Comparative study of the microbiological status of burgers of street, lower-mid, higher-mid and high level fast food shops and detection of multidrug-resistant isolates

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

Submitted by- Salma Sultana
Student ID- 11326002
August, 2016

Microbiology Program
Department of Mathematics and Natural Sciences
BRAC University
Dhaka, Bangladesh

DECLARATION

I hereby declare that the thesis project titled "**Comparative study of the microbiological status of street, lower-mid, higher-mid and high level fast food shops and detection of multidrug-resistant isolates**" submitted by me has been carried out under the joint supervision of Dr. M. Mahboob Hossain, Associate Professor, Department of Mathematics and Natural Sciences, BRAC University, Mohakhali, Dhaka and Ms. Namista Islam, Lecturer, Department of Mathematics and Natural Sciences, BRAC University, Mohakhali, 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.

(Salma Sultana)

Candidate

Certified

(Dr. MahboobHossain)
Associate Professor
Department of Mathematics and and Natural Sciences
BRAC University
66 Mohakhali, Dhaka-1212, Bangladesh
E-mail: mmhossain@bracu.ac.bd

(Ms. Namista Islam)
Lecturer
Department of Mathematics and Natural Sciences
BRAC University
66 Mohakhali, Dhaka-1212, Bangladesh
E-mail: namista@bracu.ac.bd

DEDICATION

TO

MY BELOVED

PARENTS

Acknowledgement

All praises be to the Almighty Allah.

The accomplishment of my thesis work would not be possible without the help of some accommodative people. First of all, I would like to express my deepest gratitude to Prof. A. A. Ziauddin Ahmad, Chairperson, Department of Mathematics and Natural Sciences and Prof. Naiyyum Choudhury, Chairman at Bangladesh Atomic Energy Regulatory Authority (Ex. Coordinator of the Microbiology and Biotechnology Program, Department of Mathematics and Natural Sciences) for permitting me to do this research and I would like to extend my sincere thanks to my supervisor Dr. M. Mahboob Hossain, Associate Professor, Department of Mathematics and Natural Sciences for his continuous support and kind assistance and my heartfelt thanks to my co-supervisor lecturer Ms. Namista Islam.

I am as always greatly indebted with paramount gratitude to my father, Md. Saiful Islam and my mother Mrs. Rayhana Khanam for their unconditional support and faith in me and encouraging me in every spheres of life.

I am greatly indebted to the lab officer Ms. Asma Binte Afzal for her continuous support and Mrs. Shamima Akhter Chowdhury for her assistance. I would also like to thank my wonderful friend Fatema Ahmed Konica for her assistance with the works.

With the help of these people, I was able to complete my thesis work easily and they made my thesis a comfortable one.

Abstract

Fast foods and street foods are a part of life of the people of Dhaka city nowadays. Through these foods, food borne diseases are also increasing in an alarming rate. This study was conducted on chicken burgers of the Gulshan area of Dhaka city as it is one of the most consumed fast foods. Total viable count (TVC), coliform and enteric pathogens count (CEC), *Staphylococci* count (SC), and also the presence of urinary tract infection causing pathogens, *Salmonella* spp., *Shigella* spp. were observed in this study.

In the present study, it was observed that the total viable count, enteric pathogen and coliform count were very high in almost every level of restaurant foods. This might be due to the street dust, unhygienic water, unclean dishes, improper food handling etc for the street side foods. In higher-middle level and high level restaurants, this result might be due to unhygienic handling of foods, improper storage temperature and storing and uses of unhygienic and expired raw materials.

Bacterial count in Nutrient Agar (NA) isolated from street level, lower-mid level, higher-mid level and high level restaurants ranged from $3.3X10^5$ to $2.2X10^7$ CFU/ml, $1.9X10^6$ to $2.0X10^6$ CFU/ml, $7.0X10^5$ to $2.3X10^7$ CFU/ml and $7.5X10^6$ to $2.3X10^7$ CFU/ml respectively. Bacterial count in MacConkey agar isolated from street level, lower-mid level, higher-mid level and high level restaurants ranged from nil to $5.48X10^5$ CFU/ml, $9.55X10^5$ to $1.8X10^6$ CFU/ml, nil to $1.0X10^5$ CFU/ml and nil CFU/ml respectively. Bacterial count in Manitol Salt Agar (MSA) of street level, lower-mid level, higher-mid level and high level restaurants ranged from nil to $1.6X10^6$ CFU/ml, nil to $8X10^5$ CFU/ml, nil to $4.33X10^5$ CFU/ml and $2.1X10^6$ to $2.2X10^7$ CFU/ml respectively.

Antimicrobial sensitivity results of the bacteria *Raoultella planticola* isolated from a higher mid-level restaurant showed resistance to 4 antibiotics out of 7 antibiotics and *Bacillus coagulans* isolated from a street level restaurant showed resistance to 3 antibiotics.

This study specifically highlighted the level of microbial loads and multi-drug resistant (MDR) bacteria found in burgers. Finally, this study recommended some preventive measures, which the government and food-maker together should follow and should also maintain the standard hygienic procedure for preparing and handling foods.

Contents

List of tables

List of figures

Abbreviations

mm : Millimeter

μm : Micrometer

mg : Milligram

g : Gram

Kg: Kilogram

e.g. : For example

et al.: And others

pH: Negative logarithm of hydrogen ion concentration

CFU: Colony Forming Unit

spp.: Species

%: Percentage

^{o}C: Degree Celsius

NA: Nutrient Agar

Mac: MacConkey agar

MSA: Mannitol salt agar

XLD: Xylose lysine deoxycholate agar

TCBS: Thiosulfate-citrate-bile salts-sucrose agar

TSI: Triple sugar iron agar

MIU: Motility indole urease agar

CEC: Coliform and enteric pathogen count

SC: *Staphylococci* count

TVC: Total viable count

MDR: Multidrug resistant

INTRODUCTION

1.1 Introduction

Life is getting very fast nowadays. People barely have time to have their meals properly. Therefore, they have foods from the streets which are known as street foods. Street foods and fast foods are becoming very popular in the developing countries like Bangladesh nowadays. People of every age and class are fond of these foods. Food and Agriculture Organization, (FAO 1986) defines street foods as "ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers especially in street and other similar public places". Fast food is a kind of snack, which is already prepared before and just fried or cooked just after the order of the customers for quick serving of the foods to them. These are not processed foods. In many of the shops these foods are displayed on the racks after preparing in the kitchens. These foods are becoming an important part of people's life as it saves time and energy and are also cheap. A lot of people, especially from middle class and lower class have their breakfasts and snacks from these street food shops.

There are different types of street and fast foods. Among them chotpoti, fuchka, puri, singara, potato chop, chicken fry, french fries, sandwich, pizza, burger etc are common. These are very appealing due to their taste, texture, flavor, colour, aroma and appearance. People easily get attracted to these foods because of these characteristics.

There are different types of restaurants or shops selling these foods. Some are placed on the streets openly, some are mid level shops and some are very high qualified restaurants such as, KFC, BFC, Pizza Hut, Pizza Inn etc. These are very well known restaurants for these foods especially fried chicken, French fries, pizza etc.

Though people of all classes are eating these foods, very few people are aware of the nutrition facts and hygienic condition of these foods. Those few people, who know about the harms, also have to eat these foods to save time and money.

Almost all of the street and fast foods lack necessary nutrition and the pathogenic microbial load is also higher in most of them. In the streets, foods are kept open and due to that, dust and microbes can easily contaminate them and grow. In the mid-level restaurants, the foods are kept in the racks but open and also kept for long time and sometimes for some days, which is preferable for microbial growth. In the high level shops, they sometimes use frozen meats or the

chickens/beefs, which are cooked a long time ago. However, with all these reasons, handling of food is also a very important issue. The cooks sometimes do not wash their hands properly and do not use proper cautions for handling the foods. Sometimes the chickens/beefs are not properly cooked. Because of all these reasons, many people suffer from diarrheal or abdominal diseases.

Therefore, to figure out the harms made by these foods, especially, with the microbes present here, this thesis work was done. Some other goals of the thesis were, to compare the street, lower-mid level, higher mid-level and high level restaurants food quality, to figure out the microbial loads in these foods, the harms made by those microbes, whether the microbes were antibiotic resistant or not and to detect the probable identity of the species of those microbes. Foods were collected aseptically and analyzed at BRAC University microbiology lab. Total viable count, enteric pathogen and coliform count were observed. Besides these counts, selective media were used to observe the presence of urinary tract infection causing pathogens, *Vibrio* spp., *Salmonella* and *Shigella* spp. This was done for food quality analysis. After observing microbial growth and biochemical tests, the test results were put into ABIS online software which has shown four most probable names of the bacteria. According to these results further analysis were done.

1.2 Literature review

Different studies and experiments were performed to analyze the microbial quantity of the street and fast foods of Gulshan area of Dhaka city.

1.2.1 Comparative microbial quality analysis among street, homemade and restaurant foods

A study on microbiological quality of street foods in comparison with home-prepared and hotel-restaurant foods was carried out at Hai Ba Trung and Hoan Klein districts in Hanoi, Vietnam from December 1994 to March 1995. Ninety samples of the most common meals were collected and 35 food handlers were interviewed. The microbiological analyses were carried out according to methods of enumeration of mesophilic aerobic bacteria and enumeration of coliform bacteria at the Microbiological Laboratory of the National Institute of Nutrition (NINVietnam). Greatest numbers of microbial counts were found in the samples obtained from street source. Microbiological quality of hotel-restaurant foods was superior to the street foods.

Ha Thi Anh Dao in 1995 reported that in Hanoi-Vietnam large numbers of microbial counts were found in street foods compared to homemade and restaurant foods. However, there was no significant difference between microbial counts of homemade and restaurant foods. Moreover, restaurant foods had lower microbial counts. Five star restaurant foods were not always safe for consumption compared to homemade and restaurant foods, reported by Kampenin 1998 in Jakarta.

However, there was a tendency that hotel-restaurant dishes had lower microbial counts. This study had suggested that the importance of using potable water and proper food handling practices were viewed as essential. Given that street foods were consumed by the mainstream working people in Hanoi, there were urgent needs to improve the microbiological quality of street foods. Actions should have been taken to introduce the knowledge on food hygiene priority to street vendors and their customers. More research is necessary to identify the causes of the problem using HACCP (Hazards Analysis and Critical Control Points).

1.2.2 Microbial analysis of street foods

Most of the studies done on street foods in India and other countries had indicated that these foods were not meeting the microbiological standards and were contaminated with various pathogens like *E.coli*, *Vibrio*, *Salmonella*, *Listeria* etc. (Chiou et al., 1996; Ryu et al., 1998; Mosupy and von Holy, 1999; Fang et al., 2003; Lewis et al., 2006). Sandeep and associates (2001) had reviewed about the food borne illnesses associated with the consumption of street foods. These food borne illnesses were leading cause of morbidity and mortality worldwide (Bryan 1988). The microbiological status of the food had been reported to be dependent on several factors like quality of raw material (Jones et al., 1991; Thunberg et al., 2002), handling and processing of food (Jones et al., 1991), microorganisms that survive the preservation and storage treatment (Gimenez and Salgaard, 2004), post process contamination (Long et al., 2002; Meena et al., 2004). Sometimes, the food was not covered and the foods got infested by flies and other insects. The water used in the food and to wash the materials was of poor quality.

Beside direct health consequences, these food borne illnesses can reduce the productivity and economic output, and also impose substantial stress on health care system (Martins and Anelich, 2000; Mosupye and von Holy, 2000). For these reasons, identification of these microbes is necessary to stop health problems.

1.3 Objectives of the study

1.3.1 General objectives

To conduct the thesis work, Gulshan area of Dhaka city was selected. From this area, 4 types of food shops were chosen. These were street, lower-middle, higher-middle and high level restaurants/shops. From each shop, 3 samples were collected with a gap of one week. Total 12 samples were tested. The foods were blended, diluted, grown in different types of media, isolated, stocked, antibiotic resistant tests were done and then only multi-antibiotic resistant isolates were gone through the different biochemical tests and finally the detection of microorganisms were done by ABIS online bacterial identification software.

1.3.2 Specific objectives

The specific objectives of this study were:

- To find out the microbial loads of those burgers.
- To detect which organisms were present in these.
- To find out the antibiotic resistant organisms from these.
- To compare between the street, lower-mid, higher-mid and high level restaurant foods.

MATERIALS AND METHODS

2.1 Materials

2.1.1 Study area and sample collection

A total of 12 samples of burgers from 4 different shops in 3 different days were purchased from Gulshan, Dhaka. Shops were mainly one street, one lower mid-level, one higher mid-level and one higher level. The food samples were taken in sterile plastic bags and kept here till analysis.

2.1.2Media

Different types of media were used. Some of them were suitable for total viable count; some of them were suitable for total coliform count; some for enteric pathogen; some for identifying vibrio etc. These were Nutrient agar, MacConkey agar, Mannitol Salt agar (MSA), Thiosulfate-citrate-bile salts-sucrose (TCBS) agar, Xylose lysine deoxycholate (XLD) agar and HiCrome agar.

Nutrient agar

Nutrient agar was used as a general purpose medium which supported the growth of a wide range of non-fastidious organisms.

MacConkey agar

MacConkey agar was used as a selective and differential culture medium for bacteria. It was designed to selectively isolate Gram-negative and enteric (normally found in the intestinal tract) bacilli and differentiate them based on lactose fermentation. It contained crystal violet and bile salts, those inhibit the growth of gram-positive organisms and allowed for the selection and isolation of gram-negative bacteria.

Mannitol Salt agar

Mannitol salt agar was used as a selective and differential growth medium. It encouraged the growth of a group of certain bacteria and inhibits the growth of other bacteria.

Thiosulfate-citrate-bile salts-sucrose (TCBS) agar

Thiosulfate-citrate-bile salts-sucrose (TCBS) agar was a selective agar which was used in microbiology laboratories to isolate *Vibrio* spp. TCBS Agar was highly selective for the isolation of *V. cholera* and *V. parahaemolyticus* as well as other *vibrios*.

Xylose lysine deoxycholate (XLD) agar

Xylose lysine deoxycholate agar (XLD) agar was a selective growth medium. It was used for the isolation of *Salmonella* and *Shigella* species from clinical samples and from food.

HiCrome agar

HiCrome UTI Agar was used as chromogenic differential medium. It was used for identification, differentiation and confirmation of enteric bacteria from specimens such as urine, water, or food which may contain large number of *Proteus* species as well as potentially pathogenic Gram-positive organisms. Each bacterial strain gave different colour in this medium.

2.1.3 Enrichment broth

Alkaline peptone water

Alkaline Peptone Water was used for the enrichment of *Vibrio cholerae* and *Vibrio* species from food, water and clinical samples. The pH of the broth was higher than usual broth which was 8.6±0.2.

Selenite cystine broth

Selenite Cystine Broth was a selective enrichment medium. It was used for the isolation of *Salmonella* from foods, water and other materials of sanitary importance.

2.1.4 Stock culture media

Tryptic soy broth glucose-glycerol (TGG)

When inoculated into Tryptic Soy Broth glucose-glycerol and frozen at or below -40°C, suspensions of bacteria remain viable for several months. It was used for stocking.

Skim milk tryptic soy broth glucose-glycerol (STGG)

It was also used as a stock media. The preparation was same as TGG broth, only skim milk was added extra here.

2.1.5 Media for biochemical tests

Simmons citrate agar

It is an enrichment media which was used to observe the citrate using ability of a microorganism.

Triple sugar iron (TSI) agar

Triple sugar iron (TSI) agar was made into slant and butt by keeping the autoclaved agar lying on something. Utilization of sucrose, dextrose and lactose sugars and also hydrogen sulfide production and gas production were observed in this media. Hydrogen sulfide production was detected by blackening of media due to the presence of thiosulfate and ferrous sulfate in the media. A bacterium that was a non-lactose fermenter and fermented glucose, made the agar yellow, some bacteria used thiosulfate as an electron acceptor and reduced it to hydrogen gas which might lift the agar from the butt of the tube or fracture the agar.

Methyl red and Voges-Proskauer (MRVP) broth

It was a single broth but used for both methyl red and voges-proskauer test. After incubation with colonies of same bacteria, the broth was divided into two test tubes for those tests.

The methyl red test detected production of acids, which were formed during metabolism using mixed acid fermentation pathway using pyruvate as a substrate. The pH indicator methyl red was added and a red colour appeared at pH lower than 4.2, indicating a positive test meant mixed acid fermentation was used. The solution remaining yellow at pH = 6.2 or above, indicated a negative test, meaning the butanediol fermentation was used.

The VP test was used to test for the presence of acetyl methyl carbinol indicator (acetoin). After adding both reagents, the tube was shaken vigorously and then allowed to sit for 5-10 minutes. A pinkish-red colour at the upper part of the broth indicated a positive test, meaning the 2,3-butanediol fermentation pathway was used.

Motility indole urease (MIU) agar

Motility of microorganisms, indole production and urease enzyme activity were observed in this media.

Nitrate broth

Nitrate reduction activity was observed in this broth; where change in broth colour after addition of nitrate test reagents detected the presence of nitrate utilization enzyme.

Phenol red dextrose broth

Utilization of dextrose was observed in this broth, where pH change in media caused colour change of the broth.

Phenol red lactose broth

Utilization of lactose was observed with this broth, where pH change in media caused change of colour in the broth.

Phenol red sucrose broth

Utilization of sucrose was observed in this broth, where change in pH of the media caused colour change of the broth.

Skim milk agar

Casein hydrolysis was observed in this agar, which was seen by a clear zone.

Starch agar

Alpha-amylase enzyme of bacteria breakdown starch into simple glucose molecules on starch agar and created a clear zone.

Blood agar

Lysis of blood cells by microorganisms was observed in blood agar. Alpha hemolysis, beta hemolysis and gamma hemolysis were types of blood cell lysis pattern by microorganisms in blood agar.

2.2 Methods

2.2.1 Sample collection

Sample was collected and immediately put into sterile tip bag to avoid any type of outer contamination. If it could not be done immediately, sample should have been kept in the refrigerator until sampling was done.

2.2.2 Sample preparation

After collecting the sample, it was blended in the blender machine. However, before using the blender machine it was freed from microbes by repeated washing with sterile water. After blending the sample, three sterilized beakers were taken and 1 g of sample was taken in each of the beaker by measuring in the weighing machine. Later, in one beaker 9 ml of saline was poured for spreading. In another beaker 9 ml of alkaline peptone water and in another one 9 ml of selenite cystine broth was poured and kept in the incubator for 4-6 hours for enrichment. The time and name of the broth was written immediately to maintain the time and not to lose track of progress.

2.2.3 Sampling

The sample was serially diluted up to six times in saline solution and each of the test tubes was labeled properly. All the agar plates were also labeled properly and only the dilution from 10^{-3}-10^{-6} were spread in all of the media, which were Nutrient agar, MacConkey agar, MSA agar and were done two plates for reconfirmation. In UTI HiCrome only 10^{-3} was spreaded. In XLD agar the enrichment broth of selenite cystine broth was streaked and in TCBS agar the enrichment broth of alkaline peptone water was streaked. After plating, all the plates were kept in incubator for 24-48 hours for growth.

2.2.4 Observation and reading

After 24 hours of incubation, all the plates were observed and all the colonies in each plate were counted for total viable count except for the TCBS, XLD and HiCrome agar plates. In these plates presence of specific microorganisms were observed according to the microbial growth pattern. *Vibrio* species gave yellow and green colony on TCBS agar. On XLD agar *Salmonella* gave black centered pink/red colony, *Shigella* gave pink/red colony and *E. coli* gave yellow colony. On HiCrome agar different microorganisms gave different coloured colonies such as, *Enterococcus faecalis;* blue-green, small, *Escherichia coli*; pink-purple, *Klebsiella pneumonia;* blue-purple mucoid, *Pseudomonas aeruginosa*; colourless, *Proteus mirabilis*; light brown, *Staphylococcus aureus*; golden yellow colonies respectively.

After 48 hours of incubation, the plates were again observed and counted. Then different coloured colonies from selective media were subcultured on fresh NA plates.

2.3 Preservation, biochemical tests and antibiotic tests

2.3.1 Preservation

After 24 hours of incubation in the NA medium, the colonies were taken in tryptone soya broth (TGG and STGG) culture media. They were again kept in incubator for 24 hours for growth and kept in the refrigerator for stocking at -4^{o}C.

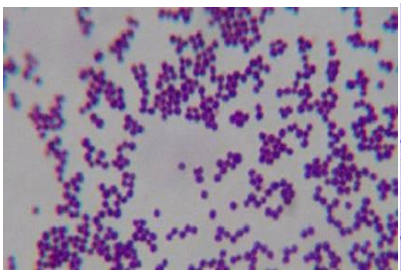
From the subcultured plates, gram staining and oxidase-catalase and antibiotic tests were done. Lawns were made on NA plates with the different colonies of different plates and antibiotic disks were kept on the agar. For gram positive bacteria, Cefepime (CPM), Ciprofloxacin (FOX), Rifampicin (RD) were used. For gram negative bacteria, Amikacin (AK), Kanamycin (K), Norfloxacin (NOR) were used. For both gram positive and gram negative, Ampicillin (AMP), Cefoxitin (FOX/CX), Chloramphenicol(C), Gentamicin (CN) were used. After putting the antibiotics on the plates, they were incubated for 24 hours. After incubation, the multi antibiotic resistant colonies were observed and found by following the antibiotic resistant table and for those bacteria biochemical tests were done.

Triple sugar iron test, motility indole urease test, Simmons' citrate test, nitrate test, methyl red and Voges-Proskauer test, casein utilization test, starch utilization test, hemoglobin utilization test, carbohydrate (sucrose, dextrose, lactose) utilization tests were performed for each type of multi antibiotic resistant colonies. Biochemical test results were noted on a chart and then all the results were put on online Advanced Bacterial Identification software (ABIS) on the basis of gram staining results. After putting the biochemical test results, the software gave 4 most probable names of the bacteria, which approximately matched the test results. If detail option was applied after these probable results, then it would suggest which test was positive/negative for these four bacteria among the biochemical tests which were performed and also would suggest more biochemical tests for specification of bacterial species if needed.

2.3.2Biochemical tests

- ✓ **Gram staining**

One small bacterial colony was taken and smeared on a glass slide and hit fixed. Then crystal violet, grams iodine, absolute ethanol and saffranin were added respectfully one after another and kept for one minute each and every time was washed. Then, the slide was seen under the microscope to differentiate bacteria into two large groups which were Gram positive and Gram negative; based on their cell wall structure.



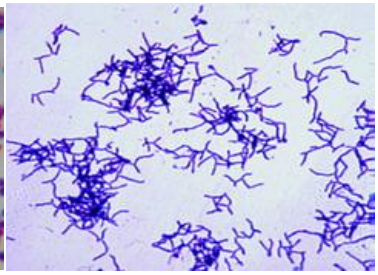


Fig 2.1: Gram positive cocci Fig 2.2: Gram positive rod

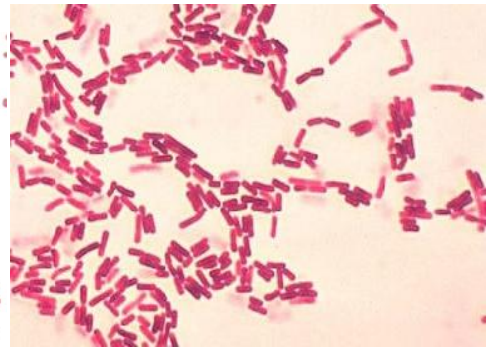


Fig 2.3: Gram negative cocci Fig 2.4: Gram negative rod

- ✓ **Oxidase**

One drop of oxidase reagent (*N,N,N′,N′*-tetramethyl-*p*-phenylenediamine) was added on the Whatman filter paper and then one bacterial colony was taken by the sterile loop and smeared over the paper. Positive reactions turned the colour from violet to purple within 1-30 seconds. Delayed reactions were not acceptable.

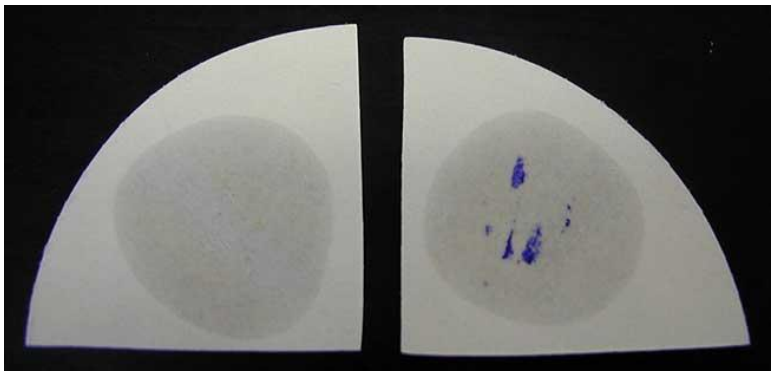


Fig 2.5: Oxidase negative (Left), Oxidase positive (Right)

- ✓ **Catalase**

A drop of catalase reagent (hydrogen peroxide) was taken on a glass slide. A small bacterial colony was taken by a sterile loop and put on the drop and mixed. A positive result gave oxygen production which was seen by bubble production within 10 seconds. On the other hand, a negative result did not give any bubble production.

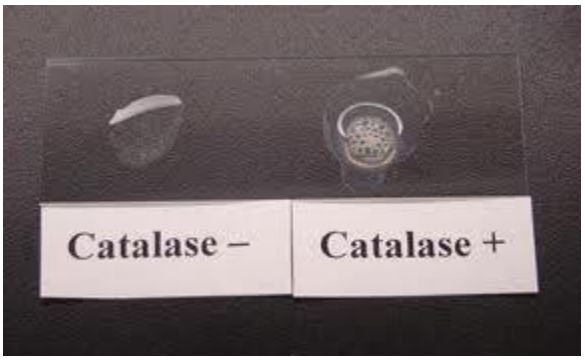


Fig 2.6: Catalase test

- ✓ **Simmon's citrate test**

A small bacterial colony was taken by a sterile needle and streaked on the Simmons citrate agar and incubated at 37^{o}C for 24 hours. After incubation, bacteria which could utilize citrate meant a positive result which changed the agar colour from green to blue where a negative result did not change the colour.

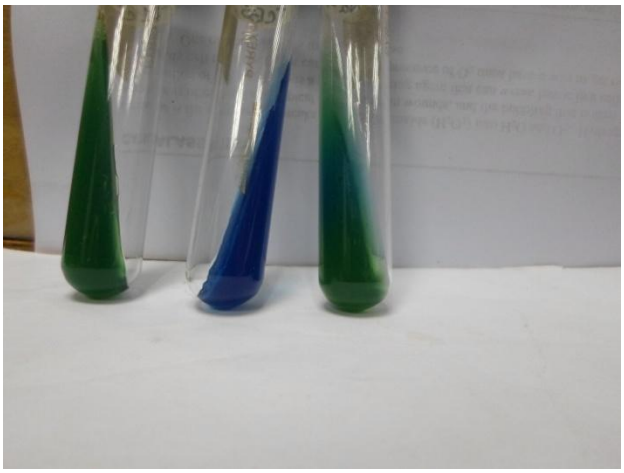


Fig 2.7: Simmons citrate test

- ✓ **Triple sugar iron test**

A small bacterial colony was taken by a sterile needle and stabbed in the TSI agar and then streaked on the agar slant and incubated at 37^{o}C for 24 hours. After incubation, the tubes were observed. Hydrogen sulfide production was detected by blackening of media due to the presence of thiosulfate and ferrous sulfate in the media. A bacterium that was a non-lactose fermenter and fermented glucose, made the agar yellow, some bacteria used thiosulfate as an electron acceptor and reduced it to hydrogen gas which might lift the agar from the butt of the tube or fractured the agar.

Fig 2.8: Triple sugar iron test

- ✓ **Methyl red and Voges-Proskaure test**

A bacterial colony was inoculated in the MR-VP broth and incubated at 37^{o}C for 24 hours. After incubation, the broth was divided into two tubes. One for methyl red test and another for voges-proskaure test. The pH of the medium was tested by the addition of five drops of MR reagent. Development of red colour was taken as positive. MR negative organism produced orange colour.

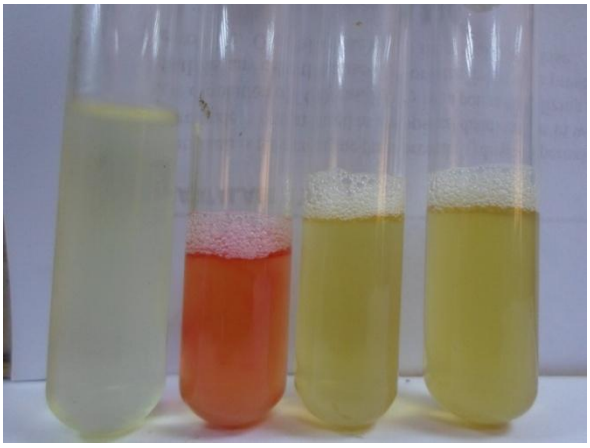


Fig 2.9: Methyl red test; Red color was the positive Methyl red test and others were negative

For VP test, Barritt's reagent A was added to the test broth and shaken. Barritt's reagent B was added and the tube was allowed to stand for 15 minutes. Appearance of red colour was taken as a positive test and no colour development was taken as negative test result.

Fig 2.10: Voges-Proskaure test

- ✓ **Peptone water test**

A small bacterial colony was taken by a sterile loop and mixed in the peptone water and incubated at 37^{o}C for 24 hours. After incubation, kovac's reagent was added. Positive result gave a cherry red colour of the reagent whereas a negative result gave no colour change. Peptone water test is also known as indole test.

Fig 2.11: Indole test; Ring like cherry red color shows the positive Idole test

- ✓ **Motility indole urease**

A small bacterial colony was taken by a sterile needle and stabbed carefully in the agar without touching the bottom. After overnight incubation, the tubes were observed for the presence of motile organisms which made the stab line spreaded and made the agar of the tube turbid. Pink colour appeared where urea was utilized by urease enzyme.

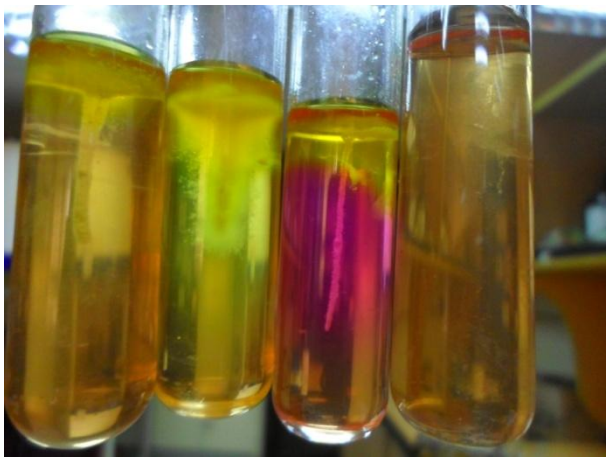


Fig 2.12: Motility indole urease test; Second tube from the left shows positive result for motility

- ✓ **Nitrate reduction test**

Nitrate broth was inoculated with an isolate from each sample plates and incubated for 48 hours.Then reagent A and reagent B were mixed carefully. If the bacterium produced nitrate reductase, the broth would turn into a deep red colour within 5 minutes. If no colour change was observed, then the result was inconclusive. Then a small amount of zinc was added to the broth. If the solution remained colourless after the addition of Zinc then both nitrate reductase and nitrite reductase were present. If the solution turned red, nitrate reductase was not present.

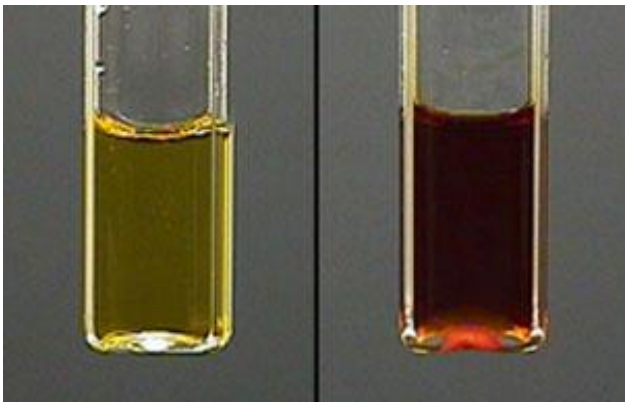


Fig 2.13: Negative nitrate test (Left), Positive nitrate test (Right)

- ✓ **Carbohydrate utilization test**

All the samples were inoculated in all three different carbohydrates, which were sucrose, dextrose and lactose. After inoculation, the tubes were incubated for 24 hours at 37^{o}C. Following incubation, the tubes showed either of the results: acid production, acid and gas production or no fermentation at all. The presence of acid and gas changed the medium into a yellow colour indicating a positive result. Gas production could be detected by the presence of small bubbles in the inverted durham tubes. The broth retaining the red colour was an indication of the absence of fermentation.

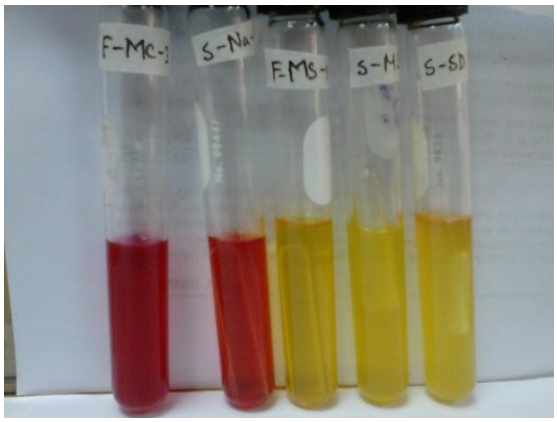


Fig 2.14: Carbohydrate utilization test

- ✓ **Starch hydrolysis test**

All the inoculums from pure cultures were streaked on sterile plate of starch agar. The inoculated plates were incubated at 35-37^{o}C for 24 hours. Iodine reagent was then added to flood the growth. Presence of clear halos surrounding colonies were positive for their ability to digest the starch and thus indicated presence of alpha-amylase.

Fig 2.15: Negative test (Left), Positive test (Right)

- ✓ **Casein hydrolysis test**

All the inoculums from pure cultures were streaked on sterile plate of milk agar. The inoculated plates were incubated at 35-37^{o} C for 24 hours. Casein activity was observed after incubation. The clear and colourless colonies referred that casein was digested. No clear zone meant, no digestion of casein.

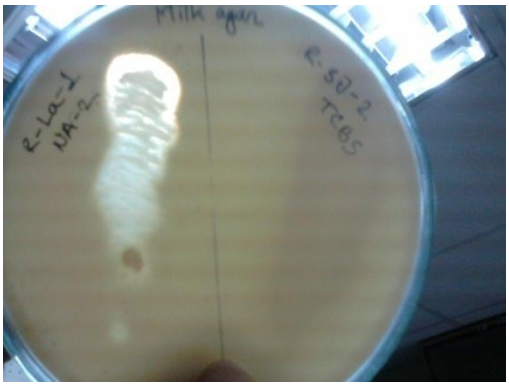


Fig 2.16: Casein hydrolysis test; Left side with clear zone showed positive result and right side without clear zone showed negative result

- ✓ **Blood hemolysis test**

Specific bacterial colonies were streaked on sheep blood agar. After overnight incubation when alpha hemolysis (α-hemolysis) was present, the agar under the colony was dark and greenish and no clear zone around it, lightened (yellow) and transparent clear zone appeared if the bacteria broke blood cells completely which was beta hemolysis and no clear zone appeared if the bacteria was not able to break down blood cells which was indicated as gamma hemolysis.

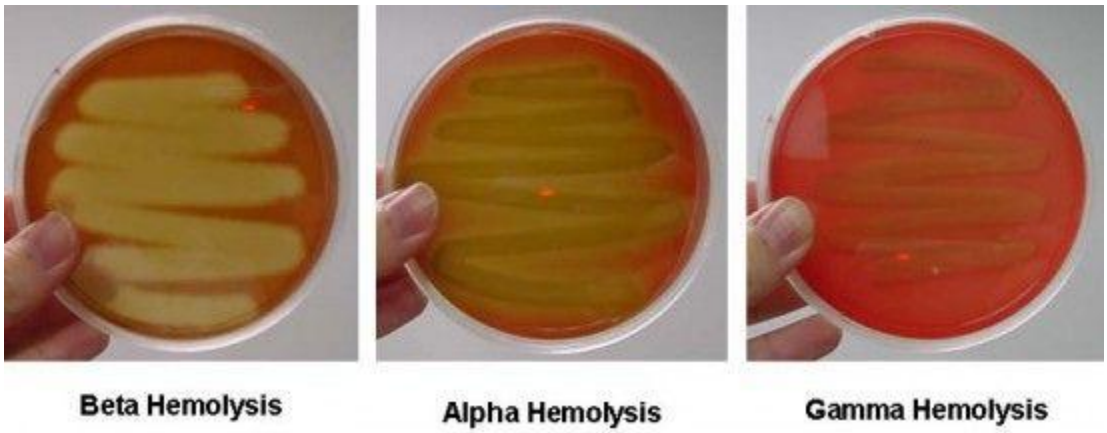


Fig 2.17: Blood hemolysis test

2.3.3 Antibiotic susceptibility test

1. A portion of specific bacteria were collected by loop from fresh sub cultured media and inoculated into 0.9% saline solution and mixed well by vortex machine.
2. The turbidity of the bacterial suspension was compared with standard MacFarlane solution.
3. A cotton swab was dipped into the turbid saline solution and made lawn on Nutrient agar (NA).
4. Specific antibiotic disks were placed on the inoculated NA media through forceps and disks were slightly pressed on the agar to place it well.

5. After 18-20 hours of incubation either different clear zones or no clear zone appeared on inoculated NA plates according to the susceptibility and resistance to specific antibiotics around antibiotic disks.
6. Clear zone of specific diameter indicated susceptibility of bacteria to the specific antibiotic and no clear zone indicated resistance to the antibiotic.

Fig 2.18: Antibiotic susceptibility test

2.4 Bacteria Identification Software: ABIS (Analysis of Bacteria Identification Software) online software

ABIS software gives probable bacterial names based on their morphological, biochemical and cultural characteristics, growth conditions, ecology and pathogenecity data. This database contains an encyclopedia dedicated to a plethora of bacterial species as well as the Kauffman-White scheme for the identification of *Salmonella* serotypes and an antibiogram interpreter.

2.4.1 How to search

- ✓ ABIS online software is typed in Google search bar.
- ✓ ABIS online bacterial identification option is selected.
- ✓ A web page appeared.
- ✓ A user name is inserted and biochemical test is chosen.
- ✓ Then the respective taxons are chosen and then the biochemical test results are inserted and pressed continue button.
- ✓ Probable four organisms names are appeared but the probability and accuracy are different for each bacterium, a high probable bacterium is listed first and then less probable bacteria.
- ✓ A tab is included beside each bacterium name; this option is linked with encyclopedia. By clicking this option an elaborate description will be appeared for specific bacteria.

RESULTS

3. Result

3.1. Microbial loads of different food items

This study was based on statistical analysis of microbial loads of fast food items of street, lower mid-level restaurant, higher mid-level restaurant and high-level restaurant. The work had been done at Gulshan area of Dhaka city.

3.1.1. Qualitative Result

Selected food samples were plated on different selective, non-selective and differential media. After 24 hours and 48 hours of incubation microbial counts were recorded. Total count on Nutrient Agar (NA), coliform count in MacConkey agar (Mac) and *Staphylococci* count on mannitol salt agar (MSA) were recorded. A table format of the results is presented in the next section.

3.1.2. Quantitative Result:

Some selective media were used to observe the presence of some specific microorganisms; Thiosulfate-citrate-bile salts-sucrose (TCBS) agar for *Vibrio* spp., Xylose lysine deoxycholate (XLD) for *Salmonella* spp. and *Shigella* spp. and HiCrome agar for the bacteria which were responsible for urine infection; different bacteria exhibited different colour. The qualitative result is also presented in the next sections.

Quantitative result

Table- 3.1: Total viable count of chicken burger of street, lower mid-level, higher-mid level and high level restaurants

Restaurant type		**NA** CFU/ml	**MAC** CFU/ml	**MSA** CFU/ml
Street	**1**	$2.2X10^7$	$5.48X10^5$	$1.6X10^6$
	2	$4.5X10^6$	Nil	$1.5X10^6$
	3	$3.3X10^5$	$3.3X10^5$	Nil
Lower-mid	**1**	$1.95X10^6$	$9.55X10^5$	$8X10^5$
	2	$2.0X10^6$	$1.8X10^6$	Nil
	3	$1.9X10^6$	$1.0X10^6$	Nil
Higher-mid	**1**	$2.3X10^7$	Nil	$4.33X10^5$
	2	$7.0X10^5$	Nil	$4.3X10^5$
	3	$1.4X10^7$	$1.0X10^5$	Nil
High	**1**	$2.0X10^7$	Nil	$2.1X10^6$
	2	$7.5X10^6$	Nil	$2.2X10^7$
	3	$2.3X10^7$	Nil	$1.9X10^7$

Out of the three burger samples from street food shop, one did not show any microbial growth on MacConkey agar and another sample on MSA. Out of the three burger samples from lower-mid level food shop, two did not show any growth on MSA. Whereas out of the three burger samples from higher-mid level food shop, two did not show any growth on MacConkey agar and one in MSA. All the three burger samples from high level food shops, showed no growth on MacConkey agar.

In NA burger samples collected from street level, lower-mid level, higher-mid level and high level showed bacterial count ranged from $3.3X10^5$ to $2.2X10^7$ CFU/ml, $1.9X10^6$ to $2.0X10^6$ CFU/ml, $7.0X10^5$ to $2.3X10^7$ CFU/ml and $7.5X10^6$ to $2.3X10^7$ CFU/ml respectively.

In MacConkey agar burger samples collected from street level, lower-mid level, higher-mid level and high level showed bacterial count ranged from 0 to $5.48X10^5$ CFU/ml, $9.55X10^5$ to $1.8X10^6$ CFU/ml, 0 to $1.0X10^5$ CFU/ml and 0 CFU/ml respectively.

In MSA burger samples collected from street level, lower-mid level, higher-mid level and high level showed bacterial count ranged from 0 to $1.6X10^6$ CFU/ml, 0 to $8X10^5$ CFU/ml, 0 to $4.33X10^5$ CFU/ml and $2.1X10^6$ to $2.2X10^7$CFU/ml respectively.

Table-3.2: Presence of various types of microorganisms in burgers in different types of selective media

	Colony colour in different selective media		
	Hicrome (colony colour)	**TCBS (colony colour)**	**XLD (colony colour)**
Street	Blue-green (small), golden yellow, Purple-magenta	Yellow	Yellow, red with black center
Lower-mid	Blue-green (small), golden yellow, Purple-magenta	Green, yellow	Yellow, red with black center
Higher-mid	Blue-green (small), golden yellow, colourless(greenish pigment)	Yellow	Yellow with black center
High	Blue-green (small), golden yellow, colourless(greenish pigment)	No growth	No growth

Selective media support the growth of one group of organism while inhibit all other. These media were used to identify microorganisms with the colour they showed.

3.2 Antibiotic susceptibility test

Total twenty isolates of bacteria were selected for antibiotic susceptibility test. Seven antibiotics were tested for each type of bacteria to see the sensitivity and resistance towards those antibiotics. Two tables were prepared according to Gram negative and Gram positive bacteria and showed sensitivity and resistance according to the clear zone diameter. For gram negative bacteria kanamycin, gentamicin, amikacin, cefoxitin, nofloxacin, ampicillin and chloramphenicol antibiotics were used. For gram positive bacteria ampicillin, cefoxitin, cefepime, chloramphenicol, ciprofloxacin, gentamicin and rifampicin were used.

Some bacteria did not show any clear zone which meant they were resistant to that specific antibiotic and some showed very small diameter of clear zone which also was an indicator of antibiotic resistance. The clear zone diameter scale when matched or exceeded the susceptibility diameter scale then the bacteria was considered as sensitive to that specific antibiotic. The standard diameter scale was organized as a chart by antibiotic manufacturing companies and the test outcome was then matched with this chart to interpret the result.

Table-3.3: Antibiotic susceptibility test results of gram negative bacteria

Sample no.		Names of antibiotics						
	Gram negative bacteria (Bacteria from agar plates)	Kanamycin	Gentamicin	Amikacin	Cefoxitin	Nofloxacin	Ampicillin	Chloramphenicol
1	MSA (Y) (Street)	S	S	R	S	S	S	S
2	Hi (Y) (High-mid)	S	S	S	S	S	S	S
3	Hi (V) (HIGH-mid)	S	S	S	S	S	R	S
4	MAC(P,R) (High-mid)	R	S	R	S	R	R	S
5	MAC(P,I) (High-mid)	S	S	S	R	S	R	S
6	XLD(R/B) (HIGH-mid)	S	S	S	R	S	R	S
7	MAC(P) (High-mid)	S	S	S	S	S	S	S
8	XLD(Y) (Low-mid)	S	S	S	S	S	S	S
9	Hi(B/G) (Low-mid)	S	S	S	S	S	S	S
	S= Sensitive, R= Resistant, MSA(Y)- Mannitol salt agar, yellow colony; Hi(Y)- Hicrome, yellow; Hi(V)- Hicrome, violet colony; MAC(P,R)- MacConkey agar, pink regular colony; MAC(P,I)- MAC pink irregular colony; MAC(P)- MAC pink colony; XLD(Y)- Xylose Lysine Deoxycholate agar, yellow colony; Hi(B/G)- Hicrome blue-green colony.							

Antibiotic susceptibility test results for gram negative bacteria, MAC(P,R) from high-mid level restaurant burger, which was *Raoultella planticola* and it was resistant to kanamycin, amikacin, nofloxacin and ampicillin.

In this study none of the Gram negative bacteria was resistant to gentamicin and chloramphenicol. Of the 9 Gram negative isolates, 4 (44%) were found to be resistant to ampicillin, 1(11%) was found to be resistant to kanamyin and nofloxacin, 2(22%) were found to be resistant to cefoxitin.

Table-3.4: Antibiotic susceptibility test results for gram positive bacteria

Sample no.		Names of antibiotics						
	Gram positive bacteria	Ampicillin	Cefoxitin	Cefepime	Chloramphenicol	Ciprofloxacin	Gentamicin	Rifampicin
1	Hi(Y) (High-mid)	R	R	S	S	S	S	S
2	XLD(Y) (high-mid)	R	S	S	S	S	S	S
3	MAC(P) (Street)	R	S	S	S	R	S	S
4	Hi(B/G) (Street)	R	R	S	S	S	S	R
5	Hi(V) (High-mid)	S	R	S	S	S	S	R
6	XLD(R,B) (Street)	S	S	S	S	S	S	R
7	TCBS(Y) (High-mid)	S	S	S	S	S	S	S
8	MSA(Y) (High)	S	R	S	R	S	S	S
9	Hi(B/G) (High)	S	R	S	R	S	S	S
10	XLD(Y) (Low-mid)	S	R	S	S	S	S	R
11	Hi(B/G) (Low-mid)	S	S	S	R	S	S	R
	S= Sensitive; R= Resistant; Hi(Y)= Hicrome agar, Yellow colony; XLD(Y)= Xylose Lysine Deoxycholate agar, yellow colony; MAC(P)= MacConkey agar, pink colony; Hi(B/G)= Hicrome agar, blue/green colony; Hi(V)= Hicrome agar, violet colony; XLD(R,B)= Xylose Lysine Deoxycholate agar, Red with black centered colony; TCBS(Y)= Thiosulfate Citrate Bile Salts Sucrose agar, yellow colony; MSA(Y)= Mannitol Salt agar, yellow colony.							

According to the antibiotic susceptibility test results of gram positive bacteria, Hi(B/G) from street level restaurant burger, which was *Bacillus coagulans* and it was resistant to ampicillin, cefoxitin and rifampicin.

Of the Gram positive organisms studied in this investigation, none of the isolate was resistant to cefepime and gentamicin. Of the 11 Gram positive isolates, 4(36%) were found to be resistant to ampicillin, 6(55%) were found to be resistant to cefoxitin, 3(27%) were found to be resistant to chloramphenicol, 1(9%) was resistant to ciprofloxacin and 5(45%) were resistant to rifampicin.

After the antibiotic tests were done, there were five bacteria those were resistant to none of the antibiotics, four were resistant to only one antibiotic and nine were resistant to two antibiotics, one was resistant to three antibiotics and one was resistant to four antibiotics. Bacteria those resisted three or more antibiotics were multi-antibiotic or multi-drug resistant.

3.3 Biochemical Test Results of Isolates

As one of the main purposes of this research was to find out multi-drug resistant bacteria, therefore, the biochemical tests were done only for those bacteria. Gram staining, Carbohydrate utilization test, Citrate utilization test, Nitrate test, Peptone water test, Methyl-red and Voges-proskaure test, Motility indole urease test, Casein utilization test, Starch utilization test and Hemolysis tests were done for finding out the characteristics of those bacteria. Biochemical test results were then put into online software which analyzed the results and gave four most probable names of bacteria. According to this probable microorganism's name, result tables were prepared. The most highly probable microorganism's name and its biochemical test results were put into the table.

Biochemical test results revealed that *Raoultella planticola* was observed in two samples of higher-mid level restaurant; *Bacillus coagulans* was found in one sample of street level food shop; *Staphylococcus arlettae* was observed in one sample of lower-mid level restaurant; *Streptococcus acidominimus* was found in one sample of higher-mid level restaurant; *Bacillus niacini* and *Bacillus nealsonii* were observed in two different samples of street level food shop; *Paenibacillus validus* was found in another sample of higher-mid level restaurant; *Bacillus firmus* was observed in one sample and *Bacillus megaterium* was observed in another sample of high level restaurant. All isolates were not biochemically tested as the sample size was large and different types of biochemical tests had to be performed.

Table-3.5: Results of standard biochemical tests for identification of unknown microorganisms

Sample name	Gram Stain	Citrate	Nitrate	Motility	Indole	Urease	TSI	Methyl red	Voges-Proskauer	Dextrose	Lactose	Sucrose	Oxidase	Catalase	Blood agar	Milk agar	Starch	Suspected Organism
MAC(P/I) (High-mid)	-ve bacilli	-	-	-	+	+	AG+	+	+	AG+	AG+	AG+	-	+	α-hemolysis	-	-	*Raoultella planticola*-76%
MAC(P/R) (High-mid)	-ve bacilli	-	-	-	-	+	AG+	+	+	AG+	AG+	AG+	-	+	α-hemolysis	-	-	*Raoultella planticola*-76%
Hi(B/G) (Street)	+ve coccobacilli	-	-	-	-	-	AG+	+	+	AG+	AG+	AG+	-	+	α-hemolysis	-	-	*Bacillus coagulans*- 89%
MAC(P) (Low-mid)	+ve cocci	-	-	-	-	+	AG+	+	-	AG+	AG+	AG-	-	+	α-hemolysis	-	-	*Staphylococcus arlettae*- 99%
XLD(R,B) (High-mid)	-ve cocci	-	-	-	-	-	AG+	+	+	AG+	AG+	AG+	-	+	α-hemolysis	-	-	*Streptococcus acidominimus*- 99%
Hi(Y) (Street)	+ve bacilli	+	-	-	-	+	AG-	+	+	AG+	AG+	AG+	-	+	α-hemolysis	-	-	*Bacillus niacini*- 81%
MAC(P) (Street)	+ve bacilli	-	-	-	-	-	AG+	+	+	AG+	AG+	AG+	-	+	α-hemolysis	-	-	*Bacillus nealsonii*-85%
XLD(Y) (High-mid)	+ve bacilli	-	-	-	+	+	AG+	+	+	AG+	AG+	AG+	-	+	α-hemolysis	-	-	*Paenibacillusvalidus*- 82%
Hi(B/G) (High)	+ve bacilli	-	-	-	-	-	AG-	+	-	AG-	AG+	AG-	-	+	β-hemolysis	+	+	*Bacillus firmus*- 84%
MSA(Y) (High)	+ve bacilli	-	+	-	-	-	AG-	+	-	AG-	AG+	AG-	-	+	β-hemolysis	+	+	*Bacillus megaterium*- 81%

MAC(P/I)= MacConkey agar, pink and irregular colony; MAC(P/R)= MacConkey agar, pink and regular colony; Hi(B/G)= Hicrome agar, blue/green colony; MAC(P)= MacConkey agar, pink colony; XLD(R,B)= Xylose Lysine Deoxycholate agar, red with black centered colony; Hi(Y)= Hicrome agar, yellow colony; XLD(Y)= Xylose Lysine Desoxycholate agar, yellow colony; MSA(Y)= Mannitol Salt agar, yellow colony; 'A'= Acidic; 'G'= Gas; '+'= Positive; '-'= Negative.

DISCUSSION

4. Discussion

Based on the research study, it was observed that total viable count, enteric pathogen and coliform count was relatively higher in street and higher-middle level restaurant burgers and the highest in high level restaurant burgers. This was due to the street dust, unhygienic water, dishes, food handling etc for the street side foods. In higher-middle level and high level restaurants, this result was seen due to unhygienic handling of foods, improper storage temperature and storing and uses of unhygienic and expired raw materials. Therefore, overall the lower-middle level restaurant burgers were less in microbial load than street, higher-middle level and high level restaurant burgers.

In Washington State in January1993, an *E. coli* outbreak caused severe illness in 144 people, many of whom ate undercooked hamburgers prepared by Jack-in the- Box fast-food restaurants. A majority of the seriously ill were young children, who had to undergo kidney dialysis for weeks. Although media reports indicated that the outbreak killed four children, health officials could only link one of those deaths to the hamburger from the restaurant chain (Garrett, 1994).
All these incidents happened because of the improper handling of food, improperly washed utensils, unhygienic cooking materials, raw materials etc.

From the quantitative result it was observed that out of the three burger samples from street food shop, one sample did not show any growth on MacConkey agar and one sample did not show any growth on MSA. Out of the three burger samples from lower-mid level food shop, two samples did not show any growth on MSA. Among the three burger samples from higher-mid level food shop, two samples did not show any growth on MacConkey agar and one sample did not show any growth on MSA. Amidst of the three burger samples from high level food shops, all three samples did not show any growth on MacConkey agar. This indicated that, sample those did not give any growth on MacConkey agar might not contain any gram negative bacteria and sample those did not give any growth on MSA might not contain any *Staphylococcus*.
Rayan and Ray (2004) found that upon prolonged refrigeration, lactic acid bacteria, micrococci, enterobacteria, bacillus and yeast may grow and form slime on burgers. Human contact may sometimes introduce a few *Escherichia coli* or *Staphylococcus aureus* which was indicated by the growth of yellow colonies on MSA.

After conducting all the biochemical tests, the results were put into the ABIS online software. According to the biochemical test result interpretation *Raoultella planticola* was observed in two burger samples of the higher-mid level restaurant; *Bacillus coagulans* was observed in one sample of the street level restaurant; *Staphylococcus arlettae* was observed in one sample of the lower-mid level restaurant; *Streptococcus acidominimus* was observed in one sample of the higher-mid level restaurant; *Bacillus niacini* and *Bacillus nealsonii* were observed in two different samples of the street level restaurant; *Paenibacillus validus* was observed in one sample of the higher-mid level restaurant; *Bacillus firmus* was observed in one sample of the high level restaurant and *Bacillus megaterium* was observed in another sample of the high level restaurant. Almost all the samples had different types of bacteria. According to a survey by Consumer Reports, researchers examined 300 samples of ground beef from grocery stores in 26 cities across the United States. Although they did not detect *E. coli* 0157, they did find at least one type of bacteria in every sample they tested (Carina Storrs, 2015).

According to the antibiotic test results, almost at every level there was bacterial isolates in foods, which resisted one or two antibiotics and those were not serious and acceptable. However, comparing with other levels of foods, higher middle level and street level restaurants burgers contained bacteria which were resistant to multiple antibiotics. Those bacteria were resistant against 3 or more antibiotics, which meant they were multidrug resistant. *Raoultella planticola* from higher-middle level restaurant burger was resistant against four antibiotics and *Bacillus coagulans* from street level restaurant burger was resistant against three antibiotics. This is an indicator that, day by day bacteria are becoming more antibiotic resistant which will lead to different diseases and people will not be able to treat them by antibiotics.

Among the antibiotics used none of the gram negative bacteria was resistant to gentamicin and chloramphenicol. Of the 9 gram negative isolates, 4 (44%) were found to be resistant to ampicillin, 1(11%) was found to be resistant to kanamyin and nofloxacin, 2(22%) were found to be resistant to cefoxitin. Among the antibiotic used none of the gram positive bacteria was resistant to cefepime and gentamicin. Of the 11 gram positive isolates, 4(36%) were found to be resistant to ampicillin, 6(55%) were found to be resistant to cefoxitin, 3(27%) were found to be resistant to chloramphenicol, 1(9%) was resistant to ciprofloxacin and 5(45%) were resistant to rifampicin.

Over the years antibiotics have produced a great paradox. After their introduction into medical practice nearly 50 years ago, the drugs controlled many life-threatening diseases, reduced death and illness, and increased the life expectancy of Americans. Since then, the use of antibiotics, including inappropriate uses that have little benefit to the patients, has fostered antibiotic resistance and caused many antibiotics to lose their effectiveness against some bacterial diseases. As a result, some illnesses that were once easily treatable now pose problems for patients and physicians. (Princeton education, 1995)

Conclusion

The present study was carried out to compare the microbial quality among the chicken burgers of street level, lower-middle level, higher-middle level and high level restaurants and finding out multi-drug resistant bacteria. The collected foods were handled with much delicacy to avoid any kind of contamination. All the precautions, protocols and safety instructions were followed very strictly. After finishing all the works with those samples, they were preserved safely so that if any result went wrong or anyone wanted to analyze or investigate them further, can be done.

Finally, people should become more aware in having foods from outside. People selling and preparing the foods should also become more alert. Food safety rules and implementation of food regulatory laws in food preparation, serving and preservation should be strongly maintained to avoid contamination problems and food-borne diseases.

References

Antibiotic use and resistance in the community; Princeton education (1995); Retrieved from https://www.princeton.edu/~ota/disk1/1995/9503/950305.PDF

Asma Binte Afzal. (2014); Microbiological qualities of some foods sold in the streets and in the mid-level and high-level restaurants. MSC thesis, BRAC University.

Bryan, F.L., Michanie, S.C., Alvarez, P., Paniagua, A; (1988). Critical control points of street vended foods in Dominican Republic. Journal Food Prot. **51**, 373-383.

Carina Storrs; (2015). What's bugging your burger? High rates of bacteria found in ground beef. Special to CNN.

Chiou, T.Y., Wang, M.Y., Lin, A.Y.; (1996). Sanitary indicator bacteria of the hot-keeping cooked food items in Southern Taiwan. Food Sci. **23**, 909-912.

FAO regional workshop on street food in Asia, held in Jakarta, Indonesia in 1986

Garrett, L.; (1994). *The Coming Plague: Newly Emerging Diseases in a World Out of Balance* (New York, NY: Farrar, Straus and Giroux).

Gimenez, B.C., Salgaard, P.; (2004). Modeling and Predictive the simultaneous growth of Listeria Monocytogenes and spoilage microorganisms in cold smoked acimoon, Microbiol. **96**, 96-109.

Ha Thi Anh Dao; (1995). The Microbiological Quality of Street Foods Compared to Home Prepared and Hotel Restaurant Foods in Hanoi-Vietnam. Id.-80621

Jones, F.T., Axtell, R.C., Rives, D.V., Scheideler, S.E., Tarver, F.R., Walker, R.L., Wineland, M.J.; (1991). A Survey of Campylobacter jejuni contamination in modern broiler production and processing systems. Journal Food Prouction, **54**, 259-262.

Kampen J V, Gross R, Schultink W and Usfar A.; (1998). The Microbiological Quality of Street Foods in Jakarta as Compared to Home prepared foods and foods from tourist hotels, International Journal of Food Sciences and Nutrition, **49**. 17-26.

Long, S.M., Adak, G.K., O'Beirne, S.J, Gillesple I.A.; (2002). General Outbreaks of infectious intestinal disease linked with salad vegetables and fruits, England and Wales. 1992-2000, Communicable Disease Public health. **5**, 101-105.

Mosupye, F.M., von Holy, A.; (1999). Microbiological quality and safety of ready-to-eat street-vended foods in Johannesburg, South Africa, Journal of food Protection. **62** (11), 1278-1284

Martins, J.M., Anelich, L.E.; (2000). Socio-economic features of street vending, hygiene and microbiological status of street foods in Gauteng, 2000, Technical Cooperation Programme (TCP) Project on Improving Street Foods in South Africa.

Meena, C.A., Meida Carni, L., Teixeira, P., Hogg, T., Gibbs, P.A.; (2004). Incidence of Listeria monocytogenes in different food products commercialized in Portugal, Food Microbiol **21**, 213-216.

Ray, C. George; Ryan, Kenneth J.; Kenneth, Ryan.; (2004). Sherris Medical Microbiology: An Introduction to Infectious Diseases (4th ed.). McGraw Hill. p. 237.

Ryu, J.H., Beuchat, L.R.; (1998). Influence of acid tolerance responses of survival, growth, and cross protection of E.coli O157:H7 in acidified media and fruit juices. International Journal of Food Microbiol. **45**; 185-193.

Sandeep, M., Diwakar.A., Abhijit, G.; (2001). Microbiological Analysis of street vended fresh squeezed Carrot and Kinnow-Mandarian Juices in Patiala City, India. International Journal of Food Safety, **3**, 1-3.

Thunberg, R.L., Tran Bennett, R.W., Matthews R.N., Belay. N.; (2002). Microbial evaluation of selected fresh produce obtained at retail market. Journal of Food Prot. **65**,677-682.

APPENDICES

Appendix-1

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 121oC 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. **MacConkey Agar (Oxoid, England)**

Ingredients	Amounts (g/L)
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium Chloride	5.0
Neutral red	0.075
Agar	12.0

3. **Mannitol Salt Agar (Himedia, India)**

Ingredients	Amounts (g/L)
Proteose peptone	10.0
Meat Extract	1.0
Sodium Chloride	75.0
D-Mannitol	10.0

Phenol red	0.025
Agar	15.0

4. Thiosulfate Citrate Bile Salts Sucrose agar (Himedia, India)

Ingredients	**Amounts (g/L)**
Proteose peptone	10.0
Yeast extract	5.0
Sodium Thiosulphate	10.0
Sodium Citrate	10.0
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

5. Xylose Lysine Deoxycholate agar (Himedia, India)

Ingredients	**Amounts (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

6. **Hicrome UTI agar (Himedia, India)**

Ingredients	Amounts (g/L)
Peptic digest of animal tissue	15.0
Chromogenic mixture	26.80
Agar	15.0

7. **Alkaline Peptone water (Himedia, India)**

Ingredients	Amounts (g/L)
Peptic digest of animal tissue	10.0
Sodium chloride	10.0

8. **Selenite cystine broth (himedia, India)**

Ingredients	Amounts (g/L)
Casein enzymic hydrolysate	5.0
Lactose	4.0
Disodium phosphate	10.0
L-Cystine	0.01

9. **Simmon's citrate agar (Himedia, India)**

Ingredients	Amounts (g/L)
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	1.0
Dipotassium phosphate	1.0
Sodium citrate	2.0
Sodium chloride	5.0
Bromothymol blue	0.08
Agar	15.0

10. Triple sugar iron agar (Himedia, India)

Ingredients	Amounts (g/L)
Beef extract	3.0
Peptone	20.0
Yeast extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose monohydrate	1.0
Ferrous sulphate	0.2
Sodium chloride	5.0
Sodium thiosulphate	0.3
Phenol red	0.024
Agar	12.0

11. Nitrate agar (Himedia, India)

Ingredients	Amounts (g/L)
Peptic digest of animal tissue	5.0
Beef extract	3.0
Potassium nitrate	1.0
Agar	12.0

12. Motility Indole Urease agar (Himedia, India)

Ingredients	Amounts (g/L)
Casein enzymic hydrolysate	10.0
Dextrose	1.0
Sodium chloride	5.0
Phenol red	0.01
Agar	2.0

13. Methyl-red and Voges-proskauer media (Himedia, India)

Ingredients	Amounts (g/L)
Buffered peptone	7.0
Dextrose	5.0
Dipotassium phosphate	5.0

14. Phenol red sucrose broth (Himedia, India)

Ingredients	Amounts (g/L)
Proteose peptone	10.0
Beef extract	1.0
Sodium chloride	5.0
Sucrose	5.0
Phenol red	0.018

15. Phenol red dextrose broth (Himedia, India)

Ingredients	Amounts (g/L)
Proteose peptone	10.0
Beef extract	1.0
Sodium chloride	5.0
Dextrose	5.0
Phenol red	0.018

16. Phenol red lactose broth (Himedia, India)

Ingredients	Amounts (g/L)
Proteose peptone	10.0
Beef extract	1.0
Sodium chloride	5.0

Lactose	5.0
Phenol red	0.018

17. Skim milk agar (Himedia, India)

Ingredients	**Amounts (g/L)**
Skim milk powder	28.0
Casein enzymatic hydrolysis	5.0
Yeast extract	2.5
Dextrose	1.0
Agar	15.0

18. Starch agar (Himedia, India)

Ingredients	**Amounts (g/L)**
Meat Extract	3.0
Peptic digest of animal tissue	5.0
Starch, soluble	2.0
Agar	15.0

Appendix- 2

Reagents

Kovac's reagent

1.25 gm of para-dimethyl aminobenzaldehyde was dissolved in 18.75 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^{o}C.

Methyl red reagent

0.01 gm of methyl red was dissolved in 30 ml of 95% ethanol. Then distilled water was added to make the final volume 50 ml. This reagent was covered with aluminum foil and stored at 4^{o}C.

Barritt's reagent

Solution A

1.25 gm of alpha-naphthol was dissolved in 95% ethanol by stirring constantly to dissolve and made 25 ml of solution. This solution was covered with aluminum foil and stored at 4^{o}C.

Solution B

10 gm of KOH was dissolved in distilled water. The solution became warm. After cooling to room temperature, creatine was added and dissolved by stirring. Distilled water was added to make the final volume 25 ml. This solution was covered with aluminum foil and stored at 4^{o}C.

MacFarlane turbidity(standard no.5)

0.05 ml of 1.175% barium chloride dihydrate ($BaCl_2.2H_2O$) was mixed with 9.95 ml of 1% sulphuric acid (H_2SO_4)

Methyl red reagent

0.1g 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 4oC.

Oxidase reagent

100 mg of N,N,N1,N1-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.

Catalase reagent

35% Hydrogen peroxide

Nitrate reagent

Solution A, Sulfanilic acid 1gm of sulfanilic acid was dissolved in 125 ml of 5N acetic acid.

Solution B, Alpha-napthylamine 0.625 gm of α-napthayl amine dissolved in 120ml of 5N acetic acid.

Appendix 3

Instruments

The important equipments used through the study are listed below:

Instruments	Manufacturer
Autoclave	SAARC
Incubator	SAARC
Micropipette (10-100µl)	Eppendorf, Germany
Micropipette (20-200µl)	Eppendorf, Germany
Micropipette (100-1000µl)	Eppendorf, Germany
Oven	LG, China
Refrigerator ($4^{o}C$)	Samsung
Safety cabinet, Class II Microbiological	SAARC
Water bath	Korea
Weighing balance	ADAM EQUIPMENT™, United Kingdom
Vortex Mixture	VWR International
Light microscope	Olympus CX45

Appendix 4

List of antibiotics

Name	Concentration	Short form	Manufacturer
Ampicillin	10 µg/disc	AMP	Himedia
Amikacin	30 µg/disc	AK	Himedia
Cefoxitin	30 µg/disc	CX/FOX	Himedia/Oxoid
Cefepime	30 µg/disc	CPM	Himedia
Chlorumphenicol	10 µg/disc	C	Himedia
Ciprofloxacin	5 µg/disc	CIP	Himedia/Oxoid
Gentamicin	10 µg/disc	CN	Himedia/Oxoid
Kanamycin	30 µg/disc	K	Himedia/Oxoid
Norfloxacin	10 µg/disc	NOR	Oxoid
Rifampicin	5 µg/disc	RD	Himedia