *Coliform* ranks the second position. the high *E. coli* percentage indicates poor practice sanitary conditions during handling, and transportation of fast foods. The organisms gaining access to fast foods were not only the cause of deterioration and spoilage but also responsible for giving warning signal of indication of the presence of many food borne disease outbreaks. presence of *Salmonella* spp. in fast foods must receive particular attention, as these organisms are responsible for causing hazard.

In this particular study of sandwich sample from fast food shop HiChrome chromogenic agar was used in which he overall findings of this study suggests that though expensive, chromogenic media like HiCrome agar media, offer an excellent and time saving method for the eliable identification of most of the uropathogens and differentiation of mixed bacterial cultures in primary culture plate, and thus reduces laboratory workload (i,e. plate burden and sheep house). Moreover HiCrome agar media have an advantage over conventional media for identification of Enterococcus spp.

5.1 Conclusion

It is unfortunate that there has been not yet any application of legal standard relating to the hygienic production, processing and distribution of fast foods in Bangladesh. The microbial contamination may also come from environment, meat, salad or the dough of the sandwich sample. The present study has focused on the pre-harvest and post-harvest safety of fast food and their impact on potential health hazard arising from consumption. The result contributes in making suggestions to ensure delivering safe food product to meet the demands of consumers now and in future. The following suggestions to be ensured hygienic food safety and obtaining hygienic quality fast foods may be credible -

- Procurement of raw materials of the best possible microbiological quality.
- Prevention of undue contamination of fast foods prior to processing.
- Appropriate processing of fast foods.
- Proper measures to avoid contamination during and particularly after processing of fast foods.
- Quality packaging to keep foods fresh and get rid of health risk factors
- Adequate storage, ideal transportation, and hygienic handling of the finished product and if needed cold-chain application
- Microwave oven treatment prior to serving to consumption.

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APPENDIX-I

Media composition

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

1. **Nutrient Agar (Himedia,India)**

Ingredients	**Amounts (g/L)**
Peptic digest of animal tissue	5.0
Beef extract	1.50
Sodium chloride	5.0
Yeast extract	1.50
Agar	15.0

2. **Nutrient Broth (Oxoid, England)**

Ingredients	**Amount (g/L)**
Lab-lemco powder	1.0
Yeast extract	2.0

Peptone	5.0
Sodium chloride	5.0

3. T

ryptone soy broth, (Oxoid, England)

Ingredients	**Amount (g/L)**
Pancreatic digest of Casein	17.0
Papaic digest of soybean meal	3.0
Sodium chloride	5.0
Di-basic potassium phosphate	2.5
Glucose	2.5

4. MacConkey agar (Oxoid, England)

Ingredients	**Amount (g/L)**
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.075
Agar	12.0

5. Simmon's citrate agar (Oxoid, England)

Ingredients	Amount (g/L)
Magnesium sulfate	0.2
Ammonium dihydrogen phosphate	0.2
Ammonium phosphate	0.8
Sodium citrate	2.0
Sodium chloride	5.0
Agar	15.0
Bacto brom thymol blue	0.08

6. Peptone Water

Ingredients	Amount (g/L)
Peptone	10.0
Sodium chloride	5.0

7. MR-VP broth

Ingredients	Amount (g/L)
Peptone	7 g
Dextrose	5 g
Potassium phosphate	5 g

8. Triple sugar iron agar (Himedia, India)

Ingredients	Amount (g/L)
Peptic digest of animal tissue	10.0
Sodium chloride	5.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous sulfate	0.20
Sodium thiosulfate	0.30
Casein enzymatic hydrolysate	10.0
Yeast extract	3.0
Beef extract	3.0

9. Eosine methylene blue agar (Oxoid, England)

Ingredients	Amount (g/L)
Peptone	10.0
Sucrose	5.0
Lactose	5.0
Di-potassium phosphate	2.0

Eosin Y	0.14
Methylene blue	0.065
Agar	13.50

10. Mannitol Salt agar (Oxoid, England)

Ingredients	**Amount (g/L)**
Peptone	10.0
Manitol	10.0
Lab-lemco powder	1.0
Sodium chloride	75.0
Phenol red	0.025
Agar	15.0

11. Thiosulfate Citrate Bile Salts Sucrose agar (Difco, USA)

Ingredients	**Amount (g/L)**
Proteose peptone	10.0
Sodium thiosulfate	10.0
Sodium citrate	10.0
Yeast extract	5.0
Oxgall	8.0

Sucrose	20.0
Sodium chloride	10.0
Ferric citrate	1.0
Bromothymol blue	0.04
Thymol blue	0.04
Agar	15.0

12. Xylose Lysine Deoxycholate agar (Himedia, India)

Ingredients	**Amount (g/L)**
L- lysine	5.0
Lactose	7.50
Sucrose	7.50
Xylose	3.50
Sodium chloride	5.0
Sodium deoxycholate	2.50
Yeast extract	3.0

13. Phenol red (Lactose, Dextrose, Sucrose) Broth

Ingredients	**Amount (g/L)**
Trypticase	0.4

Lactose	0.2
Sucrose	0.2
Dextrose	0.2
Sodium chloride	0.2
Phenol red	0.00072
Final pH	7.3

APPENDIX-II

Buffers and reagents

1. Phosphate buffered saline (PBS)

PBS was prepared by dissolving 8.0 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4 and 2.0 g of KH_2PO_4 in 800 ml of distilled water. The pH was adjusted to 7.4 with HCl. The final volume was adjusted to 1 liter by distilled water. The solution was sterilized by autoclaving and was stored at room temperature.

2. Kovac's reagent

5 g of para-dimethylaminobenzaldehyde was dissolved in 75 ml of amyl alcohol. Then concentrated HCl was added to make the final volume 25 ml. This reagent was covered with aluminum foil and stored at 4^oC.

3. Methyl red reagent

0.1 g of methyl red was dissolved in 300 ml of 95% ethyl alcohol. Then distilled water was added to make the final volume 500 ml. This reagent was covered with aluminum foil and stored at 4^{o}C.

4. **Barritt's reagent**

Solution A

5 g of alpha-naphthol was dissolved in 95% ethanol. This solution was covered with aluminum foil and stored at 4^{o}C.

Solution B

40 g of KOH was dissolved in distilled water. The solution became warm. After cooling to room temperature, creatine was dissolved by stirring. Distilled water was added. This solution was covered with aluminum foil and stored at

5. **Oxidase reagent**

100 mg of N,N,N^{1},N^{1}-tetramethyl-p-phenyldiamine-dihydrochloride was dissolved in 10 ml of distilled water and covered with aluminum foil. Then the solution was stored at 4^{o}C.

APPENDIX-III

Instruments

The important equipments used through the study are listed below:

❖ Autoclave	SAARC
❖ Freeze (-20°C)	Siemens
❖ Incubator	SAARC
❖ Micropipette (10-100µl)	Eppendorf, Germany
❖ Micropipette (20-200µl)	Eppendorf, Germany
❖ Oven, Model:MH6548SR	LG, China
❖ pH meter, Model: E-201-C	Shanghai Ruosuaa Technology company, China
❖ Refrigerator (4^{o}C), Model: 0636	Samsung
❖ Safety cabinet Class II Microbiological	SAARC
❖ Shaking Incubator, Model: WIS-20R	Daihan Scientific, Korea
❖ Vortex Mixture	VWR International
❖ Water bath	Korea
❖ Weighing balance	ADAM EQUIPMENT™, United Kingdom